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(54) Title: INTESTINAL UPTAKE OF MACROMOLECULES

(57) Abstract: The invention relates to the field of gut-(patho)physiology and is in particular related to the intestinal uptake of fluids, electrolytes and (macro)molecules. The invention provides a method for determining molecular transport through an intestinal wall or segment thereof comprising up- or down-regulating oxygen supply to said wall or segment. Herewith, the invention provides a methodology for evaluation of the effects of (components of) foods, drinks and speciality foods or drinks related to various different hemodynamic states of a person who consumes the food or drink.

Title: Intestinal uptake of macromolecules.

The invention relates to the field of gut-(patho)physiology and is in particular related to the intestinal uptake of fluids, electrolytes and (macro)molecules.

The mucous surface and epithelial and subsequent layers of the intestines fulfil by default an important function in uptake and molecular transport of substances in food and drink and at the same time serve to protect the body against possible invading undesired substances. At one and the same moment, fluid, electrolytes or distinct macromolecules are, selectively or by simple diffusion, transported through the mucous layer, taken up by the epithelium and transported through the intestinal wall to the lymph- and blood-vessels that transport the desired molecules further, whereas at the same time the mucous surface and epithelial layer provide solid protection against harmful and unwanted or toxic substances or molecules that are for example formed during microbial fermentation in the gut and thus protect the body against invading enteric pathogens, such as viruses or bacteria.

The tissue of the intestinal wall thus needs to actively maintain a complex functional integrity that is often under siege by external or internal influences. In several instances said integrity is challenged. For example, a major cause of death of intensive care patients in hospitals is related to an increase of intestinal permeability for macromolecules. Especially after shock, e.g. as seen with blood loss after trauma or surgery, the intestines in general function less than desirable in maintaining said integrity; oral food intake than often results in the intestinal uptake of large measures of (not yet digested) macromolecules and/or relative little uptake or even loss of water and salts resulting in a decreased or even negative net-absorption of essential fluid and electrolytes. Oral feeding protocols bear thus the risk of disturbing the crucial fluid balance of the patient which may result in additional shock or disease, sometimes leading to death. Therefore, to avoid for example the risks of oral food intake in post-operative care, intensive care patients often remain longer on parenteral feeding than strictly deemed desirable from the viewpoint of restoring intestinal function.

Another example where the functional integrity of the intestinal surfaces is under siege is seen in the field of athletics, especially with those sports, such

as long-distance running or cycling, where endurance is tested. With a large number of athletes, intestinal disturbances are seen during or after exercise that are related to the phenomenon that during mid- or long-term exercise most part of the blood supply is directed to the muscles, thereby leaving the intestines at least partly ischaemic. The ischaemic state of the intestine may lead to acidosis, which can cause damage to the intestinal epithelial cells and loss of epithelial integrity, again resulting in decreased or even negative net-absorption. The loss of epithelial integrity increases permeability leading to increased macromolecular transport over the epithelial barrier which means that unwanted compounds wind up the tissue of the intestinal wall or even in the bloodstream. It can also influence water or electrolyte transport over the intestinal wall, leading to intestinal fluid loss generating diarrhoea, in the worse cases macro-molecules can leak back into the intestine, whereby intestinal protein or even intestinal blood loss is seen causing proteinaceous or even bloody diarrhoea. Food digestion under these circumstances is sub-optimal which prevents replenishment of energy supply during exercise and increases the time necessary for recuperation after exercise.

Yet another example of lost functional integrity of the intestinal surfaces is related to age. Often, elderly people have a stomach ache after eating. It is thought that said stomach ache is related to a discrepancy between the increased need for oxygen in the intestinal tissues after eating, and the (due to age decreased) ability of the blood supply to the intestines to fulfil that need. Again, a relative intestinal ischaemia is thought to be related to intestinal malfunction. These intestinal problems related to age are often compounded in people with arteriosclerosis who have an overall reduced blood supply.

Above sketched intestinal problems illustrate a need for speciality food products tailored to for example the consumptive needs of intensive care patients, athletes such as long-distance runners, the very young, the diseased or the elderly. However, the rational design of such speciality foods is hampered because about the effects of food and its components on the intestine and transport through the intestinal wall only little is known. In particular the evaluation of the (patho)physiological effects of (various compounds of) foods or speciality foods on the intestinal wall is hampered by the absence of reliable and objective test methods. It is clear that such testing can in general only be done with a live individual since a functioning intestine is required; in general, such

testing is done by feeding various test diets to groups of individual volunteers and determining its effect on said individual's well-being. However, due to the inherent subjectivity of testing for well-being and innate variability which is caused by selecting individuals of often unknown background or physiological status, test results often vary greatly, which can only be countered by using large, financially often prohibitive numbers. Analysis of the intestinal effect of food is further complicated by the varying response dependent on each individual haemodynamic state related to age, health status, fitness, physical activity (e.g., exercise and sports) and genetic background. Furthermore, no one in his right mind contemplates using intensive care patients for such experiments, thus, where insight in the preferred composition of oral food for intensive care patients is needed, little or no possibilities exist to test experimental food compositions or compounds therefor. Clearly, to select and obtain better speciality foods tailored for various needs experimental models are needed.

The invention provides a method for determining molecular transport through an intestinal wall or segment thereof comprising up- or down-regulating oxygen supply to said wall or segment. Herewith, the invention provides a methodology for evaluation of the effects of (components of) foods, drinks and speciality foods or drinks related to various different hemodynamic states of a person who consumes the food or drink.

Others, such as Corson et al., Eur. J. Vasc Surg 6:158-163, 1992 and Bastidas et al., j. Surg. Res. 48:427-434, 1990 have investigated the relationship between I/R and gut permeability in models of multiple organ failure (MOF) in general, involving extreme ischemia such as by tourniquet of limbs. The present invention, however, allows for precise modulation of I/R at the level of the intestine only, furthermore, it allows for study of intestinal I/R in a physiological environment, without confounding effects of detrimental treatment of other organs (eg limbs). Similarly, other researchers use isolated intestinal segments *in vitro* as model for testing of the influence of luminal compounds on intestinal function. The present invention, to the contrary, provides an *in vivo* environment and thus allows for testing of compounds on intestinal function in an intact physiological environment (e.g. intact intestinal circulation, enervation), measuring intestinal permeability separately.

In one embodiment, the invention provides a test method for determining molecular transport through an intestinal wall or segment thereof comprising regulating perfusion of blood vessels in said wall or segment. Perfusion can be regulated by several methods. It is for example provided to test an experimental animal or a volunteer before, during or after resting or exercise. During exercise, the blood distribution of the body is in general lead away from the intestines, for example in favour of supply to the muscles, thereby creating a phase of relative ischaemia in at least parts of the intestines. The invention furthermore provides testing experimental subjects in artificially compromised haemodynamic states, for example after having been provided with above normal haematocrite values, as for example can be observed in athletes having trained at high altitude or having been provided with erythropoetin or with intensive care patients having for example suffered extensive blood plasma loss, or having been provided with below normal haematocrite values, as for example can be observed during anaemia, during kidney malfunction or after excessive fluid loss therapy. Furthermore, the invention also provides testing experimental subjects having been provided with erythrocytes having above or below normal capacity for uptake, transport or delivery of oxygen.

In one embodiment, the invention provides a method according to the invention further comprising rendering at least part of said intestinal wall or segment at least partly ischaemic, that is deplete of normal oxygen levels. Ischaemia herein refers to local tissue anaemia for example caused by reducing arterial influx or venous efflux of blood to or from said tissue or by other causes of reduced oxygen supply. For example, the invention provides a method according to the invention wherein a blood vessel leading to (such as one of the branches of the *arteria mesenterialis* or *arteria renalis*) or from (such as one of the branches of the *venae portae*) said part is at least partly clamped. For example, the invention provides use of one or more of the following test methods: the testing by small intestinal segment perfusion (SISP) for evaluation of food uptake, evaluation of food uptake in different haemodynamic states; ischaemic reperfusion (IR)-SISP, or evaluation of intestinal epithelial integrity and permeability in a diffusion chamber (DC) alone or after (IR)-SISP.

Use of a method as provided by the invention allows rational design of food formulas tailored to different metabolic states. Said use finds its application in the formulation of speciality foods or drinks such as probiotics, sport foods, or

medical (par)enteral food. Furthermore, the invention provides said use for evaluation of compounds or components modifying the permeability of the epithelial barrier for oral vaccination, and for the induction of oral tolerance for prevention and cure of (food) allergy. In addition, a method as provided by the invention allows evaluation of compounds for non-pharmaceutical prevention of infectious enteral disease.

In a further embodiment, the invention provides a method wherein said transport is macromolecular transport. For example, the blood circulation of the intestine of an experimental subject such as a pig is manipulated as to mimic different physiological states i.e., resting, moderate or strenuous exercise. This enables evaluation of food uptake under different haemodynamic conditions, and measurement of the influence of food on the occurrence of intestinal acidosis. By using a marker (such as a specific electrolyte, a (an)organic salt, oxygen, hydronium ions, etc., optionally labeled or identifiable by nuclear emission signals or radio-activity, horse-radish-peroxidase, fluoresceinated compounds of varying molecular mass, labeled antigens or toxins) for trans-epithelial transport of macromolecules the effect of a wide variety of influences on macro-molecular transport through the intestinal wall can be tested. The effect of food on enteral disease can for example be tested by bringing the relevant pathogen in the intestinal segment. In addition, and optionally after for example (IR)-SISP has been performed, a sample of the intestinal epithelial layer is put in a diffusion chamber (DC), and measurements on integrity and permeability are performed by electrophysiology or by using a marker for trans-epithelial transport of macromolecules.

Furthermore, the invention provides a method wherein said transport comprises fluid and/or electrolytes. For example, in a SISP (small intestinal segment perfusion) test as provided a food substance or component is perfused through part of the intestine of an anaesthetized experimental subject such as a pig. The difference between the input and outflow of fluid and/or electrolytes from the intestinal segment is measured and for example expressed as net absorption, or as differences between the sodium/potassium ratios in the intestinal lumen or the blood, in time. The effect of food composition on net absorption can be measured. Segments can for example be infected with viral and bacterial intestinal pathogens, and the effect of the pathogens on net absorption can be studied in detail. Furthermore, the effect of food components on enteral infection

can be evaluated. This enables a rational approach for food design, especially for enteral disease patients and individuals at risk.

The invention also provides use of a method according to the invention in selecting or obtaining at least one component for a speciality food. Use of a method provided by the invention provides information about the uptake of food and quantifies the physiological consequences of food, for example taking the physiological (haemodynamic) status of the individual into account. A striking example in this respect is the consequence of the presence of glutamine in food. Glutamine is the preferred fuel for intestinal cells. Under normal circumstances, resting or moderate exercise, glutamine is beneficial. However, more strenuous exercise, or blood loss leads to redistribution of blood away from the intestine, leading to relative ischaemia, especially prominent with athletes during recovery from exercise. A similar relative ischaemia is often seen with intensive care patients, and among many elderly. In the literature, i.e Dugan et al., JPEN 19:83-87, 1995 (and others) the contention is that inclusion of glutamine to the diet is always beneficial, here (using the IR/SISP) we are able to show that in certain physiological (hemodynamic) states, inclusion of certain concentrations glutamine can either be harmful or beneficial.

It is now herein provided that under relatively ischaemic conditions too high concentrations of glutamine aggravate intestinal damage. The conclusion must now be that if glutamine is included in (specialty) foods, its concentration should be optimized to its specific application., for example glutamine should be avoided or at least reduced or minimized in specialty foods for these groups due to its detrimental effects on intestines, when ischaemic.

Also, when sodium-monoglutamate is ingested under ischaemic conditions, severe intestinal acidosis can occur, commonly known as Chinese restaurant syndrome. This demonstrates that the physiological effect of the same compound can be beneficial as well as deleterious, depending on the haemodynamic state of the individual. It is therefore crucial to differentiate haemodynamic states to make rational recommendation of foods and intervention possible. Use of a method provided by the invention allows rational design of speciality foods or drinks particularly suited for an intensive care patient, an athlete, a very young person, a diseased person or an elderly person,

whose intestinal integrity may from time to time be under siege due to for example relative intestinal ischaemia.

The invention thus provides a method for selecting or obtaining a speciality food or drink tailored for the needs or desires of an individual who's functional integrity of the intestinal wall is at least at times under siege comprising testing at least one candidate component for said speciality food for its effect on molecular transport through an intestinal wall or segment thereof and selecting a component for inclusion in said speciality food that has a desired effect on said molecular transport. Of course testing of an already composed or existing food or drink for its effect on said molecular transport is provided as well. It is now for example possible to select or obtain (desired components for) a speciality food aimed at the elderly who experience stomach aches after eating, to select or obtain a food or drink aimed at intensive care patients without having to fear for example for malabsorption or intestinal acidosis in those patients, or to select or obtain a sport drink tailored to the needs or desires of an athlete, or other person undertaking a longer exercise than at least the intestines in his or hers body are prepared for, without creating intestinal malfunction. Especially for those case where functional integrity of the intestinal wall is under siege due to an at least at times insufficient oxygen supply to said intestinal wall, speciality foods or drinks can now rationally be designed and obtained.

The invention also provides a speciality food or drink selectable or obtainable by a method according to the invention. As an example of such a food or drink is provided a speciality food or drink according to the invention comprising hydrolysed polysaccharides or functional equivalent thereof. Within the detailed description herein is shown that hydrolysed polysaccharides protects against increased intestinal permeability during intestinal ischaemia. Furthermore, the invention for example provides a speciality food or drink tailored for an intensive care patient or tailored for the needs or desires of an athlete who's functional integrity of the intestinal wall is at times under siege, especially wherein said food or drink is intended for use during a phase of relative intestinal ischaemia to prevent damage to the functional integrity of an intestinal wall, thereby preventing any of a wide variety of intestinal failure, such as stomach ache, fluid or electrolyte loss, or diarrhoea. One example of such a drink is a sport drink containing optimized amounts of glutamine, glutamine

in certain concentrations, as shown herein, surprisingly being found to be detrimental to an intestinal wall during a phase of relative intestinal ischaemia.

Furthermore, the invention provides use of a speciality food or drink as provided by the invention, especially wherein said food or drink is intended for use during a phase of relative or absolute intestinal ischaemia to prevent damage to the functional integrity of an intestinal wall, thereby preventing any of a wide variety of intestinal failure or loss of functional integrity of an intestinal wall, such as seen with stomach ache, fluid or electrolyte loss, or diarrhoea.

The invention is further explained in the detailed description without limiting the invention thereto.

Detailed description

Currently, the evaluation of the effects of (compounds of) foods and speciality foods is hampered by the absence of reliable and objective test methods. Hitherto, this was done by using groups of individuals, with the inherent subjectivity and variability, which can only be countered by using large, financially prohibitive numbers. Analysis of the effect of food is further complicated by the varying response dependent on the individual haemodynamic state related to health status and fitness, and physical activity (e.g., exercise, and sports). The latter problem is particularly evident in the case of endurance sports such as long-distance running. It appeared that in spite of the consumption of speciality sport foods marathon runners had blood in their stool post-performance. This response of marathon runners to food is known to be caused by the fact that exercise leads to a redistribution of blood from the intestinal tract to the muscles. This leads to relative ischaemia of the intestinal tract which can have great consequences if certain types of food is ingested. Briefly, this can lead to acidosis, which causes damage to the intestinal epithelial cells and loss of epithelial integrity. The loss of epithelial integrity increases permeability leading to increased macromolecular transport over the epithelial barrier which means that unwanted compounds wind up in the bloodstream. It can also lead to fluid loss, in the worse cases blood loss. Food utilisation under these circumstances will be at least sub-optimal (malabsorption) which prevents replenishment of energy supply during exercise and increases the time necessary

for recuperation after exercise. This example shows that despite the fact that some basic physiological principles are known, our knowledge hitherto fails to result in the design of suitable speciality food for various purposes. In conclusion, most sport speciality food is in essence formulated based on trial and error and hitherto not very successful. Food is more than mere nutrition, in recent years there is more and more awareness that food also influences the natural resistance of the system to disease (probiotics). For the same lack of an objective test method, similar problems exist with regard to other speciality foods, e.g., patients with intestinal (infectious) disease, for post-operative patients (especially intensive care), geriatric food, and baby food, in particular in the weaning and post-weaning period. In all cases, malabsorption occurs, and in the latter case, the lack of knowledge about food pertinent to the situation often leads to the occurrence of post-weaning diarrhoea, which is a life-threatening condition.

Material and methods

Note: Pigs were chosen herein as experimental subject and are the preferred species as model for the human, however, other animals, or *ex vivo* maintained intestines or parts thereof, can be selected as well.

SISP:

Briefly, pigs were sedated with 0.1 ml azaperone (Stressnil), per kg bodyweight, after 15 minutes, inhalation anesthesia was performed with a gas-mixture of 39% oxygen, 58% nitrous oxide and an initial 3% halothane; after 10 minutes 2% halothane. The abdominal cavity was opened and about 40 cm caudal from the ligament of Treitz, the first segment of 20 cm length was prepared by inserting a small inlet tube in the cranial site of a segment and by inserting a wide outlet tube into the caudal site of a segment.

A second segment, next to the first one, was similarly prepared, forming a pair of segments with the first segment located at 10% of the total length of the small intestine. Four other pairs of segments were similarly prepared at 25%, 50%, 75%, and 95% of the total length of the small intestine. Cranial tubes of segments were connected by infusion systems (Accudrop, Braun, Melsungen) to 100 ml bottles placed in a tray above the animal; caudal tubes drained into bottles placed at the level of the pig's abdomen. If the perfusate had a viscosity incompatible with the Accudrop infusion system, perfusion was performed with syringes attached to the cranial tubes, manually (8 ml/h, = 2ml/15min). Segments were then perfused with 80 ml of fluid containing 9 g NaCl, 1 g Bacto casaminoacids (Difco), and 1 g glucose per liter distilled water for 10 hours (ml/h). After perfusion was ended, fluid remaining in a segment was blown out into the collecting bottle; then pigs were euthanised by barbiturate overdose. The surface area of each segment was measured. Net absorption was defined as the difference between inflow and outflow in ml/cm^2. Intestinal pH was measured in the effluent.

IR-SISP

Manipulation of the blood circulation of the intestine of the pig to mimic different physiological states i.e., resting, moderate or strenuous exercise was performed as follows.

Pigs were premedicated with pentobarbital 20 mg/kg body weight intravenously. Then, they were orally intubated and ventilated (12 breath per minute) with a blender (SLO1; Ohio medical products, Minneapolis, MN) and a volume controlled ventilator (900 A; Siemens, Elema, Sweden). Ventilation was kept unchanged for the duration of the experiment. Body temperature was stabilized by a heating blanket. Anaesthesia was maintained by continuous intravenous infusion of pentobarbital 6 mg/kg/h in the right *vena jugularis externa* and pancurium bromide 2 mg/kg/h by a catheter placed in the left *vena jugularis externa.*

Next, the pig was placed in the lateral position and an incision was made at 2 cm parallel to the rib cage. The superior mesenteric artery (SMA) was prepared free and an ultrasonic flow sensor was placed around the vessel. A flow probe (Transonic) was placed around the SMA. After a period of equilibration the normal flow rate was established. Then, SISP segments were placed as described above, for the determination of the luminal pH, segments could be fitted with intestinal tonometer (Tonometrics, Worcester, MA). The tonometer was filled with 2.5 ml physiological saline. At regular intervals (30 min) tonometer fluid and arterial blood was collected with a blood gas syringe (Monovette LH, Sarstedt, Germany) for determination of the pCO_2 with a IL-1306 analyzer (Instrumentation Laboratories, Milan, Italy). Intraluminal pH was then calculated using the Henderson-Hasselbalch equation.
Next, a clamp was placed around the SMA, and the blood flow was reduced to a level of 75% for a period of 60 min. After this period of relative ischaemia, the clamp was removed to allow reperfusion for 2h. This mimics an ischaemic phase follows by reperfusion such as seen during and after transient exercise. Clamping could also be continued to mimic continuous relative ischaemia.

Statistical analysis of the data obtained were performed using Genstat 5 analysis of variance (Genstat 5 Committee, 1987). Experiments were designed as

completely randomized blocks. Analysis of variance of the completely randomized blocks was used to compare treatments and interactions with day and sites. Results were significant when $p < 0.05$. Segments of the intestine can be taken at various timepoints and prepared for testing in the diffusion chambre.

Diffusion Chamber

The Diffusion Chamber (DC) is a double-cell acrylic chamber (WPI, Sarasota, FL, USA) in which a living membrane is interposed separating the two cells. An oxygenated buffer solution is circulated through each cell by independent reservoirs. The pH of the solutions was maintained between 7.35 and 7.45 by manipulation of the percentage of CO_2. The reservoirs are jacked with a recirculating water bath to maintain reservoir temperature at 37°C. Two calomel and two Ag-AgCl electrodes are connected to the cells via agar bridges. This allows measurement of transmembrane potential difference (DV) and application of a fixed current. DV is proportional to total ionic flux, which is an indicator of tissue viability, and indirect measurement of cell metabolism. By measuring DV with the addition of a fixed current, resistance (R) was calculated using Ohms Law [R (Ohm) = V (volt) x I (ampere)]. R (Ohm/cm^2) reflects tissue integrity. Membranes were perfused with Hanks Balanced salt Solution.

Membranes were prepared as follows.
Pigs were anesthetized with halothane or if samples are used from IR-SISP see there. Under sterile conditions, the ileum was exposed. The mesenteric vasculature was clamped, and 4- to 6-cm ileal segments without visible Peyer's patches were quickly excised. The seromuscular layer was stripped from the submucosa and mucosa. The mucosal preparations were immediately mounted in the diffusion chamber separating the mucosal from the serosal reservoir. After mounting, membranes required 15 minutes to stabilize prior to measurement.

Following this initial stabilization, DV and R were measured repeatedly at 15 minute intervals for 2 hours.
Macromolecular permeability was measured using horse-radish peroxidase (HRP) as a model macromolecule. Briefly, 10-5 M (0.4 mg/ml) HRP was added to the chamber at the mucosal side. Samples (0.25 ml) were taken at the serosal

side at regular intervals (30 min). HRP concentrations were determined enzymatically. Macromolecular permeability was expressed as pmol HRP/cm^2/h).

Monosodium glutamate (MSG): Influence of MSG on small intestinal permeability. (MSG is implicated in the Chinese restaurant syndrome (CRS) as "vetsin".)

Material and methods. Ischaemia/Reperfusion SISP and Ussing chamber
At time zero: two samples of untreated segments were taken and transferred to the Ussing chamber.
Next: 8 SISP segments were filled with respectively saline, 1, 2 or 3 % MSG in saline (each in duplo).
Then: blood flow was reduced to 90% for 1h, followed by 90 min of reperfusion.
Then samples of the mucosa were transferred to the Ussing chamber. Mucosal strips (duplicate) of all 8 segments were tested in the Ussing chamber for macromolecular permeability for HRP over 3h. The experiment was repeated four times on different days.
Results. Pre-I/R permeability (pre) was lower than 1 pmol HRP/cm^2/3h. I/R in the presence of saline only, and with different concentrations of MSG showed a significantly higher permeability. The addition of different concentrations of MSG increased the significance of the difference when compared to the pre-I/R sample.
Discussion The results indicate that although glutamate is described to ameliorate post-damage intestinal morphology, MSG does not attenuate the post I/R damage of which the permeability is a consequence. This is consistent with the fact that an intact intestinal morphology is not necessarily related with correct functionality. However, the presence of MSG enhances the significance of the permeability difference as compared to the untreated mucosa.
This could be indicative of MSG as a causal factor in CRS. In CRS the following could be envisaged: (relative) ischaemia of the GI-tract is aggravated by large food intake. This leads to acidosis of the small intestine, which is associated with increased permeability. MSG (as vetsin) increases or persists the permeability, etc.

Hydrolyzed polysaccharides

Do hydrolyzed complex polysaccharides hPS protect against increased macromolecular transepithelial transport in situations of intestinal ischaemia/reperfusion damage?

M&M: IR-SISP was performed with 1 h of ischaemia (I-phase), and 2 h of reperfusion (R-phase). Samples of tissue from each phase were tested in the diffusion chamber. The effect of hPS (2.10-5M) was measured by adding it to perfusate during the I-phase, or the R-phase, or at the mucosal side in the diffusion chamber. Permeability was measured by the HRP method.

RESULTS: hPS protects against increased permeability when added *in vivo* during the I-phase. No effect was seen when hPS was added *in vivo* during the R-phase. When hPS was added in the diffusion chamber, it had no effect on the permeability of I-phase or R-phase derived intestinal epithelium.

Conclusions: hPS protects against increased permeability if present during the ischemic phase in the *in vivo* IR-SISP as established in the diffusion chamber (DC). When virgin isolated epithelial layers from both the I and R phase were exposed to hPS in the diffusion chamber this effect was not seen.

Specific antibodies as food component for the protection against infectious enteral disease.

M&M: SISP was performed with enterotoxic *E. coli* F4 in the perfusion buffer. Segment were placed as triplets, one containing only buffer and *E. coli*, a second one containing an additional 10% of normal plasma powder, the third contained 10% anti-*E. coli* F4 hyper immune plasma powder.

RESULTS: *E. coli* F4 reduced the net absorption as expected, anti-*E. coli* F4 hyper immune plasma powder abolished this effect.

Conclusions: anti-*E. coli* F4 hyper immune plasma powder protects against *E. coli* F4 mediated secretory diarrhea.

Blocking of macromolecular permeability during a sensitive period.

E. coli F4 and rotavirus are pathogens under certain conditions. In those situations both cause increased permeability. Juveniles have naturally a higher

permeability than adults. Adults have a higher permeability post-injury (post-infection etc.), in the regeneration phase. It appeared that both *E. coli* F4 and rotavirus attenuate the juvenile and adult regeneration permeability as demonstrated by DC. The activity is currently being identified to specific compounds (proteins and others) and fragments thereof responsible for this effect .

Modulation of macromolecular permeability.

For several purposes, modulation of permeability rather than blocking or total and/or permanent permeability is desired. For example, in the case of oral vaccination one wants the permeability to have either a selective character, i.e. only for the vaccin, or be transient, i.e. permeability only as long as it takes to enable the vaccin to pass the intestinal barrier.

M&M: Samples of intestinal tissue were tested in the diffusion chamber. The effect of DMSO (0, 1-3%) was measured by adding it to the diffusion chamber. Permeability was measured by the HRP method.
RESULTS: DMSO increased permeability in a dose dependent way.
Conclusions: DMSO increases permeability in the diffusion chamber (DC), and is a very promising agent for achieving transient intestinal permeability for its very short physiological half-life. Furthermore, DMSO is an approved pharmaceutical additive (for other purposes), without any known side effects or contra-indications.

CLAIMS

1. A method for determining molecular transport through an intestinal wall or segment thereof comprising regulating oxygen supply to said wall or segment and determining transport of a marker molecule through said wall or segment thereof.

2. A method for determining molecular transport through an intestinal wall or segment thereof comprising regulating perfusion of blood vessels in said wall or segment and determining transport of a marker molecule through said wall or segment thereof.

3. A method according to claim 1 or 2 further comprising rendering at least part of said intestinal wall or segment at least partly ischaemic.

4. A method according to claim 3 wherein a blood vessel leading to or from said part is at least partly clamped.

5. A method according to anyone of claims 1 to 4 wherein said transport is macromolecular transport.

6. A method according to anyone of claims 1 to 4 wherein said transport comprises fluid and/or electrolytes.

7. A method according to claim 6 wherein said fluid and/or electrolyte transport is measured by determining net-absorption.

8. Use of a method according to anyone of claims 1 to 7 in selecting or obtaining at least one component for a speciality food.

9. Use according to claim 8 wherein said speciality food comprises a food particularly suited for an intensive care patient, an athlete, a very young person, a diseased person or an elderly person.

10. A method for selecting or obtaining a speciality food or drink tailored for the needs or desires of an individual who's functional integrity of the intestinal wall is under siege comprising testing at least one candidate component for said speciality food for its effect on molecular transport through an intestinal wall or segment thereof and selecting a component for inclusion in said speciality food that has a desired effect on said molecular transport.

11. A method according to claim 10 wherein said component is tested using a method according to anyone of claims 1 to 7.

12. A method according to claim 10 or 11 wherein said functional integrity is under siege due to an at least at times insufficient oxygen supply to said intestinal wall.

13. A method according to anyone of claims 10 to 12 wherein said individual comprises an intensive care patient, an athlete, a very young person, a diseased person or an elderly person.

14. A speciality food or drink selectable or obtainable by a method according to anyone of claims 10 to 13.

15. A speciality food or drink according to claim 14 comprising hydrolysed polysaccharides.

16. A speciality food or drink tailored for the needs or desires of an athlete who's functional integrity of the intestinal wall is at times under siege containing no or only little added glutamine.

17. A speciality food or drink tailored for an intensive care patient containing no or only little added glutamine.

18. A speciality food or drink according to anyone of claims 14 to 17 wherein said food or drink is intended for use during a phase of relative intestinal ischaemia to prevent damage to the functional integrity of an intestinal wall.

19. Use of a speciality food or drink according to anyone of claims 14 to 17 during or prior to a phase of relative intestinal ischaemia to prevent damage to the functional integrity of an intestinal wall.