Intestinal Drug Absorption and Metabolism II: Kinetic Aspects of Intestinal Glucuronide Conjugation

WILLIAM H. BARR* and S. RIEGELMAN†

Abstract □ Mechanisms of intestinal salicylamide glucuronide formation and transport were studied in the rabbit with *in vitro* cannulated everted intestines and *in vivo* perfused and closed loops with complete mesenteric venous blood collection. Both experimental techniques indicate that glucuronide formation is capacity limited when the lumen concentration exceeds 10^{-3} *M*. The glucuronide-to-free drug ratio appearing in mesenteric blood (*in vivo*) and serosal fluid (*in vitro*) decreases with increasing lumen concentrations. Appearance of glucuronide across the basal barrier is limited by the transport step rather than the rate of glucuronide synthesis leading to accumulation of glucuronide in the tissue compartment. Transport of glucuronide appears to be a simple first-order diffusion process into the lumen contents as well as the mesenteric blood. Some implications of these findings in pharmacokinetic studies and dosage form design are discussed.

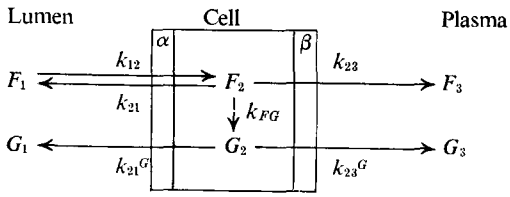
Keyphrases □ Drug absorption, metabolism—intestinal □ Salicylamide glucuronide formation, transport—intestinal □ Everted intestinal sacs—experimental technique □ Intestinal loop—*in vivo* experimental technique □ Conjugation, intestinal—salicylamide, glucuronic acid

The absorbing columnar cell of the intestine appears to be equipped with a wide variety of enzyme systems capable of metabolizing drugs. It has been shown as early as 1952 that *in vitro* intestinal slices can conjugate phenolic drugs with glucuronic acid (1). Glucuronide formation in the intestine has been shown *in vitro* for a large number of compounds including salicylic acid, salicylamide, anthranilic acid, thyroxine analogs, *p*-nitrophenol, *o*-aminophenol, testosterone derivatives, and estrogens (1–6). Sulfate conjugation also occurs in intestinal slices (8, 9). Recently, Hartiala *et al.* have shown that intestinal acetylation of sulfonamides can occur *in vitro* (10) and the work of Tapley *et al.* (6, 7, 11) indicates that reduction, hydroxylation, and oxidation of steroids can also take place in the intestinal mucosa.

The extent to which these metabolic transformations can affect the physiologic availability of an orally administered drug *in vivo* is almost completely unknown. Some information on this point has been recently obtained by use of a technique which allows complete collection of all mesenteric blood draining the absorption site of an intestinal loop. It has been shown by this method that an appreciable amount of salicylamide is absorbed *in vivo* as the glucuronide (12). That work also indicated that the appearance of the salicylamide glucuronide in the plasma was diffusion limited rather than blood flow limited and that the system could be saturated, giving capacity-limited (pseudo-zero-order) kinetics for the appearance of glucuronide in the plasma.

It is the purpose of this report to present further information on the manner in which intestinal metabolism may affect the *in vivo* absorption process using glucuronic acid conjugation of salicylamide as a model.

The mechanism of glucuronide synthesis and transport may be considered from the standpoint of the previously proposed cell compartment model (12):



where F_1,F_2,F_3 represent the amount of free drug in the lumen, tissue, and plasma (or serosal fluid *in vitro*), respectively; G_1,G_2,G_3 represent the amounts of glucuronide in the lumen, cell, and plasma (or serosal fluid *in vitro*), respectively; k_{mn} represents the rate constant for amount of free drug transferred from Compartment *m* to Compartment *n*. For example, k_{23} is the rate constant for transfer from Compartment 2 (the tissue) to the plasma or serosal fluid (Compartment 3); $k_{mn}{}^G$ represents the rate constant for the transfer of amount of glucuronide from Compartment *m* to Compartment *n*; k_{FG} represents the rate constant for the metabolism of amount of free drug to glucuronide; α and β refer to the apical and basal barriers, respectively.

EXPERIMENTAL

The methods used in this study are the same as used in the previous report in this series (12). Free salicylamide was determined by extraction into a 1,2-dichloroethane–cyclohexane mixture (65:35) from which it is reextracted into 0.2 *N* sodium hydroxide solution. The basic solution is read directly in an Aminco-Bowman spectrophotofluorometer at excitation and emission wavelengths of 350 and 430 mμ, respectively (uncorrected). Salicylamide glucuronide is determined as the total salicylamide after hydrolysis with bacterial β-glucuronidase less the free salicylamide.

All intestinal studies were done with the midileal portion of the intestine from male New Zealand rabbits weighing between 2.0 and 2.5 kg. Everted intestinal sacs for fixed time and kinetic studies were prepared as before (12) and oxygenated by an O_2–CO_2 (95:5) gas mixture bubbled directly into Krebs-Ringer bicarbonate mucosal fluid.

The *in vivo* preparation with complete venous collection was prepared by cannulating the mesenteric vein serving the intestinal loop which was either open and perfused or closed containing solutions of salicylamide in Krebs-Ringer bicarbonate solution (12).

RESULTS

Capacity-Limited Glucuronide Appearance—If metabolism in the cell follows first-order kinetics, the ratio of free drug to metabolized drug appearing in the plasma (*in vivo*) or serosal fluid (*in vitro*) should be constant and independent of the initial dose or mucosal concentration. It can be shown by both *in vitro* and *in vivo* experimental techniques that the glucuronide-to-free drug ratio is not constant and that the appearance of glucuronide in plasma is capacity limited.

Figures 1*a* and 1*b* illustrate the cumulative amounts of glucuronide and free drug appearing in the serosal fluid in everted intes-

tines containing mucosal concentrations of 10^{-4} and 10^{-3} *M* of salicylamide, respectively. When the mucosal concentration is low (10^{-4} *M*), the amount and rate of glucuronide appearing in the serosal fluid exceed the free drug. When a higher mucosal concentration is used (10^{-3} *M*), the glucuronide exhibits the same prolonged lag time but the rate and amount of glucuronide appearing in the serosal fluid are always less than that of the free drug.

Figure 2 illustrates the cumulative amounts of glucuronide appearing in the plasma in the *in vivo* intestinal loops with complete venous collection perfused with 10^{-4}, 10^{-3}, and 10^{-2} *M* salicylamide. The rates of appearance of glucuronide in the plasma as measured by the slope of the cumulative amount-time curve (or the cumulative amounts of glucuronide after 60 min.) do not differ greatly[1] even though the mucosal concentration varies over a hundredfold

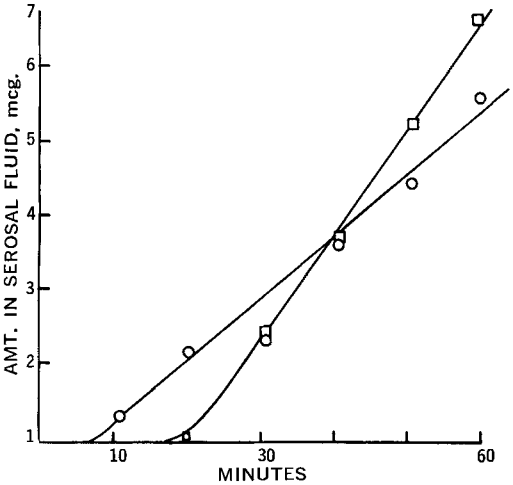


Figure 1a—*The cumulative amount of free drug* (○) *and glucuronide* (□) *in the serosal fluid of the cannulated* in vitro *everted intestine with an initial mucosal concentration of* 10^{-4} M *SAM.*

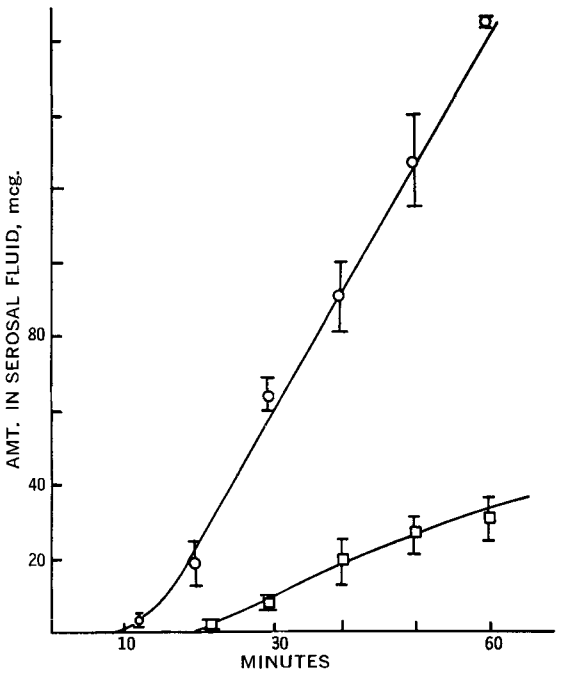


Figure 1b—*The cumulative amount of free drug* (○) *and glucuronide* (□) *in the serosal fluid of the cannulated* in vitro *everted intestine with an initial mucosal concentration of* 10^{-3} M *SAM. Bars indicate the range of two experiments.*

[1] It is difficult to exactly standardize the length of intestine and control the mean rate of blood flow in different experiments which causes some variation in the total amount of metabolite recovered in a set time. This is the probable explanation for the lower amounts of glucuronide obtained with 10^{-2} *M* perfusion shown in Fig. 2 rather than inhibition of glucuronide formation at higher concentrations of salicylamide.

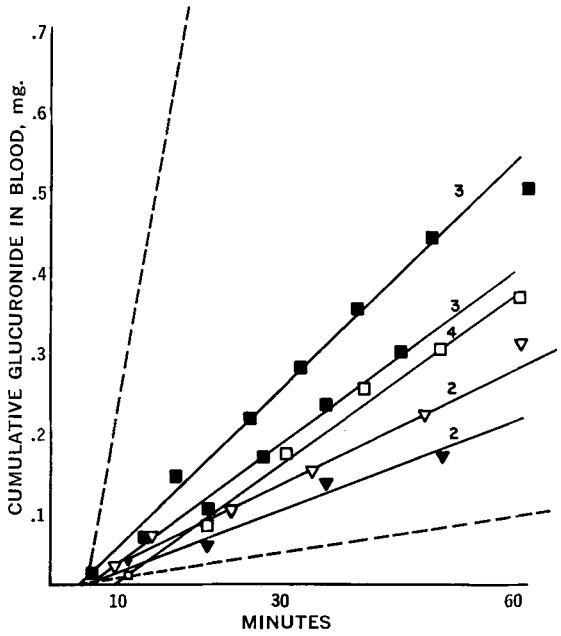


Figure 2—*Cumulative amounts of glucuronide appearing in the mesenteric blood in the* in vivo *preparation perfused with* 10^{-4} M (□), 10^{-3} M (■), *and* 10^{-2} M (△) *SAM. Broken lines show the range of amounts of free drug appearing in mesenteric blood perfused with* 10^{-4} M (*lower broken line*) *and* 10^{-2} M (*upper broken line*) *solutions of SAM.*

range. The amounts of free drug appearing in the plasma, shown by the dotted lines for comparison, do show great variations in the slope as would be expected. These points are also illustrated by Fig. 3 which shows the mean rates of appearance in plasma for free drug and glucuronide as a function of the perfusion concentration. These data provide evidence that appearance of glucuronide in the plasma or serosal fluid is capacity limited in both *in vitro* and *in vivo* experimental situations. Figure 4 compares the glucuronide-to-free drug ratio after 1 hr. in the plasma for the *in vivo* procedure and the serosal fluid for the *in vitro* procedure. The parallel lines suggest that the *in vitro* everted intestines serve well to characterize the glucuronide-to-free drug ratio that might be observed *in vivo*.

Implications of a Capacity-Limited System—The data obtained from different experimental procedures, both *in vitro* and *in vivo*, clearly indicate that the appearance of glucuronide in serosal fluid

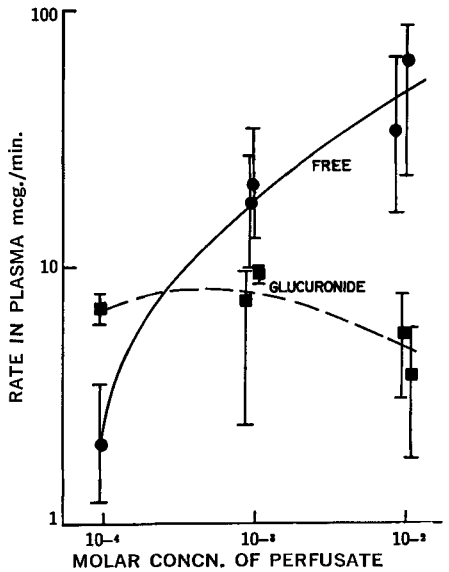


Figure 3—*Comparison of mean rates of appearance in mesenteric blood of free drug* (●) *and glucuronide* (■) *obtained with* in vivo *preparations perfused with* 10^{-4}, 10^{-3}, *and* 10^{-2} M *solutions of SAM. Each point represents the mean rate of a separate experiment. Bars represent the range of rates for each separate experiment.*

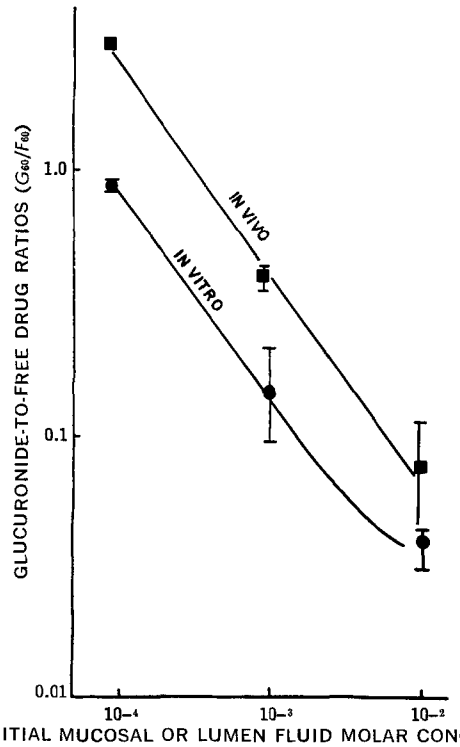


Figure 4—*Illustrates the ratios of the cumulative amounts of glucuronide to the cumulative amounts of free drug* (G_{60}/F_{60}) *appearing in the plasma after 1 hr. in the* in vivo *preparation perfused with* 10^{-2}, 10^{-3}, *and* 10^{-4} M *SAM* (■) *and the glucuronide-to-free drug ratio in the serosal fluid of the* in vitro *everted intestine with initial mucosal fluid concentrations of* 10^{-2}, 10^{-3}, *and* 10^{-4} M *SAM* (○). *Bars indicate the range of two experiments.*

or plasma is independent of the concentration of free drug in the lumen compartment at lumen concentrations greater than 10^{-3} *M*.

There are several possible physiologic mechanisms that might be postulated to account for this zero-order kinetic behavior. The two most likely possibilities are either capacity-limited glucuronide formation or capacity-limited transport of glucuronide out of the cell. The formation of glucuronide could be rate limiting if the amount of enzyme, glucuronyl transferase, responsible for coupling UDP-glucuronic acid and the phenol is limited. It is also possible that the amount of the cofactor, UDP-glucuronic acid, is limited due to small amounts present in the cell because of a slow rate of synthesis by the DPN-dependent oxidation of UDP-glucose or rapid breakdown by UDP-glucuronic acid pyrophosphatase (4).

The alternate possibility is that the transport of the polar glucuronide out of the cell is capacity limited. This would imply that some sort of carrier transport mechanism or active transport is swamped at high cellular concentrations of the glucuronide.

Appearance of Glucuronide in Lumen—Because of the extra diffusion barriers of the submucosal and muscularis layers on the basal side of the *in vitro* everted intestine, it might be expected that greater amounts of glucuronide would diffuse into the mucosal fluid than the serosal fluid if passive diffusion was the limiting factor of glucuronide transport. Representative data presented in Table I, which compares the amounts of glucuronide in mucosal fluid and serosal fluid after 1-hr. incubation, show that this is indeed the case. The amount of glucuronide in mucosal fluid is from 2 to 5 times greater than that in the serosal fluid. The relative permeabilities of the mucosal barrier and serosal barrier obtained from the *in vitro* everted intestine are probably not representative of the relative permeabilities of the mucosal and plasma barriers in the normal *in vivo* absorption process where the drug does not traverse the submucosal and muscularis layers. The fact that significant amounts of glucuronide formed in the cell actually appear in the lumen may be indicative of the *in vivo* process, which would be of considerable importance. If this phenomenon occurs in the normal absorption process, a reduction in the net amount of drug absorbed would occur since the drug present as the polar glucuronide is not

Table I—Comparison of Amounts of SAM Glucuronide Appearing in the Mucosal and Serosal Fluid of Everted Intestines after 1-hr. Incubation at 37°

No.	Initial Mucosal Concentration	Amount (mcg.) in Serosal Fluid	Amount (mcg.) in Mucosal Fluid	Ratio (S/M)
1	10^{-4} *M*	36.3	151.0	0.24
2	10^{-4} *M*	29.8	50.3	0.59
3	10^{-4} *M*	28.5	168.0	0.17
4	10^{-3} *M*	49.1	156.0	0.31
5	10^{-3} *M*	25.1	133.0	0.19

absorbed unless hydrolyzed (3, 13, 14). These considerations prompted the following studies to determine the relative amounts of glucuronide which appear in the lumen fluid *in vivo*.

***In Vivo* Lumen-Plasma Glucuronide Ratios**—To obtain an indication of the amount of lumen glucuronide and the relative permeabilities of the apical and basal barriers *in vivo*, the cannulated preparation with complete venous collection was employed. There are certain difficulties in obtaining true *in vivo* glucuronide ratios even with this preparation. First, hydrolysis of the glucuronide in mucosal fluid and possibly in the plasma complicates quantitation of the true total amount of glucuronide delivered to each fluid. Second, the concentration of glucuronide in the blood depends on the distribution ratio between plasma and red blood cells which is not known. Salicylamide (free) is equally distributed between the erythrocytes and plasma. It is assumed, on the basis of its low permeability to other cells, that the glucuronide does not enter the erythrocyte.

The concentration of the glucuronide in the lumen fluid cannot be accurately determined by the difference assay when the ratio of free drug to glucuronide is greater than 100. For this reason, it proved impractical to use a perfused intestine which maintains high concentrations of free drug. Closed loops were therefore used, and the cumulative amount of glucuronide in the mucosal fluid was obtained by multiplying the concentration by the fixed fluid volume. The cumulative amount of glucuronide in the blood was estimated by multiplying the volume of plasma collected by the concentration of glucuronide in the plasma, assuming that no glucuronide was present in the erythrocytes. The amount of free drug in the blood was obtained by multiplying the plasma concentration by the total blood volume as the free drug was found to be equally distributed between the plasma and erythrocytes. The cumulative amounts of glucuronide appearing in lumen fluid and plasma are shown in Fig. 5. It is seen that the amount of glucuronide in the

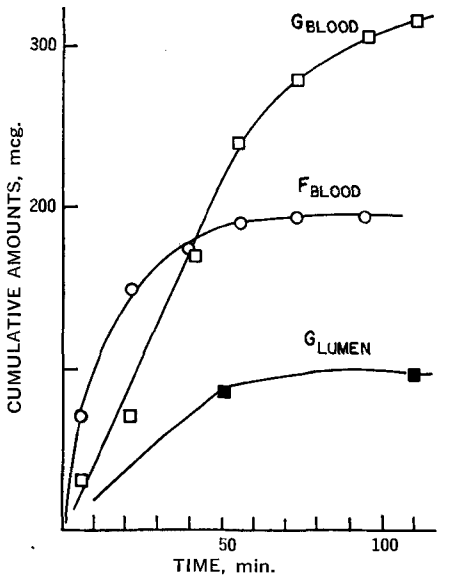


Figure 5—*Cumulative amounts of free drug in blood* (○), *glucuronide in blood* (□), *and glucuronide in lumen fluid* (■) *of a closed loop in* in vivo *preparation containing 10 ml. of* 10^{-3} M *salicylamide in Krebs-Ringer bicarbonate solution.*

mucosal fluid is significant but does not exceed the amount in the plasma. The ratio of plasma glucuronide to lumen glucuronide is from 3 to 5.

The occurrence of glucuronide in the lumen in the *in vivo* cannulated loop gives reasonable evidence that significant amounts of drug metabolites may be expected to be found in lumen during the normal absorption process in the intact animal. This may be an important consideration in explaining the presence of some drug metabolites in the lumen which has previously been assumed to be a consequence of enterohepatic cycling and biliary secretion.

It must be realized, however, that some mucosal glucuronide may be a result of mucosal cell disruption either as a result of experimental manipulation or the normal sloughing of intestinal mucosa. The latter process occurs to a high degree in normal intestinal function. The normal turnover time of intestinal epithelium is quite rapid (15).

Relative Rates of Glucuronide Synthesis and Transport—The cumulative amounts appearing in the plasma of free drug and glucuronide shown in Fig. 5 show that glucuronide is still appearing in the plasma while free drug is approaching an asymptote. This suggests that accumulation of glucuronide in the cell is occurring, which would indicate that the rate of synthesis of the glucuronide is greater than subsequent transport out of the cell ($k_{FG} \gg k_{21}^{G}$, k_{23}^{G}). Accumulation of glucuronide in the cell compartment is also suggested for *in vitro* intestinal preparations by the cumulative amounts appearing in the serosal fluid of the *in vitro* everted sacs shown in Fig. 1 and the data given by Herz *et al.* (5).

Further evidence for accumulation of metabolite in the cell, *in vivo*, can be obtained by comparing the rates of appearance in plasma of free drug and glucuronide as shown in Fig. 6. Several facts from this curve support the conclusion that the rate of glucuronide transport out of the cell rather than the rate of metabolism in the cell is the rate-limiting step for the appearance of glucuronide in the plasma. First, the linear terminal portions of the curve for both free drug and glucuronide indicate that exit from the cell is first-order and therefore proportional to the concentration in the cell. At time 60 min., the amount of free drug in the cell has dropped to 1/100th of its original concentration. The concentration of glucuronide in the cell at this time, however, is still quite high even though most of the free drug in the cell is gone. Second, if metabolism was the only rate-limiting step, the precursor-successor relationship (16) would predict that the precursor free drug curve should intersect the peak of the successor glucuronide curve and both curves would then decline with parallel slopes. The delay in the glucuronide peak requires an additional rate-limiting step in the model. The increase in the slope of the glucuronide curve compared to the slope of the free drug then reflects the slower diffusion step out of the cell for the glucuronide.

Active or Passive Transport of Glucuronide—The data presented in previous sections do not require an active transport process for the glucuronide. All data seem adequately explicable by rapid first-order or zero-order cellular glucuronide formation followed by a slow diffusion process proportional to the amount of glucuronide in the cell. This does not rule out an active transport system entirely. If such a process was present but saturated at low levels, the result would be a simultaneous mixed zero-order–first-order process, which at high concentrations of glucuronide in the cell would give the appearance of an apparent first-order process. Further work is necessary to determine the mechanisms involved in the transport of glucuronide out of the cell.

DISCUSSION

If the results of these studies on intestinal glucuronide formation and absorption of salicylamide in rabbits apply to man, there would be several important pharmaceutical and clinical implications to be considered. There are reasons to believe that these results may, in fact, be applicable to man. It has been shown that *in vitro* human intestinal slices produce comparable amounts of salicylic acid glucuronide to that formed by rabbit intestine (3). It has also been shown *in vivo* that human intestine forms steroid glucuronides (17). Analysis of the kinetics of glucuronide formation following oral doses of salicylamide in man suggests that intestinal metabolism may be a factor in the low plasma levels of free drug obtained after oral administration, even though absorption appears to be complete. Furthermore, as in rabbits, the process appears to be capacity limited at higher doses (18).

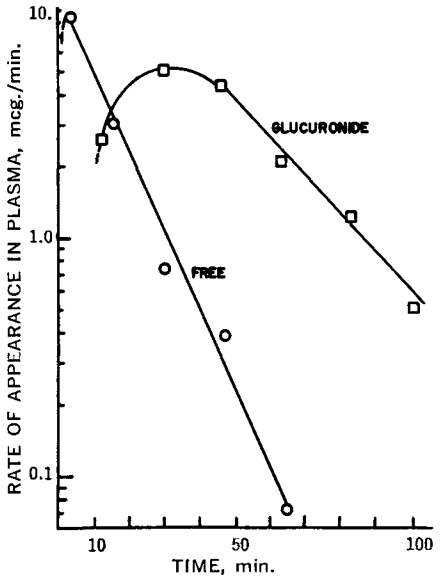


Figure 6—*Comparison of the rates of appearance in plasma of free drug* (○) *and glucuronide* (□) *in a closed loop* in vivo *preparation. These data are from the same experiment shown in Fig. 5.*

The extrapolation of this work to man offers many interesting implications.

(1) Intestinal metabolism may offer an alternate explanation for many drugs which are said to be poorly absorbed. Drug assays involving extraction into organic solvents measure only free drug and may not detect the amount of drug absorbed as the polar metabolite. Several phenolic compounds are said to be poorly absorbed orally while their *o*-methylated or dehydroxy congeners are well absorbed, *e.g.*, morphine, phenylephrine, and isoproterenol. Decreased or erratic therapeutic effects following oral doses may be a consequence of intestinal metabolic inactivation rather than decreased absorption.

(2) Drug assays which measure only total drug in the plasma may also be inadequate to characterize the absorption process of free drug when intestinal metabolism is significant. The apparent rate constant of absorption as measured by total drug in the plasma will be decreased and the peak time delayed due to the slower diffusion of the metabolite (*i.e.*, Fig. 5).

(3) The fact that appreciable amounts of metabolite are released from the cell back into the intestine may also affect the rate and extent of absorption. A cycling process may occur in which the drug is absorbed and partially metabolized in the cell. A fraction of the metabolite is released back into the lumen where it is nonabsorbable unless it is hydrolyzed to the free drug by bacterial and endogenous enzymes. The free drug would be reabsorbed and the cycle repeated until all drug is absorbed or eliminated in the feces. If the hydrolyzing enzymes are localized in a particular portion of the intestine, hydrolysis of the metabolite may be delayed and a discontinuous absorption process observed.

(4) The total amount of drug absorbed may be decreased if the metabolite released back into the intestine is not completely hydrolyzed. Furthermore, if the metabolic process is capacity limited, one might expect that the fraction of the total dose which is absorbed into the systemic circulation, and subsequently recovered in the urine, would be less with small doses than larger doses.

(5) Several factors in formulation might be considered in light of the effect of lumen concentration on the glucuronide-to-free drug (G/F) ratio absorbed when intestinal metabolism is capacity limited. The best dosage form would be that which would give maximum availability in the shortest time such as a solution or a form with a rapid dissolution rate. A sustained-release form that delivered a low concentration over a long period of time could possibly be worthless because of the high glucuronide-to-free drug ratio seen at lower concentrations. Competitive inhibition of metabolism may occur and one drug of a combination formulation may modify the absorption of another (*e.g.*, aspirin, *N*-acetyl *para*-aminophenol and salicylamide).

(6) There are also important clinical consequences which deserve attention. The use of urinary excretion of drug glucuronides as an index of hepatic function is a common clinical procedure (19). It has recently been suggested that salicylamide would be an excellent choice for this purpose (20, 21). It is possibly unwise, however, to use salicylamide, or any other drug which is largely glucuronylated in the intestine, as a measure of the ability of the liver to form glucuronides. A further consideration is that intestinal glucuronide formation may be well developed in the intestine of the fetus and newborn before appreciable hepatic glucuronide formation is established (22). The role of the intestine in bilirubin glucuronide formation may be important but has not been clearly defined (22, 23). In cases of neonatal hyperbilirubinemia, caution should be exercised in determining hepatic conjugating ability by oral tolerance curves of compounds also metabolized in the intestine. Further information is necessary on the relative importance of intestinal and hepatic metabolism for both bilirubin and any proposed test drug.

(7) It is interesting that one of the first reports of intestinal glucuronide formation was by Hartiala (24) who found increased glucuronide in the portal blood after giving cinchophen. He used this finding to support his theory that cinchophen caused ulcers by forming glucuronides in the intestine and thus depleting glucuronic acid from the mucopolysaccharide protective layer. Actually the opposite may be true, and glucuronide formation may operate as a protective mechanism. Salicylamide forms large amounts of glucuronide in the intestine but produces no mucosal damage or ulceration (25). Aspirin forms relatively small amounts of glucuronide (3) and is well known to cause mucosal damage (26, 27).

SUMMARY

At concentrations below 10^{-3} *M*, a considerable fraction of salicylamide is rapidly conjugated with glucuronic acid in the intestinal mucosal cell and subsequently appears in the mesenteric blood in the *in vivo* preparation with complete mesenteric blood collection or the serosal fluid in the *in vitro* everted intestinal preparation. Appearance of salicylamide glucuronide in the plasma is rate limited by transport across the basal barrier rather than metabolism. Exit of glucuronide from the cell is bidirectional, appearing in the lumen contents as well as in the plasma by what appears to be first-order diffusion process.

The relative order of magnitude of the rate constants in the general compartment model of intestinal absorption for the *in vivo* preparation is $k_{FG} > k_{23} \gg k_{23^G} > k_{21^G}$. In the *in vitro* preparation greater amounts of glucuronide appear in the mucosal fluid than the serosal fluid due to the extra diffusional barriers of the submucosal and muscularis layers and $k_{23^G} < k_{21^G}$.

When lumen or mucosal fluid concentrations are greater than 10^{-3} *M*, the conjugating system is capacity limited leading to zero-order appearance of glucuronide. Some implications of these findings in dosage form design, pharmacokinetic studies, and clinical studies are discussed.

REFERENCES

(1) F. Zini, *Sperimentale*, **102,** 40(1952).

(2) K. J. W. Hartiala, *Acta Physiol. Scand.*, **31,** Suppl. **114,** 20(1954).

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

(4) T. A. Miettinen and E. Leskinen, *Biochem. Pharmacol.*, **12,** 565(1963).

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

(6) M. J. Kreek, F. G. Guggenheim, J. E. Ross, and D. F. Tapley, *ibid.*, **54,** 418(1963).

(7) F. R. Smith, D. F. Tapley, and J. E. Ross, *ibid.*, **69,** 68 (1963).

(8) W. D. Conway, H. Minatoya, M. Lands, and J. Shekowski, *J. Pharm. Sci.*, **57,** 1135(1968).

(9) H. Bostrom, *Scand. J. Clin. Invest.*, **17,** 33(1966).

(10) K. J. W. Hartiala and T. Terho, *Nature*, **205,** 809(1965).

(11) T. J. Stahl and D. F. Tapley, *Endocrinology*, **73,** 271(1963).

(12) W. H. Barr and S. Riegelman, *J. Pharm. Sci.*, **59,** 154(1970).

(13) G. Levy, L. Weintraub, T. Matsuzawa, and S. R. Oles, *ibid.*, **55,** 1319(1966).

(14) W. Vogt, G. Schmidt, and T. Dakhil, *Arch. Exptl. Pathol. Pharmacol.*, **250,** 488(1965).

(15) L. Laster and F. J. Ingelfinger, *New Engl. J. Med.*, **264,** 1138(1961).

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

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

(18) W. H. Barr and S. Riegelman, to be published.

(19) I. Snapper and A. Saltzman, *Am. J. Med.*, **2,** 327(1947).

(20) H. T. F. Barniville and R. Misk, *Brit. Med. J.*, **1,** 337(1959).

(21) K. Tareta, *Acta Med. Okayama*, **16,** 129(1962).

(22) I. H. Stevenson and G. J. Dutton, *Biochem. J.*, **82,** 330 (1962).

(23) J. L. Bollman, *Gastroenterology*, **36,** 1313(1959).

(24) K. J. W. Hartiala, P. Leikkda, and P. Savola, *Acta Physiol. Scand.*, **42,** 36(1958).

(25) "Salicylamide," S. B. Penick & Co., New York, N. Y., 1959.

(26) P. Wood, E. Harvey-Smith, and A. Dixon, *Brit. Med. J.*, **1,** 669(1962).

(27) H. F. Lange, *Gastroenterology*, **33,** 778(1959).

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