(8) W. W. Mapleson, *J. Appl. Physiol.*, **18**, 197(1963).
(9) K. B. Bischoff and R. L. Dedrick, unpublished data, 1969.
(10) A. S. V. Burgen and N. J. Goldberg, *Brit. J. Pharmacol.*, **19**, 313(1962).
(11) A. M. Guarino and L. S. Schanker, *J. Pharmacol. Exptl. Therap.*, **164**, 387(1968).
(12) J. Borsa, A. F. Whitmore, F. A. Valeriote, D. Collins, and W. R. Bruce, *J. Natl. Cancer Inst.*, **42**, 235(1969).
(13) S. E. Bradley, in "Handbook of Physiology," Section 2, 1963, Circulation, vol. 2, *Am. Physiol. Soc.*, p. 1405.

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Intestinal Drug Absorption and Metabolism I: Comparison of Methods and Models to Study Physiological Factors of *In Vitro* and *In Vivo* Intestinal Absorption

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Abstract □ Two experimental methods for use in kinetic studies on a compartment model for intestinal metabolism and absorption were evaluated. The *in vitro* cannulated everted intestinal sac and the *in vivo* intestinal loop with complete mesenteric venous collection were compared in the same region of rabbit intestine. These experimental methods were used to study the effects of metabolism, tissue accumulation, and blood flow on the transport of salicylamide across the basal barrier and provide experimental evidence to support the cell compartment model. At lower initial mucosal concentration (10^{-3} *M*), over 60% of the drug appearing in mesenteric blood is conjugated with glucuronic acid. At higher initial mucosal fluid concentrations, glucuronide conjugation appears to be capacity limited and the disappearance from the lumen-curve shows a distinct distributive phase characteristic of a cell compartment model. The rate of transport of free drug across the basal barrier is blood flow rate-limited while the transport of glucuronide is essentially independent of blood flow. Appearance of free salicylamide into mesenteric blood, *in vivo*, shows a lag time of 4 min. compared to a lag time of about 10 min. for the appearance of free drug into serosal fluid *in vitro*. The steady state rate of appearance of free drug into the plasma (*in vivo*) is five to ten times greater than the rate of appearance of free drug into the serosal fluid (*in vitro*) at similar mucosal concentrations. The *in vivo* intestinal loop with complete venous collection was found to have many advantages in studying physiological factors of intestinal drug absorption.

Keyphrases □ Drug absorption—intestinal □ Everted intestine—drug absorption, *in vitro* □ Intestinal loop, cannulated—drug absorption, *in vivo* □ Salicylamide—absorption, accumulation, metabolism □ Glucuronic acid-salicylamide conjugation—concentration effect □ Fluorometry—analysis

From the standpoint of a drug administered orally to produce a systemic effect, intestinal absorption can be considered as the amount of unchanged drug absorbed from the intestinal lumen which appears in the portal circulation or intestinal lymph. Using this definition, the amount of drug disappearing from the lumen contents is sufficient to characterize the absorption process only if it reflects the rate of appearance of unaltered drug in the blood. If one accepts the above definition, then it becomes quite important to evaluate critically the widely used (1) practice of assessing absorption by monitoring only the rate of disappearance of drug from the lumen. The condition that the rate of disappearance of free (unaltered) drug from the lumen contents is identical to the rate of appearance of free drug in the mesenteric blood is implicit in the classical lipoid barrier model of intestinal absorption which assumes that the intestinal tissue is a single barrier which does not contribute to the material balance of the system.[1] When accumulation or metabolism of drug occurs in the intestinal tissue, these rates may not be identical and a tissue compartment model for absorption is considered here as a more appropriate model to describe the entire absorption sequence.

The studies described in this report were designed to compare *in vivo* and *in vitro* experimental methods and mathematical models that might be useful in studying the effect of physiological factors, such as accumulation and metabolism of drug in the intestinal tissue and intestinal blood flow, on the appearance of free drug into mesenteric blood.

The general multiple barrier tissue compartment model that will be used to describe these physiological variables in *in vivo* and *in vitro* preparations is shown in Fig. 1. This catenary three-compartment model assumes that the intestinal tissue can be described as a homogeneous compartment, which is separated from the lumen compartment by an apical barrier (α) and from the terminal blood or serosal fluid compartment by a

[1] In compartment theory, a barrier may be defined as that rate-limiting step between two compartments which distinguishes these compartments by the fact that the rate of distribution within each compartment is sufficiently rapid, compared to the rate of transport across the barrier, that each compartment may be described by a separate volume of distribution. The barrier is considered to be only a rate-limiting step of negligible volume which does not contribute to or alter the material balance of the system. See, for example, the discussion on the concept of a barrier by Riggs (*Reference 32*, p. 188) and the concept of a compartment being represented as a volume by Resigno and Segre (*Reference 31*, p. 16).

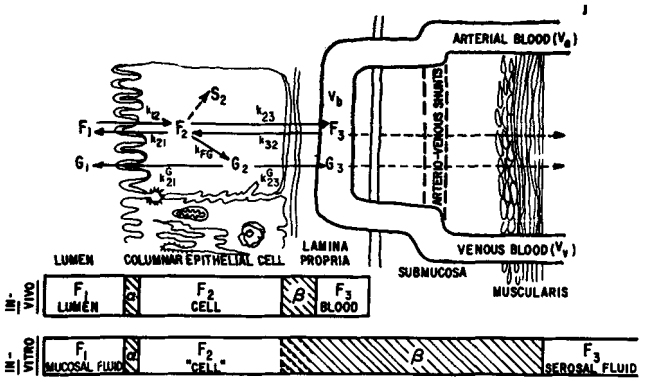


Figure 1—*General compartment model for intestinal absorption including metabolism in the epithelial cell and transport across the apical* (α) *and basal* (β) *barrier into mesenteric blood* (in vivo) *or serosal fluid* (in vitro). *Key:* F_1 *is the amount of free (unmetabolized) drug in the lumen contents;* F_2 *is the amount of free drug in the tissue;* F_3 *is the amount of free drug in the blood in* in vivo *preparations and in the serosal fluid of isolated* in vitro *intestinal preparations;* G_2 *is the amount of metabolite formed in the intestinal cell* (e.g., *glucuronide*). *For some cases, more than one metabolite may be formed as illustrated by* S_2; k_{mn} *is the rate constant for the transfer of amount from Compartment* m *to Compartment* n, e.g., k_{21} *is the rate constant for the transfer of free drug from Compartment 2 to Compartment 1;* k_{mn}^g *is the rate constant for the transfer of amount of metabolite from Compartment* m *to Compartment* n; k_{fg} *is the rate constant for the formation of metabolite.*

basal (β) barrier and that metabolism of the drug may occur in the tissue.

The anatomical entities corresponding to the α and β barriers cannot, at the present state of knowledge, be defined. There are many potential barriers that a drug may encounter during gastrointestinal absorption. It is probable that the barriers corresponding to the rate-limiting steps may be different for different preparations and drugs and possibly for different ionic species of the same drug. It is also clear that the present three-compartment model may be inadequate and additional compartments or a stochastic model may be required to describe the absorption mechanisms of some drugs.

It can be shown that the tissue compartment model will reduce to a simple apparent barrier model when metabolism is negligible and the rate constant for transfer of drug across the basal barrier is much greater than the rate constant for transfer of drug across the apical barrier. The important differences between the compartment and barrier models are summarized in Table I.

It is likely that no single experimental method will be ideal to study physiological factors of absorption and that the maximum information will often require corroborative evidence from more than one method. It was of interest, therefore, to compare different methods in the same intestinal region of the same animal. Two preparations were selected to compare *in vivo* and *in vitro* methods using the same intestinal region.

The *in vitro* preparation selected was a cannulated everted intestine which would eliminate possible variables due to blood flow and permit serial sampling from both the mucosal and serosal contents. The everted intestinal sac which was introduced by Wilson and Wiseman (2) has the advantage of simplicity and flexibility and is beginning to find use in studies on drug absorption (3–8). Everted intestinal sacs have also been used to show intestinal glucuronide formation (6) and

Table I—Comparison of Apparent Barrier and Tissue Compartment Models of Intestinal Absorption

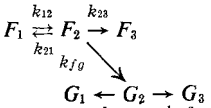
Apparent Barrier Model	Tissue Compartment Model
The intestinal tissue does not contribute to or alter the material balance of free drug in the lumen, blood, or serosal compartments.	The intestinal tissue must be considered in the material balance of free drug in the system.
No appreciable accumulation of free drug in tissue.	Accumulation of free drug in tissue may occur.
Apical barrier is rate-limiting step for appearance of free drug in plasma ($k_{12} < k_{23}$).	Basal barrier is rate-limiting step for appearance of free drug in plasma ($k_{12} > k_{23}$).
No metabolism of drug in tissue.	Metabolism of drug in the tissue may occur.
Rate of appearance of free drug in plasma can be predicted by measurements on the disappearance of drug from the lumen fluid.	Measurements on lumen fluid alone may not be sufficient to characterize appearance of free drug in plasma. Tissue or blood may also have to be sampled.
Semilog plot of amount of drug in the lumen *versus* time will appear linear with intercept near to the initial amount in lumen.	Semilog plot of amount of drug in the lumen *versus* time is biexponential showing distributive phase.

to study the mechanisms involved in glucuronide transport (7, 8).

The *in vivo* method selected for this study was a cannulated intestinal preparation with complete mesenteric venous blood collection. By cannulating the mesenteric vein and leaving the arterial supply intact, the rate of appearance of drug in the blood is measured directly without the complicating factors of tissue distribution in the body, hepatic metabolism, and urinary excretion. This type of preparation has not been generally applied to studies of drug absorption, but has been used to study intestinal metabolic processes of sugars and amino acids in the cat (9), dog (10), guinea pig and rat (11), and steroid conjugation in humans (12).

These two preparations have the advantage that the terminal compartment is closed, permitting direct evaluation and comparison of transfer across the *in vivo* and *in vitro* basal barrier.

Through the use of experimental procedures such as the "*in vivo* intestinal loop with complete venous collection," the assumptions of a closed system and irreversible transfer of drug from the tissue to the blood compartment are valid and permit considerable simplification of the general model (Model I) to the form,



Model II

which is described by the following differential equations:

$$-dF_1/dt = k_{12}F_1 - k_{21}F_2 \quad \text{(Eq. 1)}$$

$$dF_2/dt = k_{12}F_1 - k_{21}F_2 - k_{23}F_2 - k_{fg}F_2^* \quad \text{(Eq. 2)}$$

$$dF_3/dt = k_{23}F_2 \quad \text{(Eq. 3)}$$

$$dG_2/dt = k_{fg}F_2^* - k_{23}^gG_2 - k_{21}^gG_2 \quad \text{(Eq. 4)}$$

The quantity, F_2*, will vary and be equal to F_2 in the case of first-order metabolism or be fixed at a certain value in the case of zero-order metabolism. The fixed amount may be considered that amount which saturates the enzyme system so that $V_{max.}$ is reached and is equal to $k_{f_0}F_2$* which is an apparent zero-order rate constant.

Rabbits were selected as the experimental animal for several reasons. Cannulation of the mesenteric vein in smaller animals is difficult. A larger animal such as a dog would require the replacement of large amounts of blood to maintain blood flow. Furthermore, as the size of the animal increases, the thickness of the intestine precludes use for *in vitro* methods such as everted intestinal sacs or *in vitro* intestinal perfusions so that *in vivo* and *in vitro* methods cannot be easily compared in the same animal. It is stated that the general intestinal vasculature of the rabbit is very similar to that of man (13).

Salicylamide (SAM) was chosen as a suitable drug to use for the following studies on accumulation and metabolism in the cell compartment for two reasons. First, it has sufficient lipid solubility and is largely undissociated at physiological pH (pKa 8.9) (14). Second, Schachter (6) has shown that salicylamide is appreciably conjugated with glucuronic acid in *in vitro* everted intestinal sacs. Salicylamide therefore provides a model substrate to study the effect of intestinal metabolism on the *in vivo* availability of a drug.

EXPERIMENTAL METHODS

Everted Sacs for Kinetic Studies—The apparatus used, a modification of that used by Crane and Wilson (15), is shown in Fig. 2. An intestinal segment (about 10 cm.) was quickly removed from a male New Zealand rabbit under pentobarbital anesthesia, washed gently with Krebs-Ringer bicarbonate solution (16) (pH 7.4), and everted by means of a glass rod inserted through the lumen. A disposable polyethylene centrifuge tube was inserted in one end of the intestine and tied securely with umbilical tape. The opposite end was tied and 7 to 10 ml. of Krebs-Ringer bicarbonate solution introduced through the hypodermic needle inserted in the polyethylene tube. The intestine was then placed in the mucosal fluid containing 75 ml. of 10^{-4} to 10^{-2} M drug in Krebs-Ringer bicarbonate solution maintained at 37° and saturated with 95% O_2 and 5% CO_2 by directly bubbling the O_2 and CO_2 into the mucosal fluid. Serial samples of known volume were taken from both mucosal and serosal fluids by means of 1-ml. disposable plastic syringes.

Fixed-time studies used everted sacs closed at both ends which were incubated either under the conditions used by Herz *et al.* (7) for rat intestine, which was oxygenated by an O_2 and CO_2 (95% and 5%) atmosphere flowing at 122–152.5 cm.³/hr. (4–5 ft.³/hr.), or by bubbling the O_2–CO_2 mixture directly into the mucosal fluid.

***In Situ* Isolated Intestinal Preparation with Intact Arterial Supply and Complete Venous Collection**—Male rabbits weighing between 2 and 3 kg. were anesthetized with pentobarbital or urethan–pentobarbital mixture. A small midline incision allows the gentle exposure of a 20–60-cm. midileal portion of the intestine. This portion was selected because of its accessibility and suitable vasculature to facilitate cannulation. The mesenteric arcades to adjacent portions were carefully tied off. The intestine was cut and cannulated with Tygon tubing for either perfusion (Fig. 3*A*) or a closed loop (Fig. 3*B*). The mesenteric vein was cannulated with an appropriate size of Silastic or polyethylene tubing and all venous blood collected in heparinized, calibrated centrifuge tubes. The mean-flow rate was determined directly by recording the quantity of blood collected in successive intervals, usually 10 min. The blood lost from the mesenteric vein was continuously replaced with an equal volume by an intravenous infusion, *via* the jugular vein, of heparinized blood–saline mixture or whole blood previously collected from donor animals. The carotid artery was also cannulated for systemic arterial samples.

The isolated intestine was kept warm and moist by frequent application of warm (37°) saline to a gauze pad covering the intestine

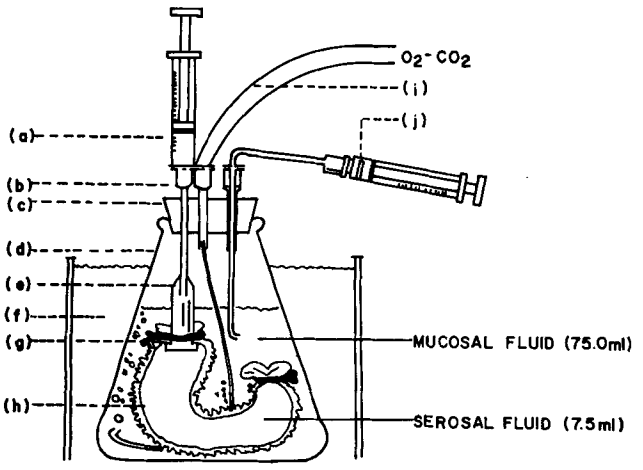


Figure 2—*Diagramatic illustration of apparatus used in the* in vitro *cannulated everted intestine preparations. Key:* (a) *1-ml. disposable plastic syringe used to collect serosal fluid;* (b) *large hypodermic needle (14 gauge);* (c) *rubber stopper;* (d) *125-ml. conical flask;* (e) *disposable polyethylene centrifuge tube;* (f) *water bath (37°);* (g) *umbilical tape used to fasten intestine to centrifuge tube;* (*h*) *everted intestine;* (i) *inlet for gas mixture (O_2–CO_2, 95%–5%);* (j) *1-ml. disposable plastic syringe used to collect mucosal fluid.*

SCHEMATIC DIAGRAM ILLUSTRATING
THE IN VIVO INTESTINAL PREPARATION WITH COMPLETE
VENOUS COLLECTION

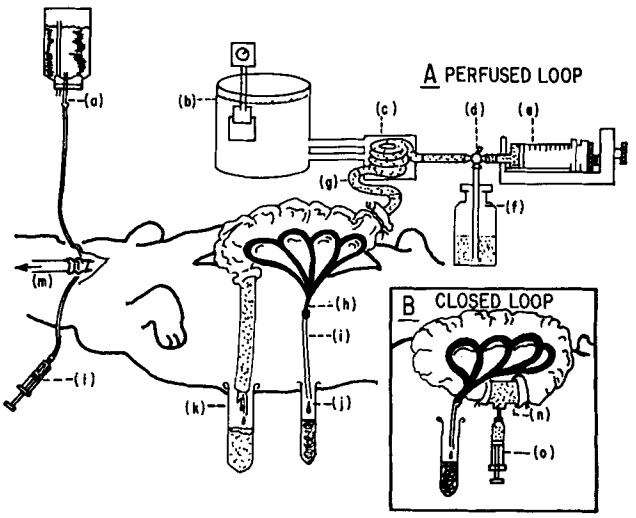


Figure 3—In vivo *intestinal preparation with complete venous collection.* A, *perfused intestine;* B, *closed intestinal loop. Key:* (a) *blood (or blood-saline mixture) infused into jugular vein;* (b) *constant temperature water bath;* (c) *heat exchange coil for perfusate;* (d) *three-way stopcock to refill infusion pump* (e) *with perfusate* (f); (g) *Tygon tubing connecting the perfusion system to the isolated intestinal loop;* (h) *mesenteric vein draining ileal segment;* (i) *polyethylene cannula draining mesenteric venous blood;* (j) *calibrated centrifuge tube (15 ml.);* (k) *centrifuge tube to collect perfusion effluent (50 ml.);* (l) *carotid cannulation for arterial sample;* (m) *attachment to animal respirator;* (n) *Tygon tubing attaching both ends of intestine to form closed loop;* (o) *1-ml. syringe used to collect samples of lumen fluid and also periodically to mix lumen fluid.*

which in turn was covered by a dental rubber dam. A small lamp placed over the area was sufficient to maintain the preparation at 37°.

Spectrophotofluorometric Assay of Salicylamide (SAM) and Its Metabolites in Plasma and Biological Fluids—The usual colorimetric method of assaying salicylamide (SAM) by a ferric ion complex proved too insensitive to be applicable to these studies. Salicylamide (SAM) is fluorescent in basic solution. This property was used to develop a fluorescence assay in which free SAM (pKa 8.9) (14) was extracted from an acidified or buffered (pH 4.3) biological fluid into an ethylene dichloride–cyclohexane (65:35) mixture and reextracted into an aqueous 0.2 *N* sodium hydroxide solution. The fluorescence of SAM in the aqueous basic solution was determined directly by an Aminco-Bowman spectrophotofluorometer at the maximum activation and emission wavelengths of 350 and 430 mμ, respectively (both uncorrected). This procedure provided a sensitive and reproducible assay capable of estimating concentration of free SAM as low as 0.5 mcg./ml. Concentrations of 1 to 50 mcg./ml. were easily assayed with an average standard error of the mean usually less than ±3%. SAM glucuronide is expressed as the free SAM released after hydrolysis with bacterial β-glucuronidase. Total SAM (free, glucuronide, and sulfate) can be determined as free SAM after acid hydrolysis.

Analog Computer Simulation of Model—An analog computer[2] program corresponding to Eqs. 1–4 is shown in standard analog computer symbols in Fig. 4. The steps for subsequent metabolite transport, k_{21}^{g}, k_{23}^{g}, have been omitted in this program as they will not affect transport of free drug which is of interest here.

When the effect of metabolism in the cell is evaluated ($k_{fg} > 0$), metabolism in the cell proceeds by a first-order process at the variable rate, $k_{fg}F_2^*$, where F_2^* is equal to F_2. When the variable input into the electronic comparator (F_2) equals the selected fixed reference voltage of the electronic comparator (F_2^* *ref.*), the comparator operates through a digital output to an electronic switch which changes the rate of metabolism to a fixed (zero-order) rate[3] equal to k_{fg} (F_2^* *ref.*). When metabolism is negligible, the potentiometer representing k_{fg} is set to zero.

RESULTS AND OBSERVATIONS

Influence of Intestinal Region and Method of Oxygenating on *In Vitro* Glucuronidation—In order to compare intestinal metabolism in *in vitro* everted intestines and *in vivo* perfused intestines in the same region, preliminary studies were made to determine the region of intestine which produced maximal glucuronide formation and experimental factors influencing final glucuronide concentrations.

Closed rabbit everted sacs were incubated with 10^{-4} *M* salicylamide under a closed O_2 and CO_2 (95%–5%) atmosphere flowing at 122–152.5 cm.³/hr. (4–5 ft.³/hr.) similar to the conditions used by Herz *et al.* (7) to study conjugation of thyroxine analogs in rat everted intestines. All regions of the intestine had essentially the same final salicylamide glucuronide concentrations and glucuronide-to-free drug ratios in the serosal fluid as shown in Table II, Rows 1–8. The ileum appeared to have slightly greater amounts of glucuronide and was selected as the standard region for this and other reasons. It is the most convenient region for cannulation in the *in vivo* preparation and is the region usually used when minimal fluid flux is desired (17).

When the O_2 and CO_2 mixture was bubbled *directly* into the mucosal fluid, two effects were noted. First, peristaltic contractions of the intestine were increased, leading to variable amounts of fluid remaining in the sac. The final serosal concentrations in the bubbled preparation of both glucuronide and free drug showed greater variation due to the fluid loss. Second, both glucuronide and free drug serosal concentrations were generally higher than those found in the O_2 and CO_2 atmosphere conditions. The increase in serosal glucuronide was greater than the increase of serosal free drug, however, as is reflected by increased serosal glucuronide-to-free drug ratios. These data are shown in Table II where Rows 1–8 are under O_2–CO_2 atmosphere conditions and Rows 9–14 are with bubbled O_2–CO_2 conditions.

[2] The analog computer used was a TR-20 with a series 1133 Variplotter recorder, manufactured by Electronics Associated Inc., Long Branch, N. J.

[3] Although this scheme has the disadvantage that the rate of metabolism shifts from first-order to zero-order instantaneously rather than following the more complex true enzyme kinetics, it is adequate to illustrate the points for which it is intended in this paper.

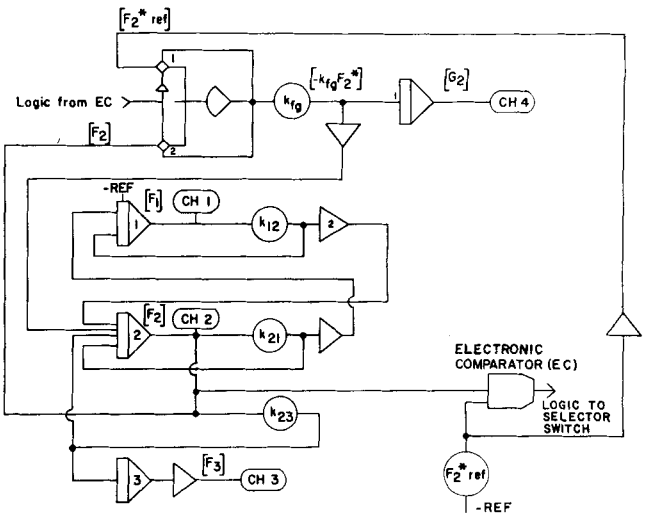


Figure 4—*Analog computer program for the model* $F_1 \rightleftharpoons F_2 \rightarrow F_3$, $F_2 \rightarrow G_2$ *where metabolite formation may be first-order or zero-order when* F_2 *reaches the reference voltage of the comparator* (F_2^* ref.). (*See text for discussion.*)

It was subsequently found that the loss of fluid due to peristaltic squeezing can be satisfactorily prevented by leaving an open syringe in the cannulating needle to act as a reservoir for serosal fluid pushed up during contraction. This procedure improves the reproducibility and usefulness of this preparation and permits the use of directly bubbled oxygen in kinetic studies.

Figure 5 shows the typical linear plot obtained in the cannulated everted sac when appropriate volume corrections are made and the amount of drug reaching serosal fluid is plotted against time. The volume corrections are as follows.

Samples of equal volume are removed from the serosal fluid every 10 min., so that the volume decreases linearly with time. Since the mucosal concentration is essentially constant, a constant amount is delivered to the serosal fluid per unit time while the serosal concentration increases inordinately, due to the decrease of

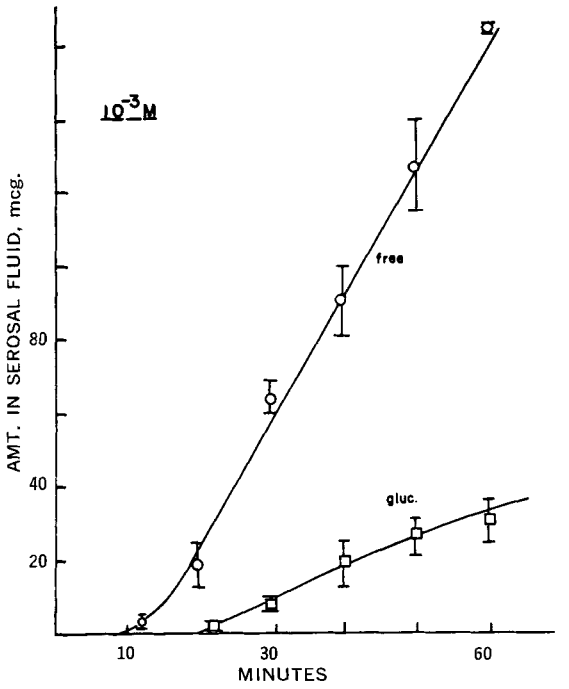


Figure 5—*The amount of free drug* (○) *and glucuronide in the serosal fluid* (□) *of the cannulated* in vitro *everted intestine as a function of time (initial concentration of* 10^{-3} M *SAM in mucosal fluid).*

Table II—Effect of Intestinal Region and Method of Oxygenation on Glucuronide Formation[a]

Row	Region of Intestine	Rabbit[b]	Method	Serosal Volume—Initial	Serosal Volume—Final	Glucuronide, Final Serosal Concn., mcg./ml.	Free Drug, Final Serosal Concn., mcg./ml.	G/F[c]
1	Duodenum	D	O_2 Atmosphere	8	8	2.10	1.20	1.75
2	Duodenum	D	O_2 Atmosphere	8	8	2.16	1.29	1.67
3	Jejunum	D	O_2 Atmosphere	8	8	2.10	.90	2.33
4	Jejunum	D	O_2 Atmosphere	8	8	1.72	1.35	1.27
5	Ileum	D	O_2 Atmosphere	8	8	1.42	1.42	1.00
6	Ileum	D	O_2 Atmosphere	8	8	3.32	1.12	2.96
7	Ileum	B	O_2 Atmosphere	10	10	1.83	1.40	1.31
8	Ileum	B	O_2 Atmosphere	8	8	2.09	1.49	1.40
9	Ileum	C	O_2 Bubbled	8.0	7.4[d]	5.48	2.92	1.88
10	Ileum	C	O_2 Bubbled	7.5	7.4[d]	9.74	3.29	2.96
11	Ileum	D	O_2 Bubbled	8.0	4.7[e]	2.44	.52	4.69
12	Ileum	D	O_2 Bubbled	8.0	3.0[e]	10.21	2.25	5.54
13	Ileum	K	O_2 Bubbled	8.0	7.5	3.98	.82	4.85

[a] Comparison of concentrations of SAM (free) and SAM glucuronide in the serosal fluid after 1-hr. incubation at 37° with an initial mucosal concentration of 10^{-4} *M* SAM. Comparisons are made of different regions of the intestine (duodenum, jejunum, ileum) with the same oxygenating system or the same region (ileum) with different oxygenating systems [95% O_2–5% CO_2 bubbled in directly or in an atmosphere flowing through a Dubnoff shaker at 122–152.5 cm.^{3} (4–5 ft.^{3})/hr.]. [b] Each different letter indicates a particular experiment using a different rabbit. Intestinal segments come from the same animal if they have the same letter (*e.g., D*). [c] The ratio of the concentrations of glucuronide and free in serosal fluids after 1 hr. [d] Fluid loss due to one sample taken at 30 min. [e] Fluid loss due to peristalsis.

serosal volume, resulting in a convex concentration-time curve requiring the following correction.

When each sample aliquot of equal volume (v) is removed from the initial volume (V^0), the amount of drug in the serosal fluid (F_t), at a given time (t), corresponding to sample number (n), of concentration (C_{Fn}), is given by

$$F_t = C_{Fn}[V^0 - v(n-1)]$$

and the cumulative amount of free drug, $\sum F_t$, which has reached the serosal fluid at time t, including the amount which has been removed during previous sampling is

$$\sum F_t = C_{Fn}[V^0 - v(n-1)] + v\sum_{1}^{n-1} C_{Fn}$$

It is possible that even corrected-amount plots will be curved upwards if metabolism is significant and becomes capacity limited within the range of mucosal cell concentrations obtained during the course of the experiment.

Evaluation of *In Vivo* Intestinal Loop with Complete Venous Collection—The technique of collecting all venous blood draining from the region of absorption was developed to provide an *in vivo* preparation with intact circulation where the mesenteric venous blood could be treated as a closed compartment. The most important criterion for the usefulness of this preparation, therefore, is that all free drug and drug metabolites which are absorbed into the capillary blood must be collected in the venous effluent and not reach the general circulation. Since arteriovenous anastomoses in the gastrointestinal circulation are present (18, 19), the following study was performed to establish that the amount of drug which reaches the systemic circulation is negligible. A closed intestinal loop with complete venous collection was filled with 10 ml. of 10^{-3} *M* SAM (1.37 mg.). After 2 hr. the intestine was rinsed and 10 ml. of a 10^{-2} *M* solution (13.7 mg.) was placed in the lumen. The venous blood was collected and assayed for free drug and glucuronide. The carotid artery was sampled periodically during the absorption process. The resulting concentrations of free drug and glucuronide in carotid arterial plasma and the mesenteric venous effluent as a function of time are shown in Fig. 6.

The concentrations in arterial blood were only very slightly above the arterial blood blank and were insignificant compared to venous effluent concentrations. Arterial blood concentrations were checked periodically throughout the experiments and never found to be significant. In some cases, total urine collection was also assayed at the end of the experiment and found to contain negligible amounts of free drug or glucuronide. These results show that the primary criterion for the usefulness of this preparation—*viz.*, complete collection of all absorbed drug and drug metabolites in the mesenteric venous effluent, is quite adequately satisfied.

Effect of Metabolism, Accumulation, and Blood Flow on the Disappearance of Free Drug from the Lumen and Appearance of Free Drug in the Plasma—In the study previously described and illustrated by the plasma concentration curves in Fig. 6, the amounts of free drug in the lumen remaining to be absorbed were also determined and are shown in Fig. 7. These curves illustrate the difficulty of deducing mechanisms of absorption from measurements only on the disappearance of drug from the lumen. Curve *b*, obtained with the lower dose (1.37 mg.), appears linear throughout with an intercept equal to the initial amount in the intestinal lumen. This is exactly the type of curve predicted by a true irreversible barrier model, $F_1 \rightarrow F_3$. Curve *a*, which was obtained in the same intestinal preparation with a higher dose (13.7 mg.), shows a distinct distributive phase, however, and follows the shape predicted by a compartment model, $F_1 \rightleftharpoons F_2 \rightarrow F_3$. If the rate of appearance of free drug in the plasma (dF_3/dt) is examined for the lower concentration (1.37 mg.), it is apparent from Fig. 8 that the slope of the semilog-time-plot is not the same as the slope of the semilog-time-plot of amount remaining in the lumen. The barrier model would require that these slopes both be equal to $-k_{13}/2.303$, since $F_1 = F_1^0 e^{-k_{13}t}$, and $dF_3/dt = k_{13}F_1^0 e^{-k_{13}t}$. Thus, although the data on the disappearance of drug from the lumen suggests that the barrier model is applicable for the lower dose, the rate of appearance of drug in the plasma at this dose does not correspond to the barrier model. These apparently anomalous results can be readily reconciled by considering the effects of cellular metabolism on the amounts of free drug in the intestinal tissue.

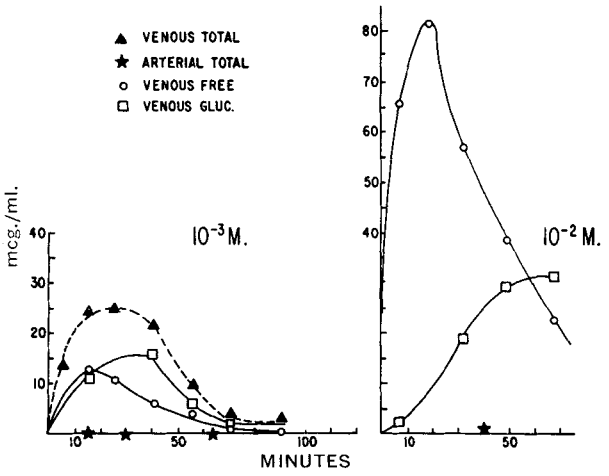


Figure 6—*Comparison of the concentrations of free drug, glucuronide, and total drug in the closed sac,* in vivo *preparation, containing* (a) *10 ml. of* 10^{-3} M *(1.37 mg.) SAM, or* (b) *10 ml. of* 10^{-2} M *(13.7 mg.) SAM.*

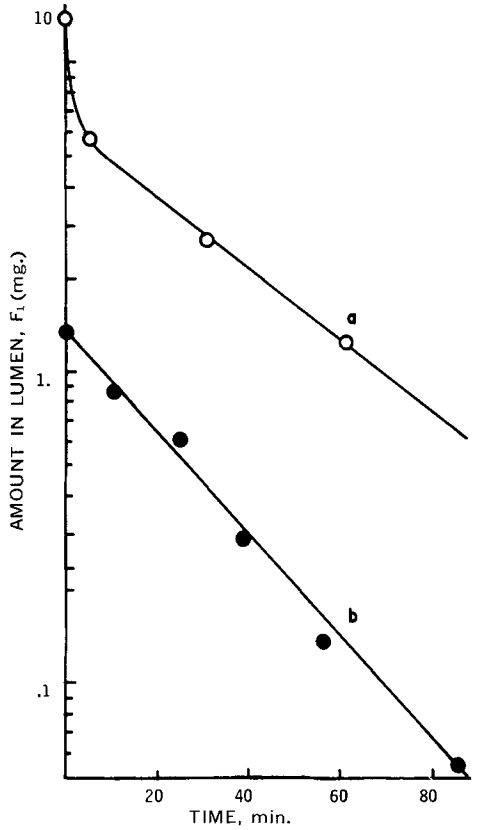


Figure 7—*Comparison of the amount of free drug in the lumen remaining to be absorbed (log scale) in the closed loop* in vivo *preparation containing 10 ml. of 10^{-3}* M *SAM* (●) *and 10 ml. of 10^{-2}* M *SAM* (○).

If it is assumed that at lower doses the free drug in the tissue is rapidly metabolized by a first-order process, the amount of free drug in the cell then remains very low and the cell compartment approximates a barrier containing negligible amounts of free drug.

At higher doses, metabolism in the cell is capacity-limited (*i.e.*, is approaching zero-order metabolism kinetics) and a smaller fraction is metabolized. This allows accumulation of the free drug in the tissue and requires distribution equilibrium to be established between the free drug in the lumen compartment and the cell compartment before monoexponential disappearance is observed. Indeed, when one examines the appearance of glucuronide in the plasma given in Fig. 6, this explanation appears quite reasonable. At 10^{-3} *M*, the fraction which appears as glucuronide in the plasma exceeds that of the free drug. At the higher (10^{-2} *M*) concentration, the fraction of drug which appears in the plasma as glucuronide is much less, particularly in the initial phase. The experimental results shown in Fig. 7 are in reasonable agreement with the types of lumen disappearance curves predicted by the analog computer program when the above assumptions on capacity-limited metabolism were made as shown in Fig. 9.

These *in vivo* studies provide experimental evidence for four important concepts: (*a*) an appreciable amount of drug absorbed from the lumen may appear in the plasma as metabolized drug; (*b*) the intestinal glucuronide conjugating system can apparently be saturated; (*c*) the intestine behaves like a compartment when the fraction of salicylamide metabolized is negligible. Approximate calculations[4] show that the apparent volume of distribution in the cell compartment is almost equal to the volume of the mucosal fluid (about 10 ml.) and (*d*) measurements on the disappearance of drug from the lumen alone are not adequate to characterize the absorption process when metabolism in the cell is significant.

[4] Present difficulties in assaying drug in the tissue allow only indirect evidence of tissue accumulation of free drug. The volume of distribution was estimated by the analog computer program. There are difficulties in curve fitting, however, as (*a*) the fraction of drug metabolized is changing and (*b*) some hydrolysis of glucuronide in the lumen fluid appears operative.

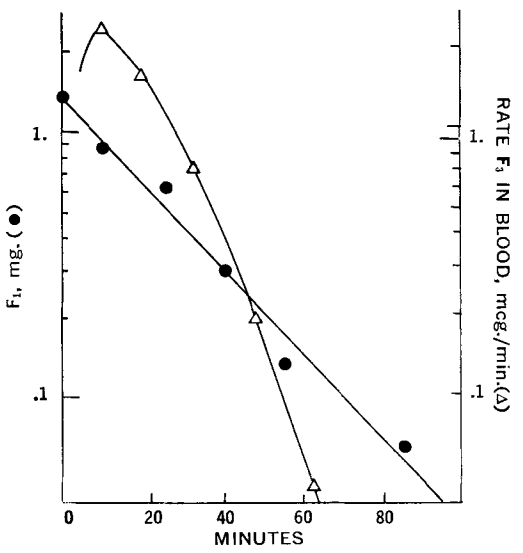


Figure 8—*Comparison of the amount of free drug* (F_1) *in the lumen* (●) *and the rate of appearance of free drug* (F_3) *in the venous plasma* (△) *obtained in an* in vivo *closed loop preparation containing 10 ml. of a 10^{-3}* M *solution of SAM.*

Effect of Blood Flow on the Rate of Appearance of Drug in Plasma—The effect of blood flow on the rate of appearance of drug in the plasma is shown by the fluctuations observed in the rates of appearance of free drug in the *in vivo* perfused intestine long after steady state should have been established. An example is given in Fig. 10 which shows the rate of appearance of free drug and glucuronide in plasma in an *in vivo* loop perfused with 10^{-3} *M* SAM. It is evident that there are considerable variations in the rate of appearance of free drug. When the rates of blood flow at the same time points are plotted, the peaks coincide. The rate of appearance of glucuronide, however, appears to be relatively constant, and independent of blood flow.

The rate of appearance of free salicylamide and glucuronide in the mesenteric plasma as a function of blood flow when the mucosal concentration is maintained constant by perfusion with 10^{-3} *M* SAM is shown in Fig. 11.

It is apparent that transport of free drug across the basal barrier is rate limited by blood flow while the more polar glucuronide is diffusion rate limited except at very low flow rates.

The role of intestinal blood flow in intestinal absorption has been controversial. Some reviewers have stated that although very little work has been done on the effect of blood flow, it may be a rate-limiting factor (20, 21). Others believe it to be unimportant because of the large rate of flow through the splanchnic region (13, 22), and that diffusion will always be rate limiting.

One must of course be cautious in extrapolating the results obtained in an anesthetized surgical preparation to absorption under normal physiological conditions. In general, the splanchnic circulation receives about 30% of the cardiac output of which about 15% goes to the intestines. For a rabbit with a cardiac output of 0.35 l./min. (0.26–0.48 by the direct Fick method), the flow through the entire intestine should be about 55 ml./min. (23). In the cannulated preparation about one-tenth of the intestine is used. Therefore, the flow rate through this segment should be about 4.0 to 7.0 ml./min. The actual flow rates achieved were somewhat less, ranging between 0.6 and 6 ml./min., but sufficiently close to indicate that similar effects might be observed in the intact animal.

Comparison of Lag Times in *In Vitro* and *In Vivo* Preparations—The existence of a lag time in the *in vitro* rabbit preparation is clearly evident from Fig. 5 and has also been demonstrated in the rat by other workers (4, 24, 25). Figure 5 shows a significant lag time of almost 10 min. for free drug and over 15 min. for the linear appearance of glucuronide into serosal fluid. The physiological significance of the lag time for transport of drug into the serosal fluid in the *in vitro* preparations has been questioned, however, because blood circulation is nonfunctional and the drug must additionally traverse the submucosa and muscularis layers which may account for a sizable drug reservoir or barrier.

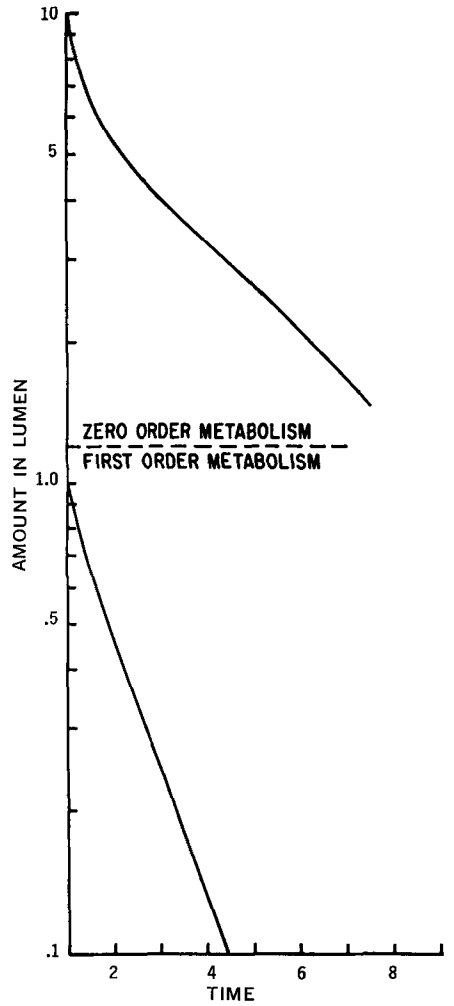


Figure 9—*Analog computor simulation of amounts of free drug remaining in the lumen when metabolism in the tissue compartment proceeds by first-order (bottom curve) at low initial dose, or zero-order at a higher initial dose (top curve). The rate constants for the analog program shown in Fig. 4 (corresponding to the symbolism in Fig. 1) were the same in both cases;* k_{12} *(0.5),* k_{21} *(0.5),* k_{23} *(0.2), and* k_{fg} *(1.2) in units of reciprocal time.* F_2* ref. *was set at 0.5 v. in the case of zero-order metabolism corresponding to a zero-order rate of 0.6 v., unit time*$^{-1}$.

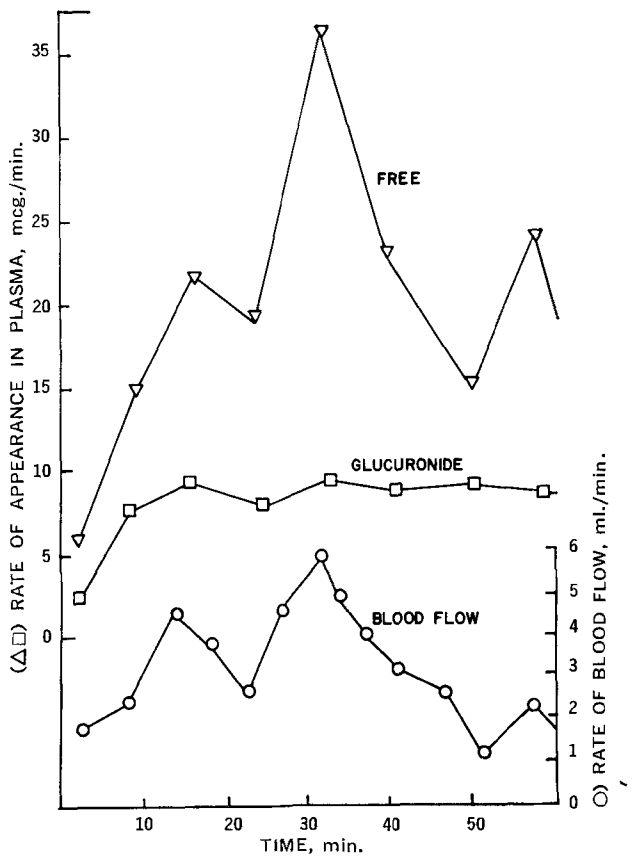


Figure 10—*The rate of appearance of free drug (▽) and glucuronide (□) in the venous plasma correlated with the rate of blood flow (○) in an* in vivo *preparation with complete mesenteric venous collection perfused with a* 10^{-3} M *solution of SAM.*

Several investigators have found it necessary to include a lag time in kinetic evaluations of drug absorption in man (26, 27). It is not possible to directly associate this lag time with the intestinal tissue, however, since in these cases other factors such as dosage form release, gastric emptying, intestinal transit times, and distribution in other tissues, *e.g.*, hepatic (27), may also be involved. Conclusive evidence for an *in vivo* lag phase due to the intestinal tissue should be evident from the *in vivo* perfused intestinal loop with complete venous collection which would not be subject to the above criticisms.

To simulate the conditions of the *in vitro* everted intestinal preparations where the mucosal concentration is essentially constant, the loops were perfused with constant concentrations of 10^{-4}, 10^{-3}, or 10^{-2} *M* SAM in Krebs-Ringer bicarbonate solution. Periodic samples of the lumen perfusate showed that the perfusion rate used (1.9 ml./min.) was sufficient to maintain a constant mucosal concentration. Since all drug is collected in the venous effluent, the plasma can be treated as a closed compartment, and the cumulative amount absorbed is easily obtained. Examples of cumulative amounts of free drug absorbed at different perfusion concentrations are given in Fig. 12. The absolute slopes vary slightly for a given concentration since no attempt was made to keep blood flow conditions and length of the loop constant. The *x*-axis intercept of the extrapolated linear portion of the curve represents the lag time. This lag time is quite consistent for all perfusion concentrations, ranging from 4 to 6 min. with an average of 4.5 ($SE \pm 0.4$) min.

These results show that intestinal lag time can be expected in a physiologically realistic preparation with intact mesenteric circulation even when dosage form delays and diffusion of drug in the lumen fluid are obviated by using a well-stirred solution of the drug. The *in vivo* lag time is, therefore, a real phenomenon attributable to the intestinal tissue.

DISCUSSION

Interpretation of Lag Time in Barrier and Compartment Models—The lag time may be interpreted either as the approach to diffusion steady state in a barrier model or the approach to apparent distribution equilibrium in a tissue compartment. The usual (29) equation describing steady-state diffusion across the intestinal "barrier" is the application of Fick's law to a plane sheet

$$-dF_1/dt = dF_3/dt = P_m A_m (C_{F1} - C_{F3}) \qquad \text{(Eq. 5)}$$

where F_1 is the amount of free drug in the mucosal fluid, P_m is the permeability constant of the intestinal barrier equal to the diffusion constant D, divided by the thickness of the barrier, x; A_m is the effective absorbing area, C_{F1} and C_{F3} are the concentrations of free drug in the mucosal fluid and serosal fluid, respectively. In the noncompartmental diffusion model for diffusion of drug through a simple[5] cylindrical barrier of inner surface area, A_1, and outer surface area, A_3, the lag time, T, is given by

$$T = (A_1 - A_3)^2/6D \qquad \text{(Eq. 6)}$$

It is difficult to assign values to the parameters of Eqs. 5 and 6 for several reasons (29). The intestine is not a simple[5] barrier but

[5] That is, a single isotropic medium.

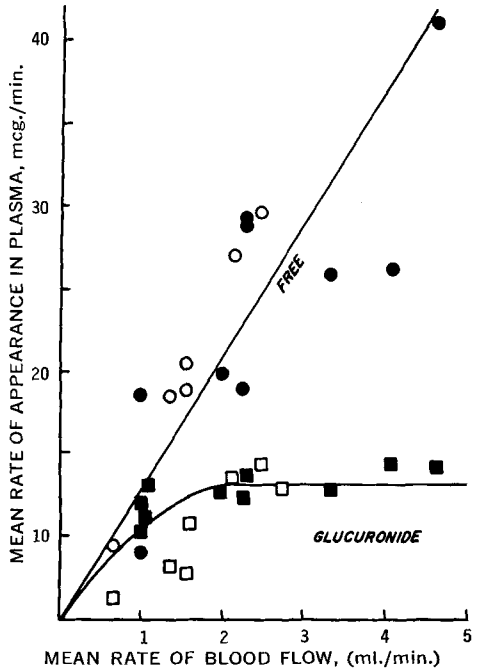


Figure 11—*The rate of appearance of free drug* (○ ●) *and glucuronide* (□ ■) *in the venous plasma as a function of the mean rate of blood flow in two* in vivo *preparations perfused with* 10^{-3} M *SAM.*

a composite barrier in which D may vary.[6] The area of the mucosal surface is much greater than the area of the serosal surface due to villi and microvilli and is difficult to estimate. Metabolism in the tissue may alter the material balance of free drug in the barrier.

When the rate out of the cell across the basal barrier is the rate-limiting step ($k_{23} \ll k_{12}$), accumulation of drug in the tissue occurs. A steady-state approximation based on negligible quantities in the intestine, implicit in a barrier model, will not be valid. Following a single dose, disappearance of the drug from the lumen will be biexponential of the form[7]

$$F_1 = A_1e^{-\alpha t} - A_2e^{-\beta t} \qquad \text{(Eq. 7)}$$

and

$$F_2 = A_3e^{-\alpha t} - A_4e^{-\beta t} \qquad \text{(Eq. 8)}$$

The rapid initial rate of disappearance from the lumen which represents the distributive phase between the lumen compartment and tissue compartment is not paralleled by a rapid rate of appearance of drug in the plasma. In fact, the opposite is true. An initial slow rate of appearance will be observed, resulting in an apparent lag time. During the distributive phase, measurements on the lumen alone will not be sufficient to characterize the rate of appearance of the drug in the blood. Accordingly, the lag time can be considered as the time to reach distribution equilibrium in the tissue compartment corresponding to the distributive phase. Apparent distribution equilibrium can be defined as follows: Apparent distribution equilibrium between two connected compartments is established during the time that the rate of change of the amount of drug in one compartment is proportional to the rate of change of the amount of drug in the other compartment. Following this definition, which will be termed "the proportional rates assumption," the following relationship exists

$$dF_2/dt = R(dF_1/dt) \qquad \text{(Eq. 9)}$$

where R is a proportionality constant.

[6] The difficulties of describing the intestine by diffusion kinetics are apparent when one considers that the simplest compartment model consisting of two barriers (α and β) would be equivalent to a diffusion model for a composite cylinder of two coaxial cylinders having different permeability constants. Crank (30) states that the extra parameters involved make numerical analysis quite formidable.

[7] A complete solution to these equations is given in *Reference 31.*

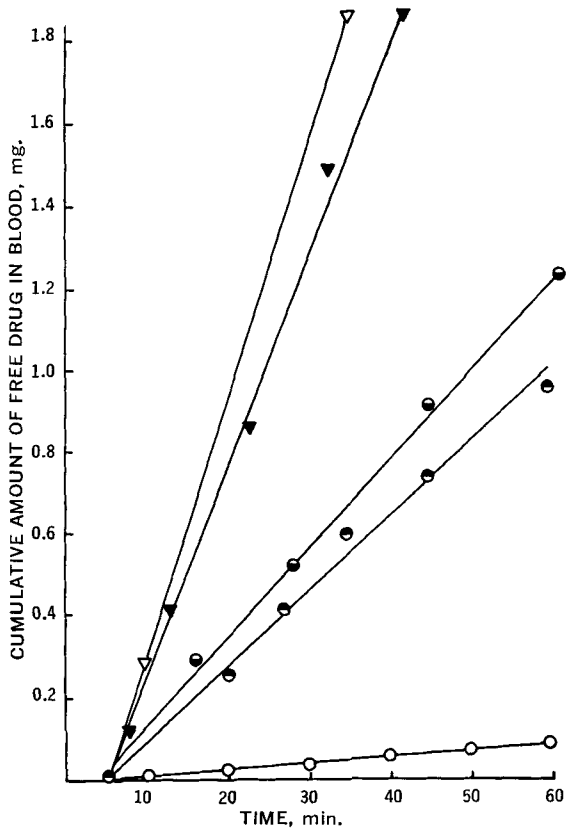


Figure 12—*The cumulative amount of free drug appearing in the blood in* vivo *intestinal loops with complete venous collection perfused* with 10^{-2} M (▽,▼, *two trials*), 10^{-3} M (◒,◓, *two trials*), *and* 10^{-4} M (○, *one trial*) *salicylamide in Krebs-Ringer bicarbonate solution.*

It might be noted that other definitions of distribution equilibrium have been proposed which are not applicable to the intestinal compartment model. Riggs (32) states that distribution equilibrium is achieved when the rate of transfer from Compartment 1 to Compartment 2 equals the rate of transfer from Compartment 2 to Compartment 1. The condition given by Riggs, $dF_{1\to2}/dt = dF_{2\to1}/dt$ for distribution equilibrium is applicable to the intestinal compartment model only when $k_{23} = 0$. Thus, if one accepts the definition of absorption used in this paper, the condition of distribution equilibrium given by Riggs would be valid only when there is no absorption. The basic relationship between the concentration in the reference region (C_1), total amount of drug in the two compartments which are in apparent equilibrium, and the volume of distribution (V_{i2}) is the same for both the condition given by Riggs and the proportional rates condition—*viz.*, $V_{i2} = (F_1 + F_2)/C_1$.

Implicit in Eq. 9, the slopes of the amount-time curves must be proportional at each point in time during apparent distribution equilibrium.

When k_{23} is less than k_{12}, the $e^{-\alpha t}$ term in Eqs. 7 and 8 will approach zero and the linear segments of the semilog plots of amount *versus* time in Compartments 1 and 2 will be parallel in the monoexponential phase. Thus,

$$R = \frac{dF_2/dt}{dF_1/dt} = \frac{\beta F_2}{\beta F_1} = \frac{\beta F_2{}^\circ e^{-\beta t}}{\beta F_1{}^\circ e^{-\beta t}} = \frac{F_2}{F_1} \qquad \text{(Eq. 10)}$$

where β is the apparent hybrid rate constant obtained from the terminal linear segment of the semilog plot of amounts *versus* time in each compartment and $F_1{}^\circ$ and $F_2{}^\circ$ are the extrapolated intercepts of these segments.

This shows that the proportionality constant, R, is equal to the constant ratio of amounts of drug in Compartments 2 and 1, after apparent distribution equilibrium is achieved.

These relationships are illustrated and verified by the analog computer plot shown in Fig. 13. Using these relationships, the identity

can be written

$$F_1 + F_2 = C_1V_1 + RF_1 \qquad \text{(Eq. 11)}$$

where C_1 is the concentration of free drug in Compartment 1 and V_1 is the true volume of Compartment 1. Rearranging Eq. 11, it is seen that the term $V_1(R + 1)$ is equal to $V_{\bar{12}}$, the apparent volume of distribution

$$V_{\bar{12}} = \frac{F_1 + F_2}{C_1} = V_1(R + 1) \qquad \text{(Eq. 12)}$$

When $V_{\bar{12}}$ is constant, it is easily shown that only measurements on the concentration of drug in the first compartment (lumen contents) are necessary to describe the rate of appearance of drug in the third compartment since

$$dF_3/dt = V_{\bar{12}}\, dC_1/dt \qquad \text{(Eq. 13)}$$

After apparent distribution equilibrium is achieved, Compartments 1 and 2 may be considered one open functional compartment with a single volume of distribution ($V_{\bar{12}}$). Thus, the rate of appearance of drug in the blood can be followed after apparent distribution equilibrium by monitoring the lumen concentration only, providing metabolism does not occur. It is important to realize, however, that the apparent volume of distribution, $V_{\bar{12}}$, must be used rather than the actual lumen volume, V_1, or the amount in the tissue will be ignored.

Interpretation of Blood Flow Effects on Drug Transference—When the intestine cannot be considered as a simple barrier, the terms P_m and A_m lose physical significance and can be replaced by an intestinal transference term (3). Transference can be defined for a given unit of tissue as the rate of transport of unaltered substance from the initial compartment to the terminal compartment per unit concentration difference between the initial and terminal compartment. For *in vitro* intestinal transfer, the terminal compartment will be the serosal fluid and transference will be identical to the tissue permeability coefficient as defined by Pappenheimer (33) in units of ml./min. per unit tissue. For *in vivo* intestinal absorption, intestinal transference will be dependent on both the tissue permeability and rate of mesenteric blood flow and is similar to the dialysis term used by Wolf to describe hemodialysis (34).

When the rate of transport of free drug is diffusion rate-limited by the basal barrier and accumulation in the tissue occurs, the transference term will include the apparent volume of distribution term $V_{\bar{12}}$ as discussed in the previous section.

The relationship between intestinal transference (3), tissue permeability (P), and mesenteric blood flow ($\dot{V}$) is given by the following equation (35):

$$\mathfrak{Z} = \dot{V}(1 - e^{-P/\dot{V}}) = kV_{\bar{12}} \qquad \text{(Eq. 14)}$$

where 3 is the transference constant in units of ml./min., k is the apparent first-order rate constant (time^{-1}) for the transfer of amount of drug $(F)_{\bar{12}}$ from the combined mucosal fluid-tissue compartment to the serosal compartment. When the mucosal or tissue concentration is constant ($\bar{C}_{F1}$) and much greater than the plasma concentration and transfer is essentially unidirectional (transference is then a clearance term), the rate of appearance (dQ/dt) of the amount of free drug (Q) in plasma will be given by

$$\frac{dQ}{dt} = V_{\bar{12}}\frac{dC_{F1}}{dt} = \bar{C}_{F1}\,\mathfrak{Z} = \bar{C}_{F1}\,\dot{V}(1 - e^{-P/\dot{V}}) \qquad \text{(Eq. 15)}$$

Thus, when P is very large relative to $\dot{V}$, the exponential term approaches zero and dQ/dt is proportional to blood flow as in the case of free drug. When $\dot{V}$ becomes large relative to P, dQ/dt will become independent of blood flow as illustrated by the glucuronide.

In view of these results, it might be assumed that intestinal blood flow will most likely influence intestinal absorption when: (*a*) the absorbed substance has a high permeability coefficient such as very lipoid-soluble drugs or pore-diffusible substances or (*b*) the blood flow through the absorbing area is low. The blood flow through the absorbing area could be altered by changes in cardiac output, redistribution, or local arterio-venous shunting. These processes may be affected by pathological conditions, emotional state, food, or in fact, the drug itself. The intestinal vasculature is governed by sympathetic tone and many drugs such as sympathomimetics, ergot derivatives, and ganglionic blocking agents could conceivably reduce the effective flow through the absorbing region and reduce their own or the absorption of other drugs given concomitantly. It is clear that the role of intestinal blood flow on intestinal absorption may be quite important and requires much further study.

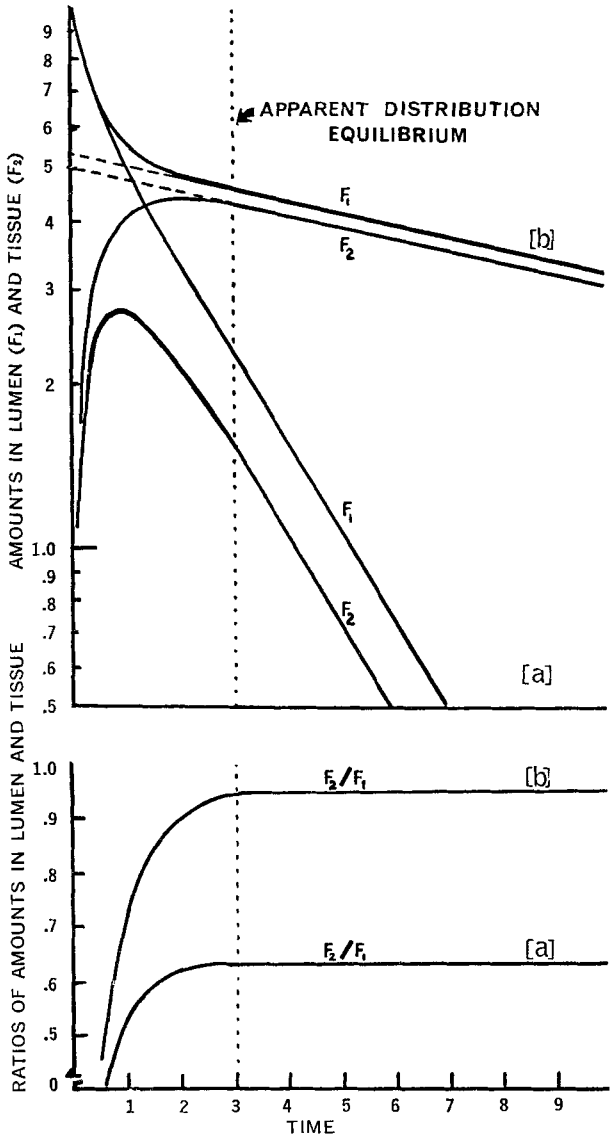


Figure 13—*Illustration of apparent distribution equilibrium by analog computer model when tissue accumulation occurs. The top curves illustrate the parallel slopes for lumen and tissue compartments in the monoexponential phase in which distribution equilibrium, defined by* $dF_2/dt = R(dF_1/dt)$, *exists where* R, *the ratio between* F_2 *and* F_1 *becomes constant as shown in the bottom curves for these cases. Rate constants (unit time*$^{-1}$*) for Case* (a) *were* k_{12} (*0.5*), k_{21} (*0.5*), k_{23} (*0.1*), *and Case* (b) k_{12} (*0.5*), k_{21} (*0.5*), k_{23} (*0.05*). *Metabolism is considered negligible* ($k_{fg} = 0$).

SUMMARY AND CONCLUSIONS

Most previous studies on mechanisms of drug absorption have used *in vivo* methods which sample only disappearance of drug from the lumen rather than appearance of drug across the basal barrier into the blood. When transport across the basal barrier has been followed, it has been with *in vitro* preparations with nonfunctional blood circulation such as the isolated perfused intestine or everted intestinal sacs.

In the present study, both the rate of appearance of free salicylamide and salicylamide glucuronide into the mesenteric blood were measured directly by cannulating the mesenteric vein of exposed rabbit intestine and collecting all venous blood draining from the absorbing region. Using this technique, it was shown that the usual *in vivo* measurements on the disappearance of drug from the lumen alone would be inadequate to characterize the appearance of free drug in the mesenteric blood due to accumulation and metabolism of drug in the tissues and the effect of mesenteric blood flow.

The effect of intestinal glucuronide conjugation was found in this study to be quite significant, *in vivo*. As much as 60% of the drug appeared in the blood as the biologically inactive glucuronide.

When significant metabolism of the drug in the tissue occurs, measurements on the lumen alone will not be sufficient to predict the rate of appearance of free drug in the blood. When metabolism is capacity limited (*i.e.*, shifts from first-order to zero-order) the apparent mechanism of absorption of free drug might erroneously appear to proceed by two different mechanisms if only disappearance from the lumen is followed. The disappearance from the lumen curves, at low doses, indicated that an apparent barrier model was applicable with no accumulation of free drug in the tissue. At larger initial doses, the glucuronide-forming system appeared to be saturated and kinetic evidence indicated accumulation of free drug, characteristic of the compartment model for absorption. Studies on tissue levels are now in progress to further evaluate this conclusion.

The mesenteric blood flow plays a significant role in the *in vivo* preparation. Comparison of the *in vitro* and *in vivo* lag times shows that the presence of an intact mesenteric blood circulation in the *in vivo* preparation reduces the lag time for appearance in the terminal compartment from about 10 min. to about 4 min. presumably by obviating the extra diffusional barriers or reservoirs beyond the lamina propria which are present in the *in vitro* preparation. In addition to circumventing the submucosa and muscularis barriers, the rate of blood flow is also shown to play a role in the rate of transport of free drug across the basal barrier. The rate of transport of free drug across the *in vivo* basal barrier was clearly blood flow rate-limited. The rate of transport of the more polar glucuronide across the basal barrier appeared to be diffusion rate-limited and independent of the rate of blood flow. The *in vivo* rates of transport of free drug was five to ten times as great as those observed at the same initial mucosal concentration in the *in vitro* preparation.

In vitro methods such as the everted intestinal sac may provide an indication of relative permeabilities. However, permeability constants obtained with *in vitro* methods will not necessarily reflect the permeabilities of the tissues *in vivo* because of the extra barriers involved. *In vitro* procedures may also give useful information on capacity of the intestinal mucosal enzymes to metabolize drugs providing attention is given to the effect of experimental variables associated with this type of preparation. For example, the method of oxygenation, serosal sampling, and fluid loss due to peristalsis were found to greatly affect serosal glucuronide concentration.

In conclusion, the barrier model, which assumes that the intestinal tissue barrier does not alter or contribute to the material balance of the system, should not be assumed in contemplating experimental design. Sampling from the lumen alone may be unsatisfactory to predict the rate of free drug in the mesenteric blood which is the most significant criterion for assessment of absorptive capacity. Methods which measure the rate of unaltered drug across the basal barrier, such as the intestinal loop with complete mesenteric blood collection, provide a useful experimental approach in studying the physiologic factors of absorption such as tissue accumulation, metabolism, and blood flow.

REFERENCES

(1) "Methods in Medical Research," vol. 9, J. H. Quastel, Ed., Year Book Medical Publishers, Chicago, Ill., pp. 255, 273.

(2) T. H. Wilson and G. Wiseman, *J. Physiol.*, **123,** 116(1954).

(3) G. Levy and W. J. Jusko, *J. Pharm. Sci.*, **54,** 216(1965).

(4) G. Levy and T. Matsuzawa, *ibid.*, **54,** 1003(1965).

(5) F. J. Dea, S. A. Maurer, and J. Biles, presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Las Vegas meeting, 1967.

(6) D. Schachter, D. J. Kass, and T. J. Lamnon, *J. Biol. Chem.*, **234,** 201(1959).

(7) R. Herz, Jr., D. Tapley, and J. R. Ross, *Biochim. Biophys. Acta*, **53,** 273(1961).

(8) M. J. Kreck, F. G. Guggenheim, J. E. Ross, and D. F. Tapley, *ibid.*, **74,** 418(1963).

(9) D. M. Matthews and D. H. Smyth, *J. Physiol. (London)*, **126,** 96(1954).

(10) R. M. Atkinson, B. J. Parsons, and D. M. Smyth, *J. Physiol.*, **135,** 581(1957).

(11) J. Y. Kiyasu and I. L. Chaikoff, *J. Biol. Chem.*, **224,** 935 (1957).

(12) E. Diczfalusy, C. Franksson, and B. Martinsen, *Acta Endocrinol.*, **38,** 59(1961).

(13) H. W. Davenport, "Physiology of the Digestive Tract," 2nd ed., Year Book Medical Publishers, Chicago, Ill., 1966.

(14) A. Agren, *Acta Chim. Scand.*, **9,** 49(1955).

(15) R. K. Crane and T. H. Wilson, *J. Appl. Physiol.*, **12,** 145 (1958).

(16) W. W. Umbreit, R. H. Burris, and J. F. Stauffer, "Manometric Techniques," 4th ed., Burges, Minneapolis, Minn., 1964.

(17) L. E. Jervis and D. H. Smyth, *J. Physiol.*, **144,** 21(1958).

(18) R. Chambers and B. W. Zweifack, *Am. J. Anat.*, **75,** 173 (1944).

(19) B. W. Zweifack, "Functional Behavior of the Microcirculation," Charles C Thomas, Springfield, Ill., 1961.

(20) L. S. Schanker, *J. Med. Pharm. Chem.*, **2,** 343(1960).

(21) D. H. Smyth, "Absorption and Distribution of Drugs," T. B. Binns, Ed., Williams & Wilkins, Baltimore, Md. 1964, p. 1.

(22) C. A. M. Hogben, *Federation Proc.*, **19,** 864(1960).

(23) "Methods of Animal Experimentation," vol. 1, W. I. Gay, Ed., Academic, New York, N. Y., 1965.

(24) R. F. Crampton and D. M. Mathews, in "Absorption and Distribution of Drugs," T. B. Binns, Ed., Williams & Wilkins, Baltimore, Md., 1964, p. 49.

(25) Y. Gressel, A. Tye, and J. W. Nelson, "An *In Vitro* Study of Intestinal Transfer," presented to the Pharmacology and Biochemistry Section, APhA Academy of Pharmaceutical Sciences, Las Vegas meeting, March 1962, reprint B-1.

(26) G. Levy, *Arch. Intern. Pharmacodyn.*, **152,** 59(1964).

(27) J. G. Wagner, "Design and Analysis of Biopharmaceutical Studies in Man," Proceedings of the Industrial Pharmacy Section, APhA Academy of Pharmaceutical Sciences, Dallas meeting, April 1966, p. 20.

(28) V. M. Whitehead and B. A. Cooper, *Brit. J. Haematol.*, **13,** 679(1967).

(29) T. H. Wilson, "Intestinal Absorption," W. B. Saunders Philadelphia, Pa., 1962.

(30) J. Crank, "The Mathematics of Diffusion," Oxford University Press, London, England, 1956.

(31) A. Resigno and G. Segre, "Drug and Tracer Kinetics," Blaisdell, Waltham, Mass., 1966.

(32) D. S. Riggs, "The Mathematical Approach to Physiological Problems," Williams & Wilkins, Baltimore, Md., 1963.

(33) J. R. Pappenheimer, E. M. Renkin, and L. M. Borrero, *Am. J. Physiol.*, **167,** 13(1951).

(34) A. V. Wolf, D. G. Romp, J. E. Kiley, and G. D. Currie, *J. Clin. Invest.*, **30,** 1062(1951).

(35) S. S. Kety, *Pharmacol. Rev.*, **3,** 1(1951).

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