AN *IN VITRO* GASTRO-INTESTINAL TRACT MODEL FOR MICROBIOLOGICAL AND NUTRITIONAL STUDIES

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1 Introduction

In vitro models are popular tools for many types of biomedical research, including nutrition, because these methods have many advantages as compared to research in humans or laboratory animals (Table 1).

*	Cheap
*	Fast
*	Not labour-intensive
*	Easy sampling
*	Reproducible
*	Allow to study variables
*	No ethical constraints

Table 1 Advantages of *in vitro* methods as compared to human and animal studies.

From these advantages one could conclude that there is no better research than *in vitro* research. However, there is one major question: what is the relevance of the results obtained from *in vitro* experiments for the *in vivo* situation? Any *in vitro* method should be thoroughly validated to ensure its applicability and to assess its limitations.

This paper focuses on the development and application of an *in vitro* model of the gastrointestinal (GI) tract.

2 The TNO Gastro-Intestinal Model (TIM)

The schematic drawing of TIM (Figure 1) shows the four serial compartments, simulating the stomach, duodenum, jejunum and ileum, connected with each other by computer controlled pump-valves. The model is made up of separate glass units, with flexible walls inside. The temperature can be controlled at 37 °C.
One of the model's striking features is simulation of the movements of the intestinal contents. The rate and strength of the mixing movements simulate those of the real stomach and small bowel. On the basis of data obtained from *in vivo* studies, our model can be programmed, using mathematical equations, in order to mimic exactly the gastric emptying curves and intestinal transit times that are observed in humans and monogastric animals.
In order to handle (semi-)solids foods without 'constipation' in the model, a special pump-valve system has been designed, so that even a viscous slurry containing solids can be transferred through the model.
Simulated gastric, biliary, and pancreatic secretions are introduced into the corresponding compartments using computer controlled pumps. In this way pH curves and the concentrations of bile salts in the different parts can be simulated.
Finally, absorption is an important feature of the small intestinal part of the model. For this purpose semi-permeable hollow-fibres are applied. In this way products of digestion, such as amino acids, and small sugars, will be absorbed. Also controlled water absorption takes place.
All parameters, such as food consumption, pH in stomach and small intestine, secretions of gastric and pancreatic juice and bile, peristaltic movements in relation to gastric and intestinal filling, gastric emptying and

intestinal transport of chyme, and absorption of water, are regulated and registered by the computer. The computer screen shows the current state of the model continuously in each compartment.

3 Physiological relevance

Compared to other models for the stomach and the small intestine, the TNO model has a number of specific advantages such as peristaltic movements, physiological pH curves, and absorption of products of digestion and water. In addition, this model is a strong dynamic system with physiological emptying patterns and gastric and intestinal transit times, which makes it more realistic than other models described in literature.

Still, this model also has its limitations. In the case of gastric emptying, for example, the model can be programmed to exactly mimic emptying curves that are observed *in vivo*. However, in man and animals gastric emptying is controlled by the central nervous system and by hormones produced in the small intestine. Although it will certainly be possible to include a number of feedback systems in the model to make it react to internal signals (on the basis of measurements), which makes the computer work like a nervous or hormonal system, real hormonal control will remain impossible.
In this respect it is important to realize that there is a serious drawback in making the system more and more dynamic and flexible. The strength of an *in vitro* experiment is that the experiments can be repeated under exactly the same conditions for a series of products. *In vivo* experiments show large physiological variation within and between individuals, which make it necessary to study relatively large numbers of animals or human volunteers in order to make the result statistically relevant. The *in vitro* model can be programmed to mimic the average physiological situation. But, if necessary, the same product can also be tested under extreme conditions, for example mimicking an individual who produces too much gastric acid or too little pancreatic enzymes. To obtain *in vivo* data in these situations it is necessary to turn to specific patient groups, which is often very complicated.

The development of an *in vitro* model that mimics as closely as possible the situation in the gastrointestinal tract will make it much easier to test many of the physiological aspects of new and existing food products. In order to ensure that the results obtained in such a system are relevant for the *in vivo* situation, validation studies are necessary in which *in vitro* and *in vivo* data are compared. These studies also have to reveal the limitations of the model. As explained above, the limitations of an *in vitro* method do not make it less useful as long as these limitations are well recognized.

4 Validation studies

An extensive programme is being carried out to validate the model and to test its applicability in several areas of research. For this purpose *in vivo* data are used from earlier and ongoing experiments with humans and farm animals carried out in our Institute, as well as data from the literature.
The accuracy and reproducibility of the model was evaluated for the simulation of *in vivo* dynamic data on pH, gastrointestinal transit, bile salt concentrations and absorption of glucose. The model appeared to reproduce acurately the various preset data in the different compartments (Minekus et al., 1994).

4.1 Survival of bacteria

The survival in the stomach and the kinetics of pyloric and intestinal delivery of viable lactic acid bacteria (LAB) in the GI model was investigated with different species and strains of *Lactobacillus*, *Streptococcus thermophilus*, and *Bifidobacterium*. The data on survival of *L. acidophilus* and *Bidobacterium* sp. in the model fitted closely those obtained with the same strains in humans during intubation studies (Marteau et al., 1994). Data of survival of *L. bulgaricus* till duodenum and ileum were also consistent with literature data. Also results of gastric survival of *Serratia* were in agreement with published data for humans.
The specific sensitivity for gastric acid, gastric emptying, and/or bile concentrations could be demonstrated. *Bifidobacterium* and *L. acidophilus* appeared more acid resistant than *L. bulgaricus* and *S. thermophilus*. Also the effects of bile stress on the survival of ingested bacteria can be studied in the model. For example some LAB survived the small in significant lower numbers under physiological bile concentrations as compared to decreased levels of bile.
These results thus strongly support the validity and scientific relevance of the model.

4.2 Digestion of food

Digestion experiments with potatoe starch showed that sixty minutes after intake of starch, high quantities of glucose units could be detected in the dialysis fluid. Only minimal levels of starch were delivered from the end of the ileum (approx. 4% of the intake). The digestibility index reached approximately 96%.
In digestion studies of processed cow milk and soya milk, the protein digestion coefficients were 93% and 83% respectively.
Experiments with several types of calf milkreplacers in the gastric compartment showed differences in the extent of coagulation, the velocity of breakdown of the coagulum, and consequently in dry matter flow and gastric emptying of proteins, depending on the type of proteins.

5 Applications

The potential applications for the model is summarized in Table 2. As mentioned above, we have started experiments in a number of areas, but there are many other interesting potential applications. Our main aim is to provide alternatives to *in vivo* animal and human studies to test aspects of (novel) foods and medical products. There is a growing market for foods and food ingredients with claims of specific health benefits, for which also evidence has to be provided. The availability of a relatively cheap *in vitro* test model to measure these effects makes it possible to apply these tests already in the phase of product development.

Methods could be used to screen the effects of different ways of production or food processing before a final product is put onto the market. In addition, in the trade of animal feeds there is a demand for reliable tests to predict the *in vivo* digestibility of protein from different sources. Furthermore, in those areas where large-scale *in vivo* testing is required, for example in toxicological and pharmaceutical research, the development of a relevant and reliable routine *in vitro* test procedure as an alternative is also very worth-while.

* digestibility of carbohydrates, proteins and fats and their interactions;
* digestibility and chemical stability of novel foods and ingredients, such as: native or modified starches, fibre fractions, oligosaccharides, constructed lipids, or other fat and carbohydrate replacers;
* slow-release characteristics of drugs
* binding of minerals, trace elements and vitamins
* binding of cholesterol and bile acids
* survival of pathogens or bacteria used as probiotics
* fate of genetically modified organisms
* effects of added enzymes and enzyme inhibitors
* drug-nutrient interactions

Table 2. Possibilities for research in the TNO gastrointestinal model.

6 Future developments

Currently we are working on the extension of the model with the large intestine. Application of the concept of peristaltic mixing will be an essential feature of this new type of large intestinal model. All existing gut flora models use magnetic stirrers or impellers for mixing, and pumps for transport. Therefore the contents of these models have to be very fluid and actually the situation during diarrhoea is imitated. Obviously, this disparity with the normal *in vivo* situation has serious consequences for the mutual interactions between bacteria and for the concentrations of nutrients, fermentation products, etc. A model based on the concept of peristaltic mixing and transport and water absorption, makes it possible to operate a model with a more realistic bacterial density. In such a model not only products of fermentation can be studied, but also changes in flora.

Besides the intestinal flora, the model so far is a purely mechanical model without living cells. In the future, however, we also plan to work with cultures of intestinal cells to study the interaction with the contents of the

intestinal tract. At present, cell cultures are already used in our Institute, e.g. to study the absorption and metabolism of nutrients or mechanisms related to colon cancer risks. The combination with a realistic *in vitro* model for the gastrointestinal tract will also increase the research possibilities in these areas.

7 Conclusions

In principle *in vitro* models for the gastrointestinal tract are a very attractive tool to study physiological events of foods and food ingredients. In the models that are currently available, however, many of the physiological aspects of the real-life gastrointestinal tract are overlooked, and the results obtained with these models therefore do not necessarily apply to humans or animals. Therefore this new type of GI-model has been developed to take into account the *in vivo* conditions as far as possible. It represents the first real dynamic *in vitro* model of the gastrointestinal tract simulating peristalsis, physiological transit characteristics, and absorption of products of digestion and water. Validation studies have been performed in several areas and specific applications are further being developed. Potential applications are for example: digestibility of carbohydrates, fats and proteins and their interactions; digestibility of novel food ingredients; slow-release effects; availability of minerals, trace elements and vitamins; survival of bacteria used in fermented foods and probiotics; fate of genetically modified organisms; effects of added enzymes or enzyme inhibitors; drug-nutrient interactions; and effects on the gut microflora and its metabolism. The model can also be applied in animal nutrition and pharmaceutical research.

8 Literature

Marteau Ph, Minekus M, Havenaar R, Huis in 't Veld J. (1994). Study of the delivery of ingested microorganisms to target sites beyond the stomach using an *in vitro* model. A pharmacokinetical approach for probiotics. submitted.

Minekus M, Marteau Ph, Havenaar R, Huis in 't Veld J. (1994). A multicompartmental dynamic computer controlled model simulating the stomach and small intestine. Submitted.

Lindwal S, Fondén R. (1984). Passage and survival of *L.acidophilus* in the human gastrointestinal tract. Int Dairy Fed Bull; 21:197

Pochart P, Marteau P, Bouhnik Y, Goderel I, Bourlioux P, Rambaud JC. (1992). Survival of bifidobacteria ingested via fermented milk during their passage through the human small intestine: an in vivo study using intestinal perfusion. Am J Clin Nutr; 55: 78-80

Peterssen L, Graf W, Sevelin V. (1985). Survival of *Lactobacillus acidophilus* NCDO 1748 in the human gastro-intestinal tract.2. Ability to pass the stomach and intestine *in vivo*. In: Hallgren B, Ed. Nutrition and the intestinal flora - Symp Swed Nutr Found XV. Uppsala: Almqvist & Wiksell: 127-30

Marteau P, Pochart P, Bouhnik Y, Zidi S, Goderel I, Rambaud JC. (1992). Survie dans l'intestin grêle de *Lactobacillus acidophilus* et *Bifidobacterium sp.* ingérés dans un lait fermenté: une base rationnelle pour l'utilisation des probiotiques chez l'homme. Gastroenterol Clin Biol; 16:25-28

Pochart P, Dewit O, Desjeux, Bourlioux P. (1989). Viable starter culture, ß-galactosidase activity and lactose in duodenum after yogurt ingestion in lactase-deficient humans. Am J. Clin Nutr; 49: 828-831

fig. 1. Schematic drawing of the TNO gastro- Intestinal Model (TIM)