NUTRIENT EFFECTS ON INTESTINAL DRUG ABSORPTION*

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The effect of oral co-administration of nutrients on intestinal drug absorption is a function of drug dissolution, gastrointestinal (GI) residence time and intestinal membrane transport. Nutrient effects on GI residence time may influence the availability of the drug for absorption when dissolution is rate controlling; alternatively, nutrient effects on membrane transport pathways may dictate variability in drug absorption when these pathways are rate limiting. This report describes nutrient effects on the absorption variability of the anticonvulsant–antiarrhythmic drug, phenytoin (PHT). Numerous reports, detailing clinical failure to maintain drug plasma levels within the narrow therapeutic index of PHT, have implicated phenytoin–nutrient interactions as (possibly) causative. Absorption variability due to limited time for drug dissolution during GI transit was studied by measuring PHT plasma concentrations with time following oral dosage in dogs. Variability in intestinal uptake from PHT solutions as a function of nutrient inclusion was studied by measuring steady-state membrane permeabilities from intestinal perfusions and initial-rate uptakes by intestinal rings in rats.

INTRODUCTION

Specific drug–nutrient interactions affecting intestinal absorption that have been reported in the scientific literature include drug–nutrient competition for active transport sites [1]; nutrient effects on drug stability or metabolism; and nutrient binding, complexation and chelation of drugs [2]. Case studies in the clinical literature which involve nutrient effects on drug absorption frequently appear for drugs possessing a narrow therapeutic index with the serious consequences of effective under- or overdosage [3]. It appears that some of these effects may be more general when drug absorption is critically rate limited by either dissolution or membrane transport.

Delay or feedback control of stomach emptying by nutrient intake results in an increase in the GI residence time of the drug, thereby permitting greater extent of drug dissolution. Phenytoin is a weakly acidic drug ($pK_a = 8.1$) possessing poor intrinsic aqueous solubility ($S_0 = 100$ mM) and a high membrane–water partition coefficient (log PC$=2.4$) at 37°C [4,5]. These physicochemical properties suggest that PHT intestinal absorption is dissolution rate controlled.

It has recently been suggested that sodium co-transport of nutrients in the small intestine enhances paracellular uptake of dissolved solutes [6] and that this process operates through the generation of local osmotic gradients [7]. Since intestinal water absorption follows sodium uptake, this is likely a consequence of both

convection-induced reduction of aqueous resistance to drug transport near the mucosal membrane and the generation of solvent drag of drug through paracellular pathways. Phenytoin's high lipophilicity suggests that transcellular membrane transport is not rate limiting for intestinal absorption. However, the drug's molecular size (M.W. 252) makes it a candidate for variable paracellular uptake as a function of nutrient input.

MATERIALS AND METHODS

The effect of increasing drug GI residence time on drug plasma levels was investigated by the oral administration of 12.5 mg/kg body weight phenytoin free acid (Sigma Chemical) in a gelatin capsule to four beagle dogs. Controls were compared to drug co-administered with an 80 calorie (20 g) oral glucose load (Col-Dex, Fernadle Labs) or 80 calories of an enteral nutrient solution (Osmolite, Ross Labs). Osmolite contains protein and lipid as well as carbohydrate calories. Total administered fluid volume in each treatment was 150 ml. Blood samples were drawn up to 8 hours post-administration and GI pH and time of stomach emptying were monitored by radiotelemetry (Heidelberg capsule, Electro-Medical Devices) [8]. An additional experiment was performed in which the above PHT and nutrient co-administration treatments were given to a dog through a duodenal fistula. Elimination kinetics were determined by intravenous (IV) infusion of sodium phenytoin (Dilantin Injection, Parke Davis — Warner Lambert) intravenously at 5 mg/kg body weight over 10 minutes and 0.5 mg/kg body weight over 1 minute in each of the dogs.

As a result of the findings in the dog studies, attempts to quantify the impact of fed-state bile salt output on PHT dissolution were undertaken. Rotating disc dissolution experiments were carried out at 37°C with varying concentrations of sodium taurocholate in the same baseline solutions that were used for control in

intestinal perfusions and ring studies.

The effect of nutrient input on intestinal drug uptake from solution was investigated by rat jejunal perfusion of 80 μM phenytoin solutions at 300 mOsm/kg and pH 6.5 as controlled by 10 mM Mes buffer (Sigma Chemical). Baseline solutions contained 100 mM sodium chloride and 5 mM potassium chloride. Solution osmolality was adjusted using choline chloride. Control solutions were compared with co-perfusion of D-glucose (Sigma Chemical) and calcium chloride with respect to the jejunal uptake of phenytoin. Jejunal lengths of 4–6 cm were perfused at 0.5 ml/min so that aqueous resistance to drug transport would not dominate membrane resistance [9] for this high-uptake drug. An aqueous diffusion coefficient was required to calculate the aqueous resistance. This parameter ($D_{aq} = 8.4 \times 10^{-6}$ cm/s) was obtained in rotating disc dissolution experiments by measuring dissolution flux versus the square root of rotational speed [10]. Water transport was monitored by the inclusion of 0.01% poly(ethylene glycol) (PEG) 4000 traced with 1,2-[^{14}C]PEG 4000 (New England Nuclear, specific activity 4.4×10^5 dpm/μmol) as a nonabsorbable marker. Steady-state drug uptake was observed by 30 minutes and phenytoin dimensionless membrane permeabilities, corrected for water transport, were calculated as previously reported [11].

Intestinal ring incubations in drug solutions were carried out to obtain initial rate uptake data. Segments of rat intestine were everted on a glass rod and cut into rings (wet weight 10–30 mg). Composition, pH and osmolality of the baseline incubation solution were the same as those solutions used for rotating disc dissolution and intestinal perfusion experiments. Uptake of 4-[^{14}C]phenytoin (Amersham, specific activity 2.2×10^6 dpm/μmol) traced solutions was linear up to 5 minutes. Co-incubations of PHT with D-glucose and calcium chloride were performed for 3 minutes and PHT uptake (normalized for tissue weight) was determined as previously reported [12].

Phenytoin plasma and perfusate concentrations were measured by an HPLC assay [13]. Radiolabeled [^{14}C]PEG 4000 in perfusate and [^{14}C]phenytoin taken up by intestinal rings, which were later dissolved in Scintigest (Fisher), were measured by liquid scintillation counting on a Beckman model 9000. Osmolality was measured by a Wescor vapor pressure osmometer. Plasma and perfusate levels of D-glucose were measured by a hexokinase assay (Sigma Chemical). Significant treatment differences in the dog pharmacokinetic and rat perfusion results were evaluated using a paired t-test in which a value of $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The IV clearance of PHT was independent of dose (elimination half-life was 3–6 hours in all dogs). The radiotelemetry-generated GI pH profiles (pH axis labeled 1 to 7) are shown with the plasma PHT concentration versus time profiles in dogs in Fig. 1. These data were obtained following oral administration of a Heidelberg capsule and a PHT capsule with 150 ml total fluid volume. In particular, dog no. 1 emptied the Heidelberg capsule immediately after drug administration in the fasted state and showed the lowest PHT plasma levels. Peak PHT plasma levels and area under the drug concentration versus time curves were positively correlated with time to stomach emptying in the fasted dog controls (Table 1). Stomach emptying following 20 gram glucose co-administration occurred at 2 hours in 3 of the 4 dogs and is consistent with a stomach–duodenal feedback loop for glucose which is known to operate at 0.7 kcal/min (0.17 g/min) in dogs [14].

The enteral feeding solution delayed stomach emptying for significantly longer periods of time. Dog no. 4 failed to show stomach emptying of the Heidelberg capsule in any of the studies, and PHT plasma levels continued to rise over the sample period for each of the treatments. The data in dog no. 4, in particular, suggest that undissolved PHT is not emptying with the liquid. This occurrence would indicate either that PHT particles are adhering to stomach mucus or are aggregating in a size greater than 1 mm in diameter [15]. The hydrophobic nature of PHT solid material has resulted in aggregation of powder mass in dissolution studies performed at low pH (16) and is consistent with these in vivo observations.

Higher PHT plasma levels correlated with nutrient-induced delay of stomach emptying in dogs nos. 1 and 2; however, dogs nos. 3 and 4 showed higher PHT plasma levels when nutrient was co-administered even though the stomach-emptying pattern was similar between treatments. In all four dogs, PHT plasma levels were highest with co-administered Osmolite, intermediate with glucose co-administration, and lowest in controls.

The dog is not a good model for phenytoin bioavailability from oral dosage in humans because of metabolic differences. The canine liver hydroxylates PHT at both the meta and para positions. The high capacity meta pathway is dominant in dogs [17]. The para pathway is secondary in dogs while it is the dominant saturable pathway in other species, including humans. As a result, elimination half-lives are much shorter in dogs than in humans [18]. Intestinal residence time is also shorter in dogs than in humans. In this regard, the dog is a good model in which to study the impact of incomplete drug dissolution on systemic availability.

The use of a dog with a duodenal fistula allowed for separation of the nutrient effect from the stomach emptying event. Dilantin (PHT free acid) suspension (Parke Davis — Warner Lambert) was infused into the duodenum at a rate designed to mimic the glucose calorie control afforded by the stomach–duodenal feedback loop in a normal dog. This study showed the same PHT plasma profile versus treatment pattern that was observed in the dogs receiving oral PHT capsules (Table 1).

(a)

PLASMA CONCENTRATION (uM)

TIME (MINUTES)

pH

(b)

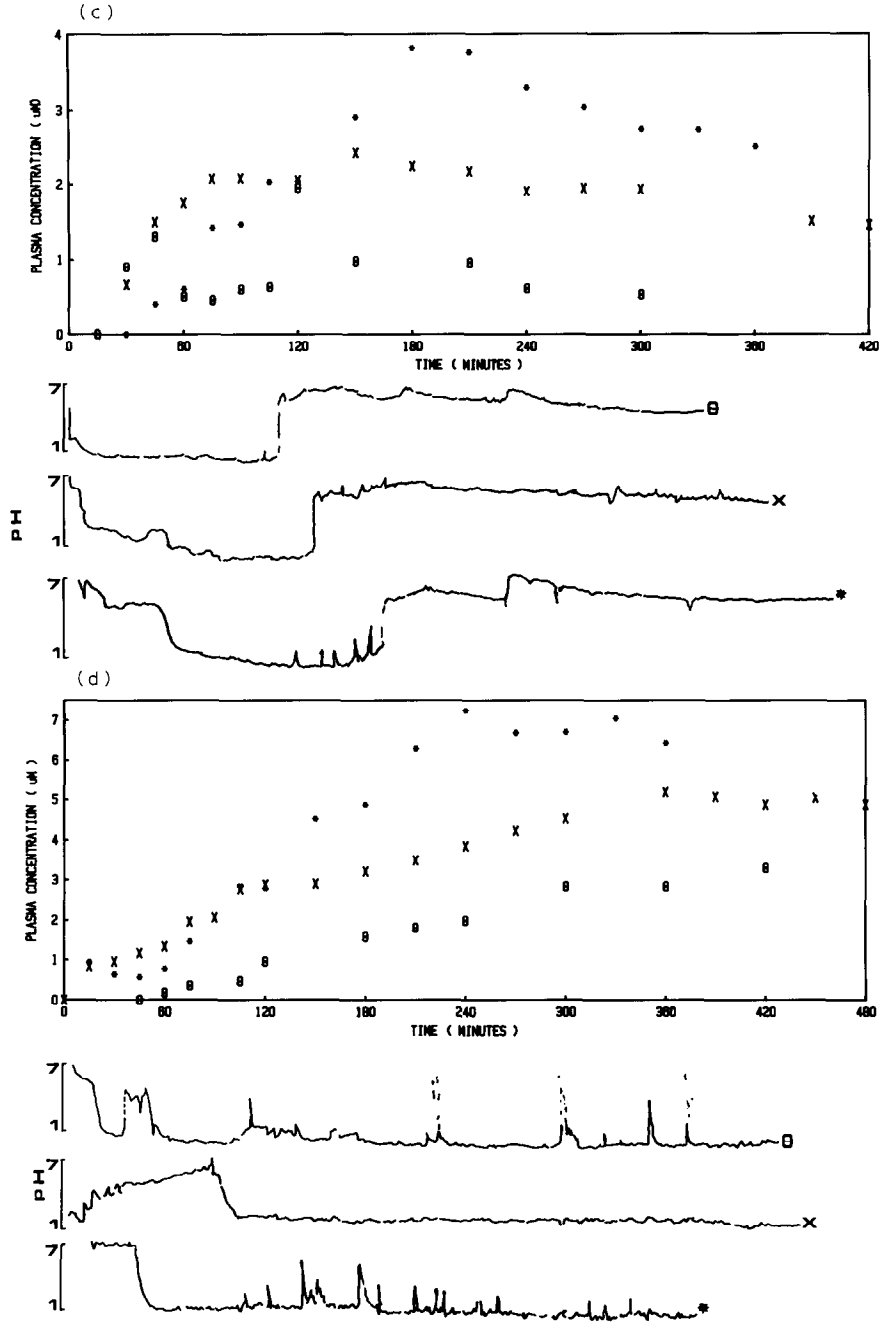PLASMA CONCENTRATION (uM)

TIME (MINUTES)

pH
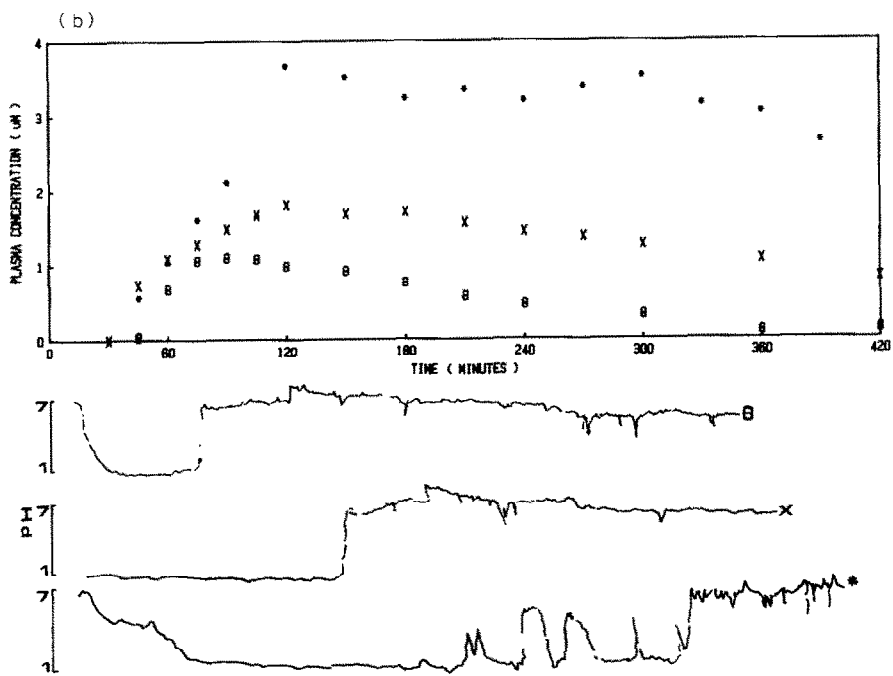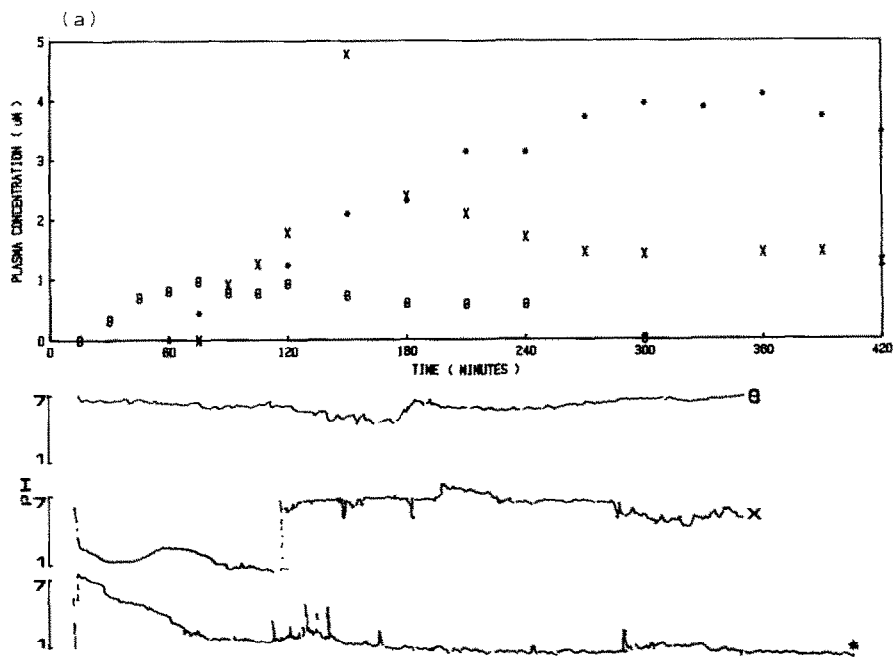
Fig. 1. PHT plasma concentrations and GI pH profiles vs. time from oral capsule dosage (15 mg/kg). Opposite page: (a) dog no. 1; (b) dog no. 2. This page: (c) dog no. 3; (d) dog no. 4. Key: O, phenytoin alone, top GI pH profile; ×, plus glucose, middle; *, plus Osmolite, bottom.

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TABLE 1

PHT plasma concentration vs. time parameters in dogs

Dog	Treatment	C_{max} (μM)	AUC to 8 h (μM h)	GET[a] (h)
1	control	1.0	2.7	0
	glucose	4.8	10.5	1.8
	Osmolite	4.1	16.8	>8
2	control	1.1	3.9	1.1
	glucose	1.8	8.5	1.8
	Osmolite	3.7	17.0	5.4
3	control	1.4	3.9	1.7
	glucose	2.4	12.4	1.9
	Osmolite	3.8	14.0	2.4
4	control	4.0	11.8	>8
	glucose	5.2	28.6	>8
	Osmolite	7.4	42.2	>8
Fistulated dog			AUC to 6 h (μM h)	
	control	3.8	11.2	—
	glucose	5.8	29.0	—
	Osmolite	8.1	35.2	—

[a]GET = gastric emptying time in hours.

The results in dogs nos. 3 and 4, as well as in the dog with the duodenal fistula, indicate that the increased plasma levels of PHT in the fed state are not solely the result of increasing gastric residence time, thereby permitting a greater extent of drug dissolution. The in vitro studies suggest that fed-state bile salt concentrations increase the dissolution rate of PHT. Sodium taurocholate concentrations above the critical micelle concentration (3–4 mM) increase phenytoin solubility and result in an increase in dissolution rate in the rotating disc experiments as shown in Fig. 2.

Although the administered fluid volume was kept constant between treatments, different nutrient treatments may have stimulated fluid secretion in the stomach to different degrees. Greater fluid volume in the stomach would provide greater dissolution capacity and result in an increase in dissolved PHT leaving the stomach with the emptying fluid. In addition, the choice of administered fluid volume (150 ml) is in a region where the fluid emptying pattern from the stomach is highly variable in dogs [19]. Experiments designed to more clearly separate the volume and nutrient effects are currently underway.

Drug and nutrient input into the dog duodenal fistula bypassed both the stomach and bile duct. Yet a nutrient enhancement of PHT plasma levels was still observed. Since the nutrient solutions were hyperosmolar, depression of intestinal motility, stimulation of intestinal secretions and increased availability of paracellular pathways may have played a role in increasing PHT uptake. In the rat intestinal perfusion studies, stomach emptying, bile input, and drug dissolution steps are bypassed by direct input of phenytoin iso-osmotic solutions into the intestine. Co-perfusion of glucose (20 and 100 mM) resulted in higher PHT permeabilities and this increase was reversible by inclusion of phlorizin, an inhibitor of active mucosal glucose transport [6] (Table 2). PHT permeabilities were less variable at 100 mM D-glucose than at 20 mM and a greater degree of intestinal water absorption was observed at the higher glucose concentration (Table 2). PHT permeabilities were depressed at high calcium chloride concentrations (15–100 mM) while they were unaffected by concentrations in the range of extracellular calcium concentrations (2–5 mM).

High incubation concentrations of calcium chloride also depressed initial rate uptake of PHT by intestinal rings while D-glucose had no effect. Since this latter result was inconsistent with the in situ perfusion data, a time course of PHT uptake by intestinal rings was conducted with, again, no effect of D-glucose on PHT uptake being detected (Fig. 3, Table 3).

The phenytoin jejunal perfusion data are consistent with a recent conjecture [6] that intestinal absorption of sodium-co-transported nutrients triggers the opening of paracellular pathways. This action would result in enhanced absorption of nutrients and may serve
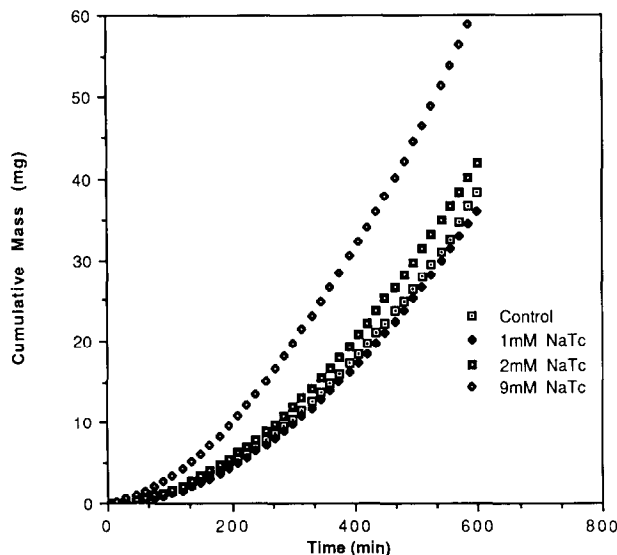
Fig. 2. Cumulative mass appearing in dissolution media with time from rotating phenytoin disc dissolution as a function of medium sodium taurocholate (NaTc) concentration. Temp. $= 37\,^\circ$C, pH $= 6.45$, rotational speed $= 200$ rpm, tablet compression $= 2500$ lbs/sq. in., and dissolution volume $= 200$ ml. Data points represent one set of experiments, $n = 1$.

TABLE 2

Dimensionless jejunal PHT membrane permeabilities

Treatment	P_m+S.E.M.	% water transport/cm[a]
Control	4.7+0.17	−0.5
+20 mM glucose	*8.8+2.58	+0.5
+100 mM glucose	*7.8+0.22	+2.0
+20 mM glucose +0.5 mM phlorizin	4.5+0.54	+0.1
+50 mM calcium	*1.9+0.50	−0.2
+2 mM calcium	4.7+1.55	−0.2

*Significantly different (t-test p value < 0.05 for $n = 8$).
[a]−: secretion; +: absorption.

TABLE 3

a. PHT jejunal ring uptake (μmol/g min)

Treatment	Uptake+S.E.M.
Control	8.0+1.59
+5 mM glucose	7.5+0.91
+20 mM glucose	7.8+0.56
+100 mM glucose	8.6+0.65

b. PHT duodenal ring uptake (μmol/g min)

Treatment	Uptake+S.E.M.
Control	9.0+0.43
+10 mM calcium	*6.0+0.43
+40 mM calcium	*6.4+0.51

Three minute incubations at shaking rate 72 rpm.
*Significantly different from controls (t-test $p < 0.05$ $n = 8$ rings in 2 rats).

as an energy-conserving mechanism. Such a mechanism was postulated to account for efficient sugar absorption at lumenal concentrations in large excess of the K_m (0.5–10 mM) of the rat jejunal sodium–D-glucose co-transporter. Increased water absorption at high nutrient concentrations suggests that local convection and solvent drag of solute may be operative, increasing solute absorption. Furthermore since all perfusion solutions were iso-osmolar, these forces could not be generated by

lumenal osmolality differences. The data appears more likely to be the result of intercellular events related to transcellular nutrient transport.

Opening of paracellular pathways in epithelia has been associated with removal of calcium from mucosal solutions [20]. With this consideration, the agreement of the in situ and in vitro

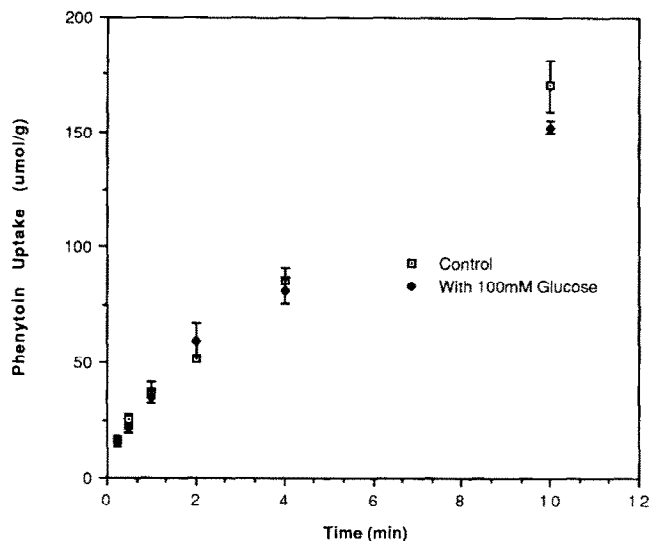Fig. 3. Rat jejunal ring phenytoin uptake with time in the presence and absence of 100 mM D-Glucose. Temp. $= 37\,^\circ$C, pH $= 6.5$, osmolarity $= 300$ mOsm/kg, shaking rate $= 90$ rpm. Values are the standard error of the mean, $n = 12$ for control and $n = 9$ for glucose.

data suggest that high calcium levels may influence the paracellular pathway from the mucosal side. The disagreement between the two systems with respect to the influence of glucose on passive transport of solute suggests that an intact blood supply is a requirement. The role of basolateral membrane controls on intestinal glucose transport and the influence on passive intestinal drug transport is currently under investigation.

The selectivity of this nutrient-controlled parallel absorption pathway should be a function of both drug molecular size and polarity. Phenytoin is a small molecule that is uncharged at perfusion pH. The high lipophilicity of the drug suggests that partitioning into the mucosal membrane should not limit transcellular transport. Reduction of the aqueous resistance near the mucosal membrane by nutrient-induced water absorption coupled with resultant solvent drag through nutrient-controlled paracellular pathways, however, may be playing a role to enhance PHT uptake.

SUMMARY

The potential *in vivo* significance of a drug-nutrient interaction can often be projected based on the physicochemical properties of the drug which dictate limits to drug absorption rate. For phenytoin, these properties suggest that interactions affecting drug dissolution should dominate absorption variability. Experimental isolation of other components of drug transport, however, indicate that nutrient effects at the level of the intestinal membrane barrier may be playing a more general role in drug absorption variability. The clinical impact of such variability for PHT may be damped in humans since metabolism is saturable within the dose range of phenytoin and may dominate absorption variability. However, the narrow therapeutic index of phenytoin suggests that absorption variability may have played a role in the case study nutrient interactions that have been reported in the clinics.

ACKNOWLEDGEMENTS

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