A Dynamic Artificial Gastrointestinal System for Studying the Behavior of Orally Administered Drug Dosage Forms Under Various Physiological Conditions

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Purpose. The purpose of this study was to demonstrate the potential of a dynamic, multicompartmental *in vitro* system simulating the human stomach and small intestine (TIM-1) for studying the behavior of oral drug dosage forms under various physiological gastrointestinal conditions.

Methods. Two model drug compounds were studied in TIM-1: a lyophilized *Lactobacillus* strain and paracetamol (acetaminophen). The *Lactobacillus* survival rate was determined by bacterial counting in the gastric and ileal effluents while simulating the conditions of the gastrointestinal tract of infants or adults. The availability for absorption of paracetamol from two oral dosage forms was investigated by measuring the drug concentration in jejunal dialysis fluid. The effect of gastrointestinal passage time and food intake on paracetamol absorption was also studied.

Results. The *Lactobacillus* survival rate in both gastric and ileal effluents was higher during simulation of the infant compared to adult conditions. We also showed that (i) paracetamol absorption was faster when it was administered as a free powder than in sustained-release tablet form, (ii) a slow passage time resulted in a delay in the absorption of paracetamol, and (iii) there was a lower rate of absorption when paracetamol was ingested with a standard breakfast as opposed to water. The *in vitro* results were consistent with *in vivo* data, showing the predictive value of TIM-1.

Conclusions. TIM-1 is a powerful tool for supplying valuable information about the effects of various gastrointestinal conditions on biopharmaceutical behavior and efficacy of drug delivery systems in the development of oral formulations.

KEY WORDS: dissolution profile; gastrointestinal model; oral formulations; paracetamol; probiotics.

INTRODUCTION

Dissolution is a critical parameter of pharmaceutical dosage forms. *In vitro* dissolution testing is used to screen formulations during the development of drug dosage forms but mostly as a quality control test in order to ensure batch-to-batch reproducibility. The fate of a new drug compound after oral administration is only studied in simple static *in vitro* systems, as described in the European and US *Pharmacopoeia*. Despite their ability to perform *in vitro/in vivo* correlation, these systems are unable to provide understanding of the possible risks related to specific gastrointestinal (GI) conditions, dose dumping, the effects of food on bioavailability, and interaction with other drugs. Moreover, these systems are not truly representative of the continuously changing variables during passage through the stomach and the gut, and they give no information about drug absorption.

A multicompartmental, dynamic, computer-controlled system that simulates the human GI tract, TIM-1 (1,2), was developed at TNO Nutrition and Food Research (Zeist, The Netherlands) and validated in collaboration with Equipe de Recherche Technologique 'Conception, Ingénierie et Développement de l'Aliment et du Médicament' (ERT CIDAM). At the present time, this *in vitro* system allows the closest simulation of *in vivo* dynamic physiological processes that occur within the lumen of the stomach and small intestine of humans. In fact, most of the artificial digestive systems developed to date have been dedicated to a single application (3–5) and include a limited number of simulated parameters. None of the *in vitro* digestive systems published so far (3–7) meet all of the following five requirements (8): (i) sequential use of enzymes in physiological amounts, (ii) appropriate pH for the enzymes and addition of relevant cofactors such as bile salts and coenzymes, (iii) removal of the products of digestion, (iv) appropriate mixing at each stage of digestion, and (v) physiological transit times for each step of digestion. However, the TIM-1 system indeed fulfills more than these requirements.

TIM-1 was designed based on parameters and data obtained from studies on human volunteers. The main parameters of digestion, such as pH, body temperature, peristaltic mixing and transit, salivary, gastric, biliary, and pancreatic secretions, absorption of small molecules (e.g., nutrients, drugs) and water, are simulated. GI passage and successive conditions are controlled to mimic parameters in humans at different life stages (infant, adult, and elderly), different food intakes and physiological or pathological conditions (such as gastric hyperacidity or pancreatic failure). This *in vitro* system offers advantages above *in vivo* studies such as accuracy, reproducibility (no biological variation), easy manipulation, and the possibility of collecting samples at any level of the GI tract at any time during digestion. In addition, it is not impeded by ethical constraints, even when toxic compounds are involved. Experiments have been performed that show that the conditions simulated in TIM-1 are reliable, reproducible, and consistent with *in vivo* data (1,9). Validation experiments demonstrate the predictive value of the system with regard to the availability for absorption of minerals (10), vitamins (11) and food mutagens (12), and the survival of bacteria (13) and yeasts (14,15) in humans.

The TNO artificial GI system could also be a useful tool in pharma-related studies for following the intralumenal fate of a drug compound or a probiotic strain under dynamic conditions. For instance, TIM-1 could be used to determine where and when a compound is released, what might influ-

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ABBREVIATIONS: CFU, colony-forming units; GI, gastrointestinal; IR, immediate release; SR, sustained-release; TIM, TNO gastrointestinal model.

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ence its release, its stability or viability and its availability for absorption, and what role the presence of food, transit time, or drug delivery systems could play in these processes. The absorption phase is simulated in TIM-1 by the use of dialysis membrane. Therefore, this system is only suited for drug compounds that are absorbed by passive diffusion and not by active transport (mucosal cells are not involved in the actual configuration of the *in vitro* system), except when the active transport through the mucosa is not a limiting step.

The aim of the current study was to show the potential of TIM-1 when applied to research on the behavior of oral drug dosage forms and the fate of the active compound. Two model compounds were chosen: a lyophilized *Lactobacillus* strain used in the treatment of diarrhea, and the common analgesic and antipyretic, paracetamol (acetaminophen), in two different dosage forms, namely as immediate release (IR) and sustained-release (SR) formulation. *In vivo*, the *Lactobacillus* strain is not absorbed in the GI tract and must survive to exert its health effect whereas paracetamol is absorbed by passive diffusion for a systemic analgesic effect. First, we determined *Lactobacillus* survival rate in TIM-1 during simulation of infant or adult GI conditions. Second, we assessed the effects of drug delivery systems, GI transit time, and food intake on the availability for absorption of paracetamol.

MATERIALS AND METHODS

Materials

Test Compounds

Lactobacillus LY/SA 1 (*Lactobacillus casei* spp. *rhamnosus*) is commercialized as a probiotic strain (Bacilor®) by Lyocentre (Aurillac, France) for the treatment of diarrhea. It was supplied as a lyophilized powder for oral suspension (1500 mg per sachet). Paracetamol was obtained from Genfarma (Maarssen, The Netherlands). The SR amylodextrin matrix tablets contains 30% active substance (16). The tablets are 9 mm in diameter.

Artificial Dynamic GI System (TIM-1)

The gastric–small intestinal system TIM-1 (1,2) consists of four serial compartments simulating the stomach and the three segments of the small intestine: the duodenum, jejunum, and ileum (Fig. 1). Each compartment is formed by two connected basic units consisting of a glass jacket with a flexible wall inside. Water is pumped from a water bath into the glass jackets around the flexible walls to control the temperature inside the units (37°C) and the pressure on the flexible walls. Changes in the water pressure enable mixing of the chyme by alternate compression and relaxation of the flexible walls. To control the transit of the chyme, a power exponential formula ($f = 1 - 2^{-(t/t_{1/2})\beta}$, where f represents the fraction of chyme delivered, t the time of delivery, $t_{1/2}$ the half-time of delivery, and β is a coefficient describing the shape of the curve) is used for gastric and ileal delivery, as described by Elashoff *et al.* (17). Chyme transit is then regulated by opening or closing the peristaltic valves that connect the compartments. The volume in each compartment is monitored by a pressure sensor connected to the computer. The pH is computer-monitored and continuously controlled by secreting either water or 1 M HCl (0.25 ml/min in total) into the stomach and either electrolytes or 1 M $NaHCO_3$ (0.25 ml/min in total) into the small intestine. Simulated gastric (0.5 ml/min), biliary (0.5 ml/min), and pancreatic (0.25 ml/min) secretions, that is, pepsin, lipase, pancreatin, and bile salts (1), are introduced into the corresponding compartments by computer-controlled pumps. The model is equipped with hollow fiber membranes (HG 400, Hospal Cobe, France, cutoff = 5800 Da) connected to the jejunal and ileal compartments. Water and small molecules (e.g., products of digestion, dissolved drugs) are removed from the lumen of the compartments by pumping dialysis fluid (10 ml/min) through the hollow fibers. This prevents product inhibition caused by the build-up of metabolites. Before each experiment, the system is washed with detergent, rinsed with water, and decontaminated by steaming at 100°C for 45 min.

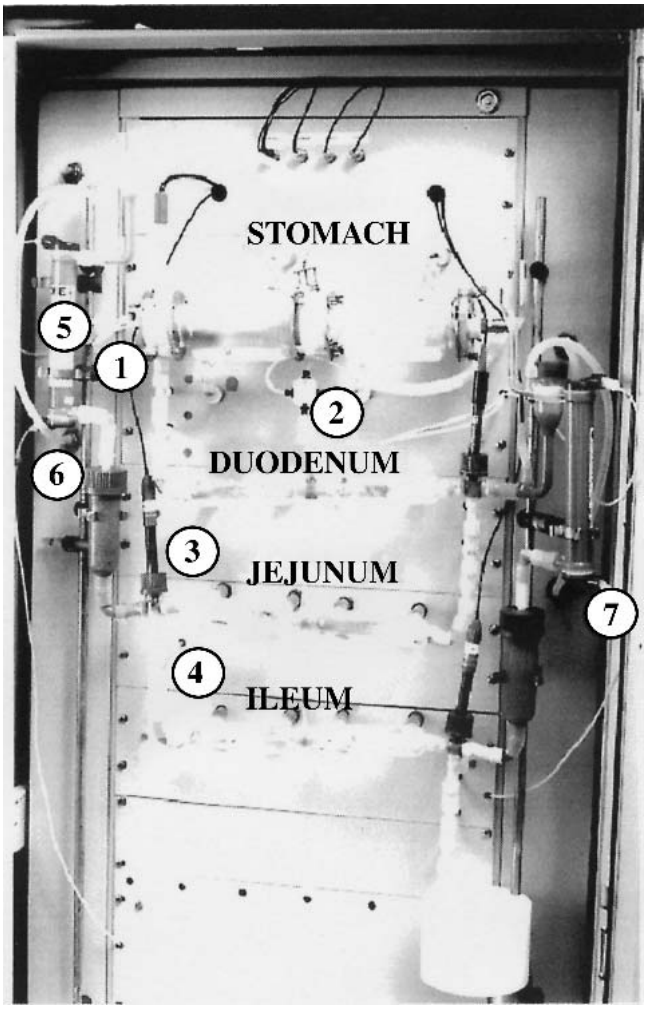

Fig. 1. TNO gastric–small intestinal model (TIM-1): 1, gastric secretions (lipase, pepsin and hydrochloric acid); 2, small intestinal secretions (pancreatic juice, bile, and sodium bicarbonate); 3, pH electrodes; 4, peristaltic valves; 5, hollow fiber membranes; 6, jejunal dialysis fluid; and 7, ileal dialysis fluid.

Methods

Survival Rate of Lactobacillus LY/SA 1

Two sachets of Bacilor® (2.8×10^9 bacterial cells) were resuspended into 300 ml of infant milk (first age, Bledina®) or

sterile water for *in vitro* experiments simulating the infant or the adult conditions, respectively. The gastric–small intestinal system was programmed with *in vivo* data to reproduce either average GI conditions of the infant after intake of formula milk or of the adult after intake of a glass of water (fast GI passage). The main parameters of *in vitro* GI conditions in the infant and in the adult are compared in Table I. In both cases, the experiments were performed either in the stomach alone (gastric digestion) or in the whole TIM-1 (gastric and small intestinal digestion).

One sample was taken before introduction of the bacterial suspension into the artificial stomach. The gastric and ileal effluents were collected on ice to inhibit the activity of digestive enzymes. For gastric digestion, the collection vessels were replaced at 15, 30, 60, 90, 120, and 180 min. In the case of the gastric and small intestinal digestion, collection vessels were changed at 60, 120, 180, and 240 min and at 60, 120, 180, 240, 300, and 360 min for the infant and the adult conditions, respectively. The volumes were measured, and samples were taken for each period.

Samples were diluted in physiological water and plated onto a selective medium for *Lactobacillus* strains (Agar MRS, pH 5). Petri dishes were incubated in anaerobic conditions at 37°C for 72 h, and the number of colony-forming units (CFU) was counted. The results were expressed as a percentage of the initial intake.

Availability for Absorption of Paracetamol

The availability for absorption of paracetamol was estimated in TIM-1 by measuring the drug concentration in jejunal dialysis fluid, following its passive diffusion through the hollow fiber membranes connected to jejunum.

To assess the effect of oral drug delivery systems on drug release, the availability for absorption of paracetamol was studied in TIM-1 after administration of the drug in powder form (IR) or as SR amylodextrin matrix tablets. The dose administered was either 500 mg of free powder or 5 tablets each containing 100 mg of paracetamol, with 200 g of water and 100 g of artificial saliva. TIM-1 was programmed to simulate the adult GI conditions after the intake of water (fast GI passage, Table I).

In order to determine the drug-nutrient interaction, the availability for absorption of paracetamol was studied in TIM-1 when it was administered with either water or a standard breakfast. To constitute the two different "meals," 500 mg of paracetamol powder was added to 100 g of artificial saliva and either 200 g of water or 200 g of a standard breakfast. The standard breakfast was a European continental breakfast for adults (18) and comprised the following: 10 slices of white bread and 10 slices of brown bread, 100 g of margarine (with 63 to 65% linoleic acid), 200 g of cheese with ~48% fat (Gouda cheese), 150 g of strawberry jam containing 45% fruit, and 1 l of water. The ingredients were mixed as described by Vaquero *et al.* (19) and stored in portions at –20°C before use. TIM-1 was programmed to simulate the adult GI conditions after the intake of water (i.e., with a fast GI passage) or the adult conditions after the intake of a semi-solid "meal" (i.e., with a slow GI passage) (Table I).

Samples were collected from the jejunal dialysis fluid every 15 min for the first 120 min of digestion then every 30 min from 120 to 240 min and every 60 min until the end of the experiment (360 min).

Paracetamol concentration in jejunal dialysis fluid was measured by reversed-phase high-performance liquid chromatography with UV detection (247 nm). Samples were analyzed on a Phenomenex (Torrance, USA) RP-18 at 25°C. Elution was performed using a mixture of phosphate buffer 50 mM (pH 5) and methanol (70:30 v/v) as a mobile phase and at a flow rate of 0.5 ml/min. The typical elution time for paracetamol was 6.5 min. Quantification of paracetamol was determined by comparison with standard paracetamol solutions.

Table I. Parameters of *In vitro* GI Conditions in the Infant and in the Adult with a Fast or a Slow GI Passage

Parameters of *In vitro* GI conditions	Infant	Adult (fast GI passage)	Adult (slow GI passage)
Gastric conditions			
Time/pH	0/6.5	0/4.5	0/5.5
	30/6.5	10/3.2	20/4.2
	150/4.5	20/2.8	40/2.8
	240/3.5	40/1.8	60/2.1
		60/1.7	90/1.8
		120/1.5	120/1.7
		360/1.5	360/1.7
Secretions	Pepsin 525 U/ml	Pepsin 600 U/ml	Pepsin 600 U/ml
	Lipase 30 U/ml	Lipase 37.5 U/ml	Lipase 37.5 U/ml
$t_{1/2}$	80 min	30 min	70 min
β	1.23	1	2
Intestinal conditions			
Compartment/pH	Duodenum/6.5	Duodenum/6.5	Duodenum/6.5
	Jejunum/6.8	Jejunum/6.8	Jejunum/6.8
	Ileum/7.2	Ileum/7.2	Ileum/7.2
Secretions	Pancreatin 2%	Pancreatin 7%	Pancreatin 7%
	Bile salts 1%	Bile salts 4% (0–30 min)	Bile salts 4% (0–30 min)
		2% (30–360 min)	2% (30–360 min)
$t_{1/2}$	200 min	160 min	160 min
β	2.2	1.6	1.6

RESULTS AND DISCUSSION

Survival Rate of *Lactobacillus* LY/SA 1

The number of living cells of *Lactobacillus* LY/SA 1 in the gastric effluents of TIM-1 did not significantly decrease during simulated infant gastric conditions (Fig. 2a). After 180 min of gastric digestion, the bacterial survival rate was 68 ± 30% (n = 3). By that time, 74 ± 20% (n = 3) of the ingested bacteria had crossed the gastric compartment and was recovered in the gastric effluents (Fig. 2b), which indicates that the *Lactobacillus* strain is highly tolerant of infant gastric conditions. When adult gastric conditions were mimicked, *Lactobacillus* LY/SA 1 survival rate decreased to 42 ± 16% (n = 2) after 60 min of digestion, and thereafter no viable bacteria were detectable in the gastric effluents (Fig. 2a). However, after the first 60 min of digestion, 38 ± 18% (n = 2) of the ingested *Lactobacillus* LY/SA 1 was recovered in the gastric effluents (Fig. 2b).

The survival rate of *Lactobacillus* LY/SA 1 in the ileal effluents of TIM-1 during infant *in vitro* digestion was lower than in the gastric effluents (Fig. 3a). The survival rate decreased to 40 ± 3% (n = 3) after 60 min of digestion and remained stable thereafter. At the end of the experiment (240 min), 32 ± 2% (n = 3) of the ingested bacteria had passed the gastric and small intestinal compartments alive and was recovered in the ileal effluents (Fig. 3b). When adult GI conditions were simulated, the *Lactobacillus* LY/SA 1 survival rate quickly decreased during the first 2 h (until 0.7 ± 0.6% at 120 min, n = 2) and remained stable thereafter until the end of experiment. After 360 min of digestion, the survival rate reached 0.5 ± 0.3% (n = 2) (Fig. 3a) and the cumulative recovery in the ileal effluents was 0.9 ± 0.7% (n = 2) of the initial intake (Fig. 3b).

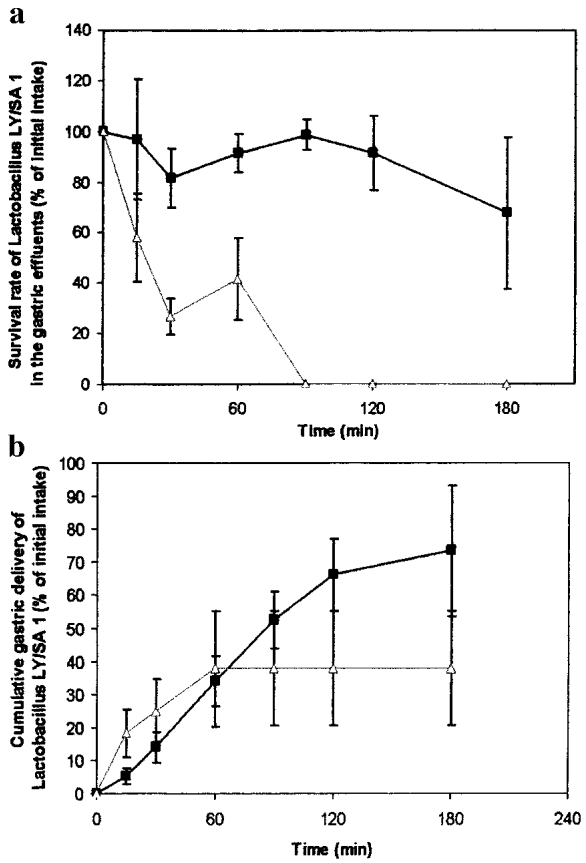

Fig. 2. Effect of the gastric conditions related to the infant (■) or the adult (Δ) as simulated in TIM-1 on the viability of *Lactobacillus* LY/SA 1. (a) Survival rate of *Lactobacillus* LY/SA 1 in the gastric effluents. (b) Cumulative gastric delivery of viable *Lactobacillus* LY/SA 1. In both cases, values are expressed as a mean percentage ± SD (n = 3 for the infant and n = 2 for the adult) of the initial intake.

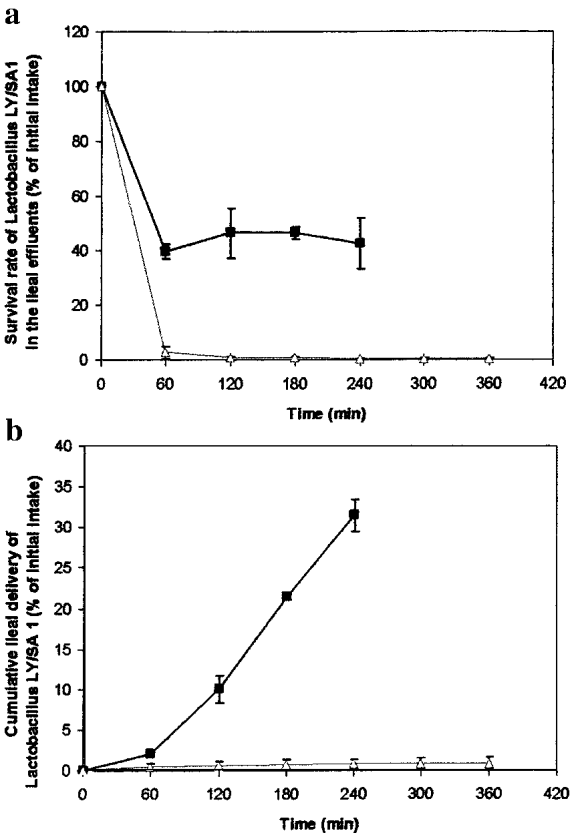

Fig. 3. Effect of the gastric and small intestinal conditions related to the infant (■) or the adult (Δ) as simulated in TIM-1 on the viability of *Lactobacillus* LY/SA 1. (a) Survival rate of *Lactobacillus* LY/SA 1 in the ileal effluents. (b) Cumulative ileal delivery of viable *Lactobacillus* LY/SA 1. In both cases, values are expressed as a mean percentage ± SD (n = 3 for the infant and n = 2 for the adult) of the initial intake.

The survival rate of *Lactobacillus* LY/SA 1 both in the gastric and in the ileal effluents was higher during GI passage simulating the infant conditions than during that of the adult. These results indicate that *Lactobacillus* LY/SA 1 is sensitive to the low gastric pH occurring during simulated adult conditions (pH was below 1.8 after 40 min of digestion) but not during simulated infant conditions (pH value was never below 3.5). Our results are in accordance with those of Goldin *et al.* (20) who showed *in vitro* that *Lactobacillus* GG and *Lactobacillus bulgaricus* survived at pH values up to 3 but were completely destroyed in 30 min when pH was under 1. The difference in the bacterial survival rates in the ileal effluents between the infant and adult conditions may also be explained by the higher concentration of bile salts simulated for the adult (Table I). The bactericidal effect of bile salts on *Lactobacillus* spp. has been previously reported (13). The higher survival rate observed during infant digestion may

have resulted from a protective effect of milk (compared to water used for the adult) due to its buffering capacity (21,22).

Despite the loss of viability of *Lactobacillus* LY/SA 1 in TIM-1, the bacterial survival rates are compatible with a probiotic effect of the strain *in vivo*, both in the infant and in the adult digestive tract. Concentrations of living bacteria in the ileal effluents ranging from 10^4 to 10^7 CFU/ml were found, which may be high enough for the bacterial strain to have a health effect on the human gut (22).

The survival rates of *Lactobacillus* LY/SA 1 in the gastric and ileal effluents of TIM-1 under simulated adult conditions were close to those obtained by other authors (21–23) studying different *Lactobacillus* spp. in adult volunteers. Conway *et al.* (21) showed that a *Lactobacillus acidophilus* strain survived from 40 to 60 min in the stomach of fasted volunteers. After a single dose of bacteria (approx. 10^{10} cfu), survival rates in the ileal fluid ranging from 0.5 to 7% were obtained, depending on the *Lactobacillus* strain (22–23). The *in vitro* and *in vivo* results are comparable, suggesting that our *in vitro* system can be used to predict the survival of *Lactobacillus* spp. in humans. With TIM-1, it is possible to determine the survival rate of bacterial probiotic strains under different GI conditions (e.g., infant or adult) and also to establish their kinetics during gastric and intestinal transit.

Availability for Absorption of Paracetamol

Effect of Oral Drug Delivery Systems

The availability for absorption of paracetamol was studied in TIM-1 after administration of free powder or SR tablets (both containing 500 mg of active compound). The results are expressed as cumulative amounts of paracetamol in jejunal dialysis fluid (Fig. 4). They show a profile characteristic of the release and absorption of a drug from a dosage form. The absorption profiles obtained depended on the drug delivery system studied. Within 6 h of GI passage, the amount of absorption was higher when paracetamol was administered as IR formulation as opposed to SR formulation (394 ± 12 mg vs. 204 ± 18 mg, n = 2). Rate of absorption was faster with the IR form, whereas a more prolonged absorption of paracetamol was observed when it was administered as a SR tablet. The rate of absorption, calculated from the unabsorbed amounts as a function of time (data not shown), can be modeled according to a first-order model for the IR formulation ($r^2 = 0.9955$) and a zero order model ($r^2 = 0.9869$) for the SR formulation.

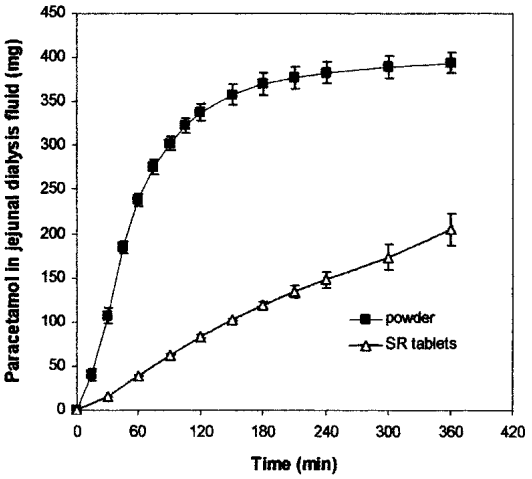

Fig. 4. Effect of oral drug delivery systems on the availability for absorption of paracetamol from the jejunal compartment of TIM-1. The paracetamol concentrations were measured in the jejunal dialysis fluid after oral administration of the drug as powder (■) or as SR tablets (Δ). Values are expressed as cumulative amounts of absorbed paracetamol (mg) ± SD (n = 2).

The profiles of jejunal absorption found *in vitro* are consistent with the *in vivo* data (24,25). Following oral administration of IR formulation, the drug is rapidly dissolved then absorbed according to a passive diffusion (Fick's law) corresponding to a first-order reaction. The limiting step of drug input in the body is the absorption. After *in vivo* administration of a SR formulation, the limiting step of drug input is its release from the dosage form (a zero-order drug release model in that case). The time-point (T_{max}) at which maximum paracetamol concentration was reached *in vivo* with the IR form (24–26) was similar to the time at which maximum rate was obtained with *in vitro* conditions. Nevertheless, no quantitative relation could be made between the maximum jejunal dialysis concentration and the maximum plasma concentration. This lack of correlation can be explained by (i) the selective uptake and metabolization of paracetamol by epithelial cells and the first-pass effect, which occur *in vivo* but not in our *in vitro* system, (ii) the much larger volume for drug dilution and distribution *in vivo,* and (iii) the removal of the drug from the blood by renal clearance. In TIM-1, SR tablets led to a more prolonged absorption of paracetamol compared to free powder. Similar effects were obtained *in vivo* when the same SR tablet (16) was administered to adult volunteers and with a different controlled-release system, the Gradient Matrix System (27).

The results obtained in our *in vitro* GI system can be related to *in vivo* conditions, showing that TIM-1 has a predictive value for studying and explaining the behavior of drug delivery systems in the human GI tract. In order to make further *in vivo*/*in vitro* correlation, absorption processes, first-pass effect, and volume of distribution and elimination should be taken into account.

Effect of GI Transit Time and Food Intake

The availability for absorption of paracetamol in powder after oral intake was studied in TIM-1 under three different conditions of the GI tract: (i) paracetamol was administered with water and subjected to a fast GI passage (fasted state), (ii) it was administered with water and subjected to a slow GI passage, and (iii) it was administered with a standard breakfast and subjected to a slow GI passage (fed state). The curves we obtained for cumulative jejunal absorption of paracetamol were characteristic of a drug absorption rate profile (Fig. 5). When paracetamol was administered with water under simulation of a fast GI passage, it was rapidly absorbed from the jejunal compartment: after 60 min of digestion; 60.5% of the total amount available for absorption was absorbed. When paracetamol was administered in the same way but subjected to the simulation of a slow GI passage time, the absorption was delayed; 20.1% being absorbed after 60 min. Nevertheless, after 120 min of digestion, the amount of paracetamol absorbed was similar under both conditions (fast or slow GI passage). When paracetamol was administered with a stan-

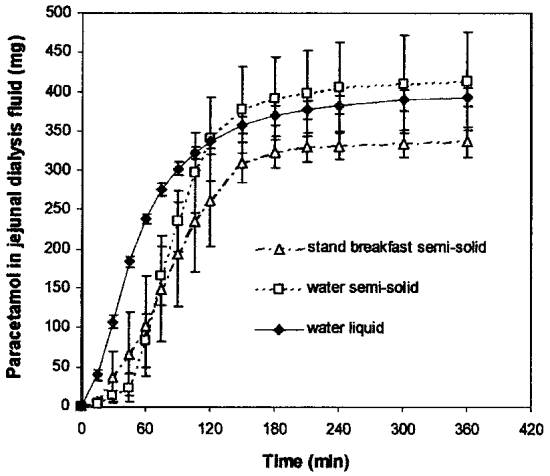

Fig. 5. Effect of GI transit time and food intake on the availability for absorption of paracetamol from the jejunal compartment of TIM-1. The paracetamol concentrations were measured in the jejunal dialysis fluid after oral administration of the drug in powder form with either water or a standard breakfast. TIM-1 was programmed to simulate a slow or a fast GI passage. Values are expressed as cumulative amounts of absorbed paracetamol (mg) ± SD (n = 2).

dard breakfast and subjected to a slow GI passage, the same decrease in rate of absorption was observed (30.4% absorbed in 60 min). In addition, related to the nature of the food intake (semisolid "meal"), a lower amount of paracetamol was absorbed within the 6 h of *in vitro* GI transit (337 ± 19 mg in semisolid "meal" matrix vs. 414 ± 62 mg in water matrix under the same GI conditions and transit time). So, the rate of paracetamol absorption, as well as the total amount of absorbed drug, were modified in presence of food. The rate is mainly determined by a difference in passage time induced by the food whereas the total amount of absorbed paracetamol is mainly determined by a food matrix effect. In the presence of food, the absorption could not be further modeled according to a first-order model.

A similar effect of food intake on paracetamol absorption was previously observed in human volunteers by Rostami-Hodjegan *et al.* (25) and Divoll *et al.* (28). They showed that, in the feeding state, the peak plasma concentration of paracetamol was lower, and the T_{max} was delayed compared to intake with water during the fasted state. According to Jaffe *et al.* (29), this lower rate of paracetamol absorption may partially be attributed to an interaction with the carbohydrates of the meal.

Hence, we have demonstrated that TIM-1 is a suitable tool for determining drug–nutrient interaction. Moreover, with this *in vitro* system, it is possible to study separately the effect of food intake and GI passage time on the availability for absorption of a drug, which is not possible in humans.

CONCLUSIONS

Using two model drug compounds, a living bacterial strain used in the treatment of diarrhea and paracetamol, a classical chemical compound, we showed that TIM-1 yields valuable information in the early stages of development of oral drug dosage forms, with a high predictive value for the *in vivo* situation. This system is a supportive tool complementary to the *in vitro* dissolution technique, but allowing more information to be collected. The effect of various GI conditions (such as different life stages, food intake, or transit time) on drug release from a drug dosage form, its stability or viability (in case of living cells), and the subsequent availability for absorption in the GI tract could be estimated in TIM-1. This gives the possibility to study the efficacy of various oral drug delivery systems very easily under controlled, reproducible, and dynamic conditions. However, because a mucosal layer is not involved in the actual configuration of TIM-1, this system cannot simulate the physiological processes of the gut wall, such as active or facilitated transport. Local cellular and humoral immune system and feedback mechanisms are also not present in the system. Particularly, the absorption phase being reproduced by the use of dialysis membrane, TIM-1 is suited for drug compounds that are absorbed by passive diffusion or when the active transport through the mucosa is not a limiting step. For greater in-depth studies on the availability for absorption of drug compounds, TIM-1 could be used in combination with cultured intestinal cells to study mucosal transport and the metabolism of drug compounds. If the fate of a living probiotic strain is studied in TIM-1, cultured cells could give information about the ability of the bacteria to adhere to intestinal cells. The use of a large intestinal system TIM-2 (30) as a complement to the gastric–small intestinal system described in this study could also be considered in the development of specific drug dosage forms such as colonic delivery or rectal (31) drug dosage forms as well as in studies on the effect of the complex microflora on the stability of drug compounds.

REFERENCES

1. M. Minekus, P. Marteau, R. Havenaar, and J. H. J. Huis in't Veld. A multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. *Atla* **23**:197–209 (1995).
2. R. Havenaar and M. Minekus. *In vitro* model of an *in vivo* digestive tract. JP, US, European Patent PCT/NL93/00225 (1994).
3. L. Babinsky, J. M. Van Der Meer, H. Boer, and L. A. Den Hartog. An *in vitro* method for prediction of digestible crude protein content in pig feeds. *J. Sci. Food Agric.* **50**:173–178 (1990).
4. H. N. Englyst, S. M. Kingman, G. J. Hudson, and J. H. Cummings. Measurement of resistant starch *in vitro* and *in vivo*. *Br. J. Nutr.* **75**:749–755 (1996).
5. J. Vatier, A. Harman, N. Castela, M. T. Droy-Lefaix, and R. Farinotti. Interactions of cimetidine and ranitidine with aluminium-containing antiacids and a clay-containing gastric-protective drug in an "artificial stomach-duodenum" model. *J. Pharm. Sci.* **83**:962–966 (1994).
6. M. Yvon, S. Beucher, P. Scanff, S. Thirouin, and J. P. Pelisssier. *In vitro* simulation of gastric digestion of milk proteins: comparison between *in vitro* and *in vivo* data. *J. Agric. Food Chem.* **40**: 239–244 (1992).
7. K. Molly, M. Van de Woestyne, and W. Verstraete. Development of a 5–step multichamber reactor as a simulation of the human intestinal microbial ecosystem. *Appl. Microbiol. Biotechnol.* **39**: 254–258 (1993).
8. A. C. Longland. Digestive enzymes in pigs and poultry. In M. F. Fuller (ed.), *In Vitro Digestion for Pigs and Poultry*, CAB International, Wallingford, UK, 1991, pp. 3–18.
9. M. J. E. Smeets-Peeters, M. Minekus, R. Havenaar, G. Schaafsma, and M. W. A. Verstegen. Description of a dynamic *in vitro* model of the dog gastrointestinal tract and an evaluation of various transit times for protein and calcium. *Atla* **27**:935–949 (1999).
10. M. Larsson, M. Minekus, and R. Havenaar. Estimation of the bioavailability of iron and phosphorus in cereals using a dynamic *in vitro* gastrointestinal model. *J. Sci. Food Agric.* **74**:99–106 (1997).
11. M. Verwei, K. Arkbage, R. Havenaar, H. Van den Berg, C. Wit-

thöft, and G. Schaafsma. Folic acid and 5-methyl-tetrahydrofolate in fortified milk are bioaccessible as determined in a dynamic *in vitro* gastrointestinal model. *J. Nutr*. **133**:2377–2383 (2003).
12. C. A. M. Krul, A. Luiten-Schuite, R. Baan, H. Verhagen, G. Mohn, V. Feron, and R. Havenaar. Application of a dynamic *in vitro* gastrointestinal tract model to study the availability of food mutagens, using heterocyclic aromatic amines as model compounds. *Food Chem. Toxicol*. **38**:783–792 (2000).
13. P. Marteau, M. Minekus, R. Havenaar, and J. H. J. Huis in't Veld. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of the bile. *J. Dairy Sci*. **80**:1031–1037 (1997).
14. S. Blanquet, S. Marol-Bonnin, E. Beyssac, D. Pompon, M. Renaud, and M. Alric. The biodrug concept: an innovative approach to therapy. *Trends Biotechnol*. **19**:393–400 (2001).
15. S. Blanquet, J. P. Meunier, M. Minekus, S. Marol-Bonnin, and M. Alric. Recombinant *Saccharomyces cerevisiae* expressing a P450 in artificial digestive systems: a model for biodetoxication in the human digestive environment. *Appl. Env. Microbiol*. **69**:2884–2892 (2003).
16. Nederlandse organisatie voor toegepast (TNO). Composition for controlled release substance and method for preparation of such a composition. European Patent 0 648 116 B1 (1996).
17. J. D. Elashoff, T. J. Reedy, and J. M. Meyer. Analysis of gastric emptying data. *Gastroenterology*. **83**:1306–1312 (1982).
18. W. G. M. Boeijen. *Nevo table. Nederlandse voedingstoffen bestand*, TNO Voeding, Zeist, 1987.
19. M. P. Vaquero, W. Van Dokkum, K. D. Bos, M. G. E. Wolters, G. Schaafsma, and J. B. Luten. *In vitro* availability of calcium, magnesium, iron, copper and zinc from white or brown bread separately or in combination with other foods. *Revista Espanola de Ciencia y Technologia de Alimentos.* **32**:47–58 (1992).
20. B. Goldin, S. Gorbach, M. Saxelin, S. Barakat, L. Gualtieri, and S. Salminen. Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. *Dig. Dis. Sci*. **37**:121–128 (1992).
21. P. Conway, S. Gorbach, and B. Goldin. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *J. Dairy Sci*. **70**:1–12 (1987).
22. P. Marteau, P. Pochard, Y. Bouhnik, S. Zidi, I. Goderel, and J. C. Rambaud. Survie dans l'intestin grêle de *Lactobacillus acidophilus* et de *Bifidobacterium spp*. ingérés dans un lait fermenté: une base rationnelle pour l'utilisation des probiotiques chez l'homme. *Gastroenterol. Clin. Biol*. **16**:25–28 (1992).
23. T. Vesa, P. Pochart, and P. Marteau. Pharmacokinetics of *Lactobacillus plantarum* NCIMB 8826, *Lactobacillus fermentum* KLD, and *Lactococcus lactis* MG 1363 in the human gastrointestinal tract. *Aliment Pharmacol. Ther*. **14**:823–828 (2000).
24. B. Ameer, M. Divoll, D. R. Abernethy, D. J. Greenblatt, and L. Shargel. Absolute and relative bioavailability of oral acetaminophen preparations. *J. Pharm. Sci*. **72**:955–958 (1983).
25. A. Rostami-Hodjegan, M. R. Shiran, R. Ayesh, T. J. Grattan, I. Burnett, A. Darby-Dowman, and G. T. Tucker. A new rapidly absorbed paracetamol tablet containing sodium bicarbonate. I A four way crossover study to compare the concentration-time profile of paracetamol from the new paracetamol/sodium bicarbonate tablet and a conventional tablet in fed and fasted volunteers. *Drug Dev. Ind. Pharm*. **28**:523–531 (2002).
26. T. Rygnestad, K. Zahlsen, and F. A. Samdal. Absorption of effervescent paracetamol tablets relative to ordinary paracetamol tablets in healthy volunteers. *Eur. J. Clin. Pharmacol.* **56**:141–143 (2000).
27. E. M. Van Bommel, M. Raghoebar, and J. J. Tukker. Kinetics of acetaminophen after single- and multiple-dose oral administration as a gradient matrix system to healthy male subjects. *Biopharm. Drug Dispos*. **12**:355–366 (1991).
28. M. Divoll, D. J. Greenblatt, B. Ameer, and D. R. Abernethy. Effect of food on acetaminophen absorption in young and elderly subjects. *J. Clin. Pharmacol*. **22**:571–576 (1982).
29. J. M. Jaffe, J. L. Collaizzi, and H. Barry 3rd. Effects of dietary components on GI absorption of acetaminophen tablets in man. *J. Pharm. Sci*. **60**:1646–1650 (1971).
30. M. Minekus, M. Smeets-Peter, A. Bernalier, S. Marol-Bonnin, R. Havenaar, P. Marteau, M. Alric, G. Fonty, and J. H. J. Huis in't Veld. A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. *Appl. Microbiol. Biotechnol*. **53**:108–114 (1999).
31. K. Venema, M. Van Nuenen, M. Smeets-Peeters, M. Minekus, and R. Havenaar. TNO's *in vitro* large intestinal model: an excellent screening tool for functional food and pharmaceutical research. *Ernahrung/Nutrition* **24**:558–564 (2000).