

Membrane Digestion and Transport Under Physiological Conditions: A Review of Available Data

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Abstract. A technique to study membrane digestion and transport in the small intestine under physiological conditions has been developed. The technique is based on a continuous perfusion of a chronically isolated loop of the rat small intestine. Membrane hydrolysis and transport of some nutrients in the rat small intestine in chronic, as well as in acute (in situ) experiments was investigated. The absorption of hexoses and amino acids has been found to be 2.5—4 times higher under physiological conditions than in acute in situ experiments. Both the active transport of glucose released from maltose hydrolysis and the hydrolysis of the latter is increased under physiological conditions. A coupling between the final stages of hydrolysis and the initial stages of transport in chronic experiments was shown to be highly efficient; practically all or nearly all glucose released is being transported without entering the luminal phase. The hydrolysis rate of starch during the perfusion of a small intestinal segment in chronic experiments is many times higher than that in acute experiments or under anaesthesia. The enzymatic and transport activities revealed using a widely accepted technique in situ, the more so, in vitro account for only a small fraction of those which are typical of undisturbed processes under conditions close to the physiological. The levels of functioning of the digestive-transport systems of the small intestine considered as natural levels developed in the process of evolution, actually reflect only residual processes and, in most cases, they account for 1/3 to 1/10 of the true level of an actual physiological process.

Key words: Membrane digestion — Intestinal transport — Apical membrane — Intestinal perfusion

Introduction

The ultimate objective of any scientific research is a reconstruction, based on the knowledge of molecular elements, properties and behaviour of real biological systems, i.e. systems which function under physiological conditions and which have resulted from prolonged evolution. This may be achieved by using two diametrical-

ly opposite approaches. One of them is based on the methodology of reductionism, with models, simplifying properties of the system under study to a maximum, down to their molecular components; the underlying principle of another approach is the methodology of integration, with experiments performed on intact systems under physiological conditions.

Paradoxically, it is a reduced system that has attracted a widespread attention of investigators. Thus, it is well known that the current fundamental concepts of membrane hydrolysis and transport in the small intestine have been formulated on the basis of information obtained using different *in vitro* methods. The possibilities and limitations of the above methods have been analysed in detail in a number of excellent reviews (Parsons 1968; Semenza 1968; Hubel and Parsons 1971; Smyth 1974; Holdsworth and Sladen 1979; Matthews and Payne 1980; Gray 1981; Kimmich 1981; Levin 1979, 1982 and others).

However, the reconstruction of a real biological system requires an ideal model allowing a precise and detailed characterization of the properties and features of the system studied in physiological conditions.

Indeed, results of chronic experiments on healthy animals, especially those carried out after the studies by Pavlov (1897) and his co-workers, have proved to be inconsistent with data obtained on isolated tissues, cells or molecular preparations. This may be accounted for by the use of different types of animals: in chronic experiments, the animal used were predominantly dogs, while in acute experiments, i.e. in *in vitro* conditions, they were more commonly rats, guinea pigs and golden hamsters. Therefore, it is not surprising that efforts of many investigators in recent years have been focused on the development of a new technique which would enable a comprehensive study of membrane hydrolysis and transport in healthy animals in physiological conditions.

The technique of acute experiment *in situ* has originally been thought to be such a kind of experiment. A number of basic functional characteristics have recently been obtained from acute *in situ* experiments on both anaesthetised and unanaesthetised animals, mainly rats, using a perfusion technique for an isolated loop of the small intestine (Iezuitova et al. 1964; Antonioli and Christensen 1968; Davidson and Leese 1977; Dennhardt et al. 1979; Garrido et al. 1979; Ponz et al. 1979; Younasai 1979; Kotler et al. 1981; Krause et al. 1981 and others).

Data derived from acute *in vivo* experiments have been assumed to contain correct information on properties of native systems, mainly as for undisturbed circulation and microcirculation which are very important for the maintaining of functional characteristics of intestinal cells (Lundgren 1973; Svanvik 1973; Svanvik and Lundgren 1977; Jacobson and Lanciault 1979; Levin 1979; Wiedeman et al. 1981 and others).

The purpose of the present study was a preliminary characterization of methodological approaches and results of investigations on membrane hydrolysis

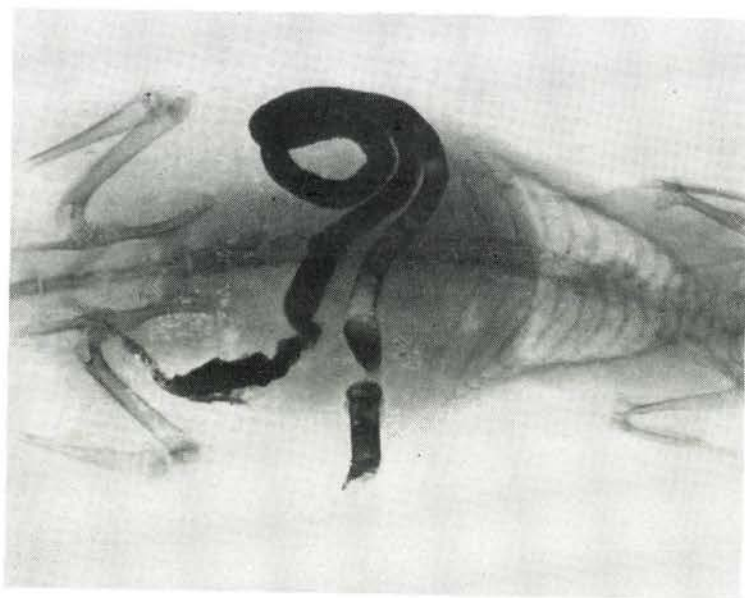


Fig. 1. X-ray picture of a rat with an isolated loop (15 cm) of the proximal small intestine filled with X-ray contrast solution two months after the operation.

and transport of some nutrients in the small intestine of healthy rats under physiological conditions, as well as their comparison with data obtained using generally accepted methodological approaches *in situ*.

Materials and Methods

The method used in our experiments has been described previously (Ugolev and Zaripov 1979; Ugolev et al. 1981). The method is based on a continuous perfusion of a chronically isolated loop of rat small intestine. The abdomen of a rat was opened under anaesthesia and a small intestinal segment (or segments) of a required length was isolated without affecting mesenterial vessels. Fistula tubes, specially designed for small animals, were inserted into both ends of the isolated intestinal segment and brought out through narrow holes in muscles and skin 2–3 cm lateral to the midline. The technique of operative intervention developed in our laboratory has permitted to prevent a prolapse of the fistulas and a formation of perifistular ways due to the additional external fixation of the fistula tubes with a synthetic material (nylon or capron nets) which did not interfere with the growth of the connective tissues. Different segments of the jejunum (or the ileum), usually 15 cm long, were isolated. X-ray picture (Fig. 1) illustrates an isolated loop (15 cm long) of the rat small intestine two months after the operation. The continuity of the digestive tube was restored by "end to end" enteroanastomosis.

Data on glucose, fructose, galactose absorption and maltose and starch hydrolysis, as well as on subsequent absorption of released glucose have been obtained using a combination of following methods: anthrone, arseno-molybdenic and glucose oxidase. In order to study soluble starch hydrolysis, the modified method of Smyth and Roy was employed (Ugolev et al. 1969).

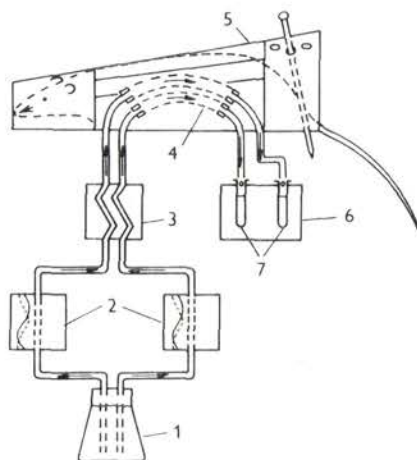


Fig. 2. Schematic representation of the experimental layout for studying digestive-transport processes in isolated small intestinal loops of small laboratory animals in chronic experiments. 1 — a vessel with substrate solution; 2 — precision chromatographic pumps; 3 — thermostat; 4 — isolated small intestinal loops; 5 — camera-“hole” for immobilization of the animal; 6 — a vessel with ice for cooling the outflowing perfusate; 7 — glass tubes to collect the outflowing perfusate.

The concentration of free glycine was determined by the method of Ugolev and Timofeeva (Ugolev et al. 1969, 1981).

The absorption rate of hexoses and amino acids (including maltose hydrolysis products) was estimated from the formula:

$$R_a = V(C_1 - C_2),$$

where R_a is the absorption rate of substrate ($\mu\text{mol/l/min}$); V is the rate of perfusion (ml/min); C_1 is the initial substrate concentration (mmol/l); C_2 is the substrate concentration in the outflowing perfusate (mmol/l).

The hydrolysis rate of maltose was estimated from the formula:

$$R_h = V(C_1 - C_2 + C_3),$$

where R_h is the hydrolysis rate of maltose; V is the rate of perfusion (ml/min); C_1 is the initial hexose concentration (mmol/l) (determined by the anthrone method); C_2 is the hexose concentration in the outflowing perfusate (mmol/l) (determined by the anthrone method); and C_3 is the glucose concentration in the outflowing perfusate (mmol/l) (determined by the glucose oxidase method).

A schematic illustration of the experimental layout for studying digestion and absorption in small laboratory animals and conditions of the chronic experiment is shown in Fig. 2. The animal was placed into a special camera-“hole”. After perfusing the isolated intestinal loops with solutions containing various substrates, by means of precision pumps, the outflowing perfusate was collected into appropriate vessels where it was cooled and the biochemical reaction was stopped. Prior to each experiment, the isolated small intestinal loop (or loops) was rinsed with the Ringer solution.

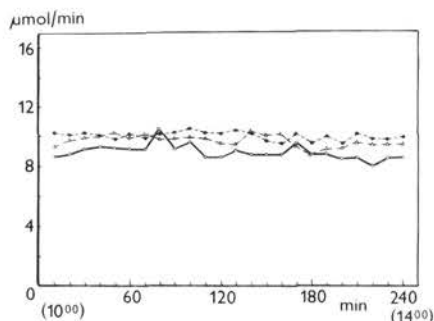


Fig. 3. The absorption rate of glucose (initial concentration 27.5 mmol/l) during the perfusion of an isolated loop of the rat proximal small intestine (15 cm in length) for 240 min. Samples for analyses were taken in 10 min intervals ($n = 3$). Abscissa: time of perfusion (min); 10.00 — start of perfusion; 14.00 — cessation of perfusion; Ordinate: the absorption rate of glucose ($\mu\text{mol/min}$); ●, ○, △ — different animals.

It is important that throughout the lifespan of an experimental animal, a substrate load was applied to the isolated intestinal loop to maintain its functional activity. For this purpose, the intestinal loop(s) was subjected to 60 min perfusion with glucose solution or other substrate solutions.

A detailed analysis of the reproducibility and accuracy of the results obtained using the above technique has shown that it has some advantages over conventional techniques as for basic experimental criteria.

Results

The mucosa of isolated non-functioning intestinal segments is known to become rapidly atrophied (Dowling and Booth 1967; Ugolev 1978; Dowling 1982; Williamson 1982; see also contributions in: *Intestinal Adaptation* (1974)*). In our experiments, atrophy of isolated intestinal loops was observed both at daily substrate loads and in the absence of loads. Two weeks later, the mass of the mucosa of isolated intestinal loops was reduced by 22.8%. Ten days after the isolation of the proximal small intestine in intact rats, the height of the villi diminished from $399.5 \pm 12.3 \mu\text{m}$ ($n = 6$) to 252.2 ± 12.4 ($n = 6$), ($P < 0.0027$), i.e. 1.5 fold at daily substrate loads, whereas the absorption rate of substrates remained unchanged for 8 months.

However, as will be shown below, despite the atrophy of the villi, the functional characteristics of the isolated intestinal segment were kept at a high and constant level.

As can be seen from Fig. 3, a high stability and close correlation of the levels of

* Proc. of Int. Conf. on Anatomy, Physiology and Biochemistry of Intestinal Adaptation. Schattauer, Stuttgart, New York

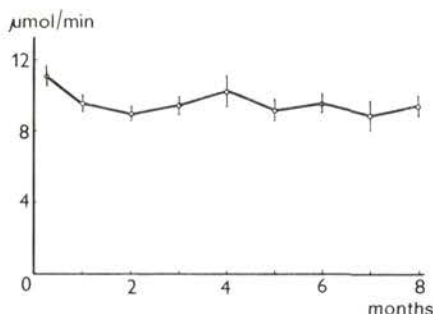


Fig. 4. The absorption rate of glucose (27.5 mmol/l) during the perfusion of an isolated loop of the rat proximal small intestine (15 cm in length) for 8 months after the operation ($n = 6$). Abscissa: months after the operation; Ordinate: the absorption rate of glucose ($\mu\text{mol/min}$).

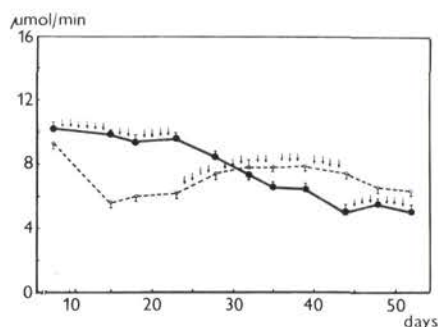


Fig. 5. The absorption rate of glucose (27.5 mmol/l) during the perfusion of two isolated loops (each 15 cm in length) of the rat small intestine under substrate loads and in the absence of loads. The first loop (●—●) was isolated distally 5 cm from the duodenum; the second loop (○—○) was isolated from a part of the small intestine directly adjoining to the first loop. Abscissa: days after the operation; Ordinate: the absorption rate of glucose ($\mu\text{mol/min}$). Arrows indicate days when the perfusion of the isolated intestinal loop preloaded with a substrate was being continued for 60 min or more.

glucose absorption were observed in the isolated small intestinal loop during a single experiment in different animals. The specific levels of the transport characteristics of the isolated intestinal loop may remain stable for many months, as shown in Fig. 4. It has already been noted above that regular substrate loads are needed to maintain this level.

A special analysis of this phenomenon was made in experiments on two isolated loops of the rat small intestine. Fig. 5 shows that the isolated loops had first nearly identical rates of glucose absorption. One of the intestinal loops was subjected to daily substrate loads, while the other was rinsed with the same volume

of the Ringer solution. The level of glucose absorption in the first loop remained stable, whereas in the second one, it was reduced almost to one half. After stopping substrate loads in the first loop and starting loading the other one, the ability to absorb glucose was reduced in the former and increased in the latter. Finally, after a new change of loads, a repeated reduction in glucose absorption in the intestinal loop in the absence of substrate load and no further decrease in glucose absorption under substrate load could be observed.

An analysis of these data has revealed a more complex dependence of the level of nutrient transport on substrate loads, than suggested earlier. In particular, glucose loads have been found to prevent a reduction of the ability of the intestine to transport glucose. In other words, the presence of glucose in the isolated intestinal loop prevents a loss of glucose-absorption capacity, it however does not restore this capacity completely. A special additional factor may exist, responsible for the initiation of the ability to absorb glucose. These findings reveal a clear-cut, though not very common, relationship between substrate loads and the ability of the isolated loop of the small intestine to transport glucose. As has been shown in Fig. 4, this ability to absorb glucose under regular substrate loads may be preserved for 8 months and longer. Results of chronic and acute experiments with the hydrolysis and absorption of different sugars in isolated loops of the rat proximal small intestine of unanaesthetised animals are summarized in Tables 1 and 2.

It should be pointed out that absorption of hexoses and amino acids is 2.5—4 times higher under physiological conditions than in acute *in situ* experiments. Table 2 shows that both the active transport of glucose released from maltose hydrolysis and the hydrolysis of the latter are increased under physiological conditions. An analysis of data presented herein has shown that the coupling between the final stages of hydrolysis and the initial stages of transport in chronic experiments is highly efficient, and that practically all or nearly all glucose released is being transported without entering the luminal phase.

What is the relation between the levels of transport and membrane hydrolysis in chronic experiment and in acute *in situ* experiment?

Theoretically, significant distinctions should not be expected between these two types of experiment, since in acute experiments circulation and other functions ensuring the proceeding of fundamental digestive and transport processes change insignificantly. Moreover, it could be suggested in view of the essential atrophy of the intestinal mucosa in isolated intestinal loops that, in chronic experiments, the absorption rate of glucose will be reduced as compared with that in acute *in situ* experiments in unanaesthetised animals, due to a decrease in the digestive-transport surface.

The evidence presented in Fig. 6 has proved to be rather unexpected. As can be seen, results obtained from acute *in situ* experiments on anaesthetised animals, originally considered to be the true characteristics of absorption and digestion of

Table 1. Absorption of different monomers in isolated loops of rat proximal small intestine in chronic and acute experiments on unanaesthetised animals

Substrate	Absorption rate of monomers ($\mu\text{mol}/\text{min}$)							
	Low concentrations				High concentrations			
	Concentration (mmol/l)	Chronic	Acute	Ratio	Concentration (mmol/l)	Chronic	Acute	Ratio
Glucose	27.5	9.5 ± 0.4	3.4 ± 0.1	2.8	110	20.0 ± 1.3	7.9 ± 0.6	2.5
Galactose	27.5	5.4 ± 0.4	1.7 ± 0.3	3.2	110	15.1 ± 1.1	4.5 ± 0.3	3.4
Fructose	27.5	2.5 ± 0.25	0.6 ± 0.07	4.2	110	7.3 ± 0.9	2.0 ± 0.2	3.7
Glycine	5.0	1.15 ± 0.2	0.35 ± 0.08	3.3	20	2.25 ± 0.1	0.6 ± 0.01	3.8

Table 2. Hydrolysis and absorption of maltose in isolated loops of rat proximal small intestine in chronic and acute experiments on unanaesthetised animals

Concentration (mmol/l)	Hydrolysis rate ($\mu\text{mol}/\text{min}$)			Absorption rate ($\mu\text{mol}/\text{min}$)			Coefficient of coupling*	
	Chronic	Acute	Ratio	Chronic	Acute	Ratio	Chronic	Acute
27.5	12.0 ± 1.1	7.7 ± 0.9	1.6	10.8 ± 1.2	4.4 ± 0.4	2.4	0.90 ± 0.09	0.57 ± 0.06
110.0	26.3 ± 2.2	16.0 ± 1.7	1.6	22.8 ± 2.1	9.3 ± 1.2	2.4	0.87 ± 0.07	0.58 ± 0.06

* The coefficient of coupling indicates the efficiency of absorption of glucose released from membrane hydrolysis of maltose as the absorption rate/hydrolysis rate ratio.

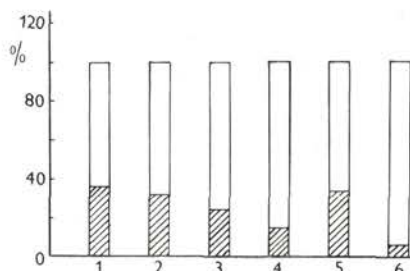


Fig. 6. The absorption and hydrolysis rates of various food substrates during the perfusion of isolated loops of the rat proximal small intestine (15 cm in length) in chronic and in acute in situ experiments on unanaesthetised animals. Ordinate: rates of processes (in per cent). The rates of corresponding processes in chronic experiments are taken as 100%. 1, 2, 3 — the absorption rates of 27.5 mmol/l glucose, galactose and fructose solutions, respectively; 4 — the absorption rate of 10 mmol/l glycine solution; 5 — the hydrolysis rate of 27.5 mmol/l maltose solution; 6 — the hydrolysis rate of soluble starch (20 g/l). Stripped squares — the rate of the process in acute in situ experiments on unanaesthetised rats.

the intestinal cells, actually are but a component of them. Thus, if in chronic experiments the level of absorption and membrane hydrolysis of various food substrates in the isolated intestinal loop of healthy rats is taken for 100%, then in acute experiments, the absorption rate of glucose at an initial concentration of 27.5 mmol/l will make up 35.8%, that of galactose 31.5%, of fructose 24%; that of glycine at a concentration of 10 mmol/l 15.2%; the hydrolysis rate of maltose at an initial concentration of 27.5 mmol/l will make up less than 40%, and that of soluble starch at an initial concentration of 2 g/l less than 10%.

The hydrolysis rate of starch during the perfusion of a small intestinal segment in chronic experiments is many times higher than that in acute experiments or under anaesthesia: 82.7 ± 2.5 $\mu\text{g}/\text{min}/\text{ml}$ for the latter vs. 538.3 ± 70.3 $\mu\text{g}/\text{min}/\text{ml}$ for the former ($n=6$, $P<0.0027$). These figures suggest a high efficiency of membrane hydrolysis of starch.

Thus, the most important result of our studies is that, under conditions close to the physiological, the ability of the small intestine to absorb and hydrolyse nutrients is several times as much as under conditions of acute experiments.

Discussion

We shall not discuss here the mechanism underlying the functions of unaffected intestinal cells, nor an attempt will be made to characterize the functional differences between intact systems and systems altered in acute experiments. We shall confine ourselves to a statement that most of the enzymatic and transport

activities in the small intestine have apparently failed to be detected by standard methods. The data presented herein indicate that the enzymatic and transport activities revealed using a widely accepted technique *in situ*, the more so, *in vitro* account for only a small fraction of those which are typical of undisturbed processes under conditions close to the physiological.

Our data suggest that the levels of functioning of the digestive-transport systems of the small intestine considered as natural levels developed in the process of evolution, actually reflect only residual processes and, in most cases, they account for 1/3 to 1/10 of the true level of an actual physiological process.

In addition, almost an ideal system of transportation of final hydrolysis products from the enzyme onto the transport systems was first discovered under physiological conditions. However, the same process in an acute *in situ* experiment is accompanied by a dissipation of nearly a half of monomers released in the bulk phase; this is consistent with data of other authors (Ugolev and Iezuitova 1982).

We should not look for too a simple explanation of the observed phenomena. For example, it would be attractive to interpret a reduction in transport processes as a disturbance in functioning of the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ in the basolateral membrane of the intestinal cells, resulting in a decrease of energization or a drop in Na gradient under the influence of other factors (Kimmich and Randles 1979, 1981; Harms and Wright 1980; Robinson 1980; Kimmich 1981; Stekhoven and Bonting 1981 and others). Indeed, there is evidence indicating that a decrease in enzyme activity due to anaesthesia gives rise to a reduction in Na gradient and, thus, in the intensity of all Na^+ -dependent processes. This hypothesis has also been supported by our results showing that adrenalectomy and aldosterone deficiency result in a progressive decrease (more than by one half) in glucose and glycine transport. Both these processes are known to be Na^+ -dependent (Holdsworth and Sladen 1979; Levin 1979; Ullrich et al. 1979 and others).

However, it must be kept in mind that under the conditions of our experiments, a reduction occurred both in glucose and glycine transport as well as in the transport of fructose, i. e. a Na^+ -independent process (Holdsworth and Sladen 1979). Moreover, a reduction occurred in the rates of maltose and sucrose hydrolysis, which are energetically Na^+ -independent processes (Ugolev 1972; Crane 1977).

Thus, the explanation of our results must relate features typical of membrane hydrolysis and transport of nutrients under physiological conditions with a definite state of the membrane (Adolf 1979). A transition from the normal to a disturbed state occurs by the principle "all or nothing". This may be confirmed by the fact that, beginning from a certain dose of nembutal, any further increasing of the dose of the anaesthetic does not enhance the inhibitory effect, prolonging its duration at the same time.

Thus, our results reveal new processes which could not be observed earlier; they may become distinct only when experimenting on animals subjected to no experimental trauma.

In conclusion, it should be emphasized that differences between acute and chronic *in vivo* experiments are in no way of less importance than those between *in vivo* and *in vitro* experiments. Here it is relevant to refer to the current valid assessments of the *in vivo* and *in vitro* methods. A comprehensive characterization of the above methods has recently been made by Levin in his brilliant review (Levin 1982). In his view, the advantage of *in vitro* methods lies in an exquisite experimental control of many parameters, allowing specific transfer-secretory mechanisms to be isolated and studied. Along with some technical problems, these methods possess disadvantages, such as the lack of blood and lymph flows, the disruption of normal motility and of hormonal and nervous control. Further, the author points to the need of studying the intestinal function *in vivo* or better *in situ* as a "final court of inquiry". However, our investigations have shown that chronic rather than *in situ* experiments in general provide the necessary and extremely important means for the assessment of intestinal functions.

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Received September 23, 1983/Accepted August 15, 1984