Persorption Of Plant Microparticles After Oral Plant Food Intake
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Citation

Abstract
INTRODUCTION: The phenomenon of persorption - a non resorptive ingestion of microparticles by the intestinal wall of mammals and human beings - is known since 1844. Previous research has been done by means of light microscopy. METHODS: We are the first group, to use a scanning electron microscope for particle detection. We compared starch particles by means of polarization light microscopy and scanning electron microscopy. RESULTS: According to recent studies we can confirm the electron microscopic existence of starch granules in human blood after oral plant food intake. The results of electron microscopic and polarization light microscopic analysis correlate significantly. Particle number can be compared with former light microscopy. Due to the improved resolution in the electron microscope, we expected a higher detection of small starch particles, which however could not be confirmed. We also studied the persorption of non-starch polysaccharides or remnants of plant material as a part of dietary fibre. We were not able to find any non-starch polysaccharide particles in human blood with our investigation method, as might be suspected via the mechanism of persorption. CONCLUSION: Scanning electron microscopy is not able to detect more persorbed particles than polarization light microscopy. Despite our expectations we could not detect smaller starch particles in human blood compared to those seen in polarization light microscopy.

INTRODUCTION
The phenomenon of non-resorptive ingestion of microparticles by the intestinal wall of mammals and human beings has been described since 1844. Particles smaller than 100 nm are called nanoparticles, those larger than 100 nm, microparticles. The ingestion of non-dissolving microparticles is called persorption or the Herbst-Volkheimer effect. Food is taken up in the bowel by 1.) active transport via membrane bound enzymes 2.) by endocytosis or micropinocytosis/cytopempsis (energy dependent active material transport) and 3.) by diffusion (water and non-dissolving materials, such as electrolytes). Starch hydrolysis is accomplished by amylase from salivary glands and the pancreas. The end products of hydrolysis are monosacharides and disacharides. Disacharides are split by membrane-bound enzymes of the apical endothelial cells (lactase, sacharase, maltase, isomaltase) to monosacharides. Only monosacharides can be absorbed by the enterocytes (active transport mechanism).

Water, electrolytes and iron are transported paracellularly along an electro-chemical or osmotic gradient (tight junctions) without energy consumption from apical to the basal side of the enterocytes.

The term “persorption” is cited in 51 publications (Medline, November 2012).

Particles in ranging from 5 µm to 150 µm in diameter are described, most of the particles are smaller than 120µm. It is propagated, so that persorbed particles slip between two defective cell connections (tight junctions) in the apical cell pole. After 10 , 100 and 210 minutes peak values of starch granules concentrations can be detected in light microscopy. There is similar curve distribution with the particle removal in the urine. The persorption rate was estimated at 1:50.000 (+/-50%), i.e. one of 50.000 particles is able to be persorbed. One gram of starch contains approximately $10^6$ starch particles.

In literature the particle admission is most described via microfold cells (M-cells) of Peyer's patch. They have good capability to transport particles through the cytoplasm. M-Cells have a bigger volume than absorptive enterocytes. The relation between M-cells and absorptive enterocytes is given at 1:12. A close contact to macrophages inside the lymph follicles was found. Absorption time is dependent on particle size and the transport of the particles is organized by the abdominal lymph system. It is unquestionable that the M-
cells of Peyer's patch play the key role in the transport of microparticles. The epithelium of absorptive villi and crypts play a subordinate role.

Another possible transit mechanism of non-dissolving particles could be achieved through the controlled apoptosis of villous columnar epithelial cells. Apoptosis in the epithelium of the bowel is a physiological repair mechanism which after 3-4 days causes controlled cell death. In the case of sheep red blood cells, their passage from apical (=luminal) to the basal cell borders could be observed in histological sections. The transport takes place in the form of controlled cell destruction intracytoplasmatically, the tight junctions remain intact.

A paracellular particle transfer can be observed in chitosan, starch granules and hydrophilic polymers. The essential function of intact tight junctions between enterocytes is the retention of fluids and electrolytes (in both directions). Tight junctions are membrane-bound proteins with a central pore, just 5 nm in diameter. Small macromolecules (octapeptides) with a molecular mass of 5500 Daltons are able to pass by solvent drag. Electric charge, size, shape and surface tension of nanoparticles smaller than 5 nm are ideal for passing the tight junctions. In order of defect tight junctions (apoptosis, inflammation, toxines) the apical cell contact of enterocytes is broken and larger particles can pass.

The smaller the nanoparticles, the better their passage. This was observed at least in vivo in polystyrene microspheres and colloidal gold particles.

In previous studies the peristaltic contractions of the muscularis mucosae and transferred oscillations of the pulsations of endothelial blood vessels was a simple explanation of the particle transfer. Microparticles are then funneled through the basal membranes of endothelial cells into the lymph vessels and mesenteric veins, this is shown by experimental investigations (light microscopy in histological sections) of the last century. Non drug induced sleep, cigarette consumption, coffee consumption, young age and body movement increased the quantity of persorption rate. A classical resorption by transcellular phagocytosis of the enterocytes (pinocytosis) can take place merely with particles, smaller than 3 µm.

Persorbed particles can be detected in urine, blood, cerebrospinal fluid, peritoneal fluid, breast milk, in the blood of foetuses and in the umbilical cord blood. Persorption was also observed in the respiratory epithelium. Typical persorbed microparticles are pollens, fungal spores, bacteria, starch granules from plant food like maize, rye, wheat, natural and industrially induced dust, diatoms and non-dissolving crystalline structures, bismuth particles, carrageenan, liposomes, Candida albicans, polyvinyl particles, metallic iron particles, foreign DNA, gold nanoparticles and pulverized charcoal. Fast food contains a large portion of alpha-cellulose as filler. In diet recommendations, a high fibre content is required over and over again to avoid recurrence of diverticulitis. In a WHO expert committee, no injurious limit value called for the consumption of cellulose, because "no problem of persorption arises". A potentially harmful effect has been described by the ingestion of starch in children who suffer from glycogenosis, because a substantial amyluria was observed. Similar mechanisms are also known to the skin (particles of lead, titanium, chitosan) and lung.

Plant derived fibres (carbohydrates) can be classified in the following types:

1. simple sugars (glucose, fructose, lactose, maltose, sucrose), 2. oligosaccharides (3-10 glucose molecules joined together), 3. starch polysaccharides - digestible and indigestible “resistant” starch and 4. non-starch polysaccharides (xylose, arabinose, mannose, etc.). In our daily meals we find soluble fibre and non-soluble fibre. Soluble fibre include pectins, gums, mucilage and oligosaccharides. Insoluble fibre include cellulose, hemicellulose (arabinoxylanes), lignin, mixed linked beta glucanes, xylaglucanes, waxes, cutin, phytate, saponins, tannins and suberin. Dietary fibre is the preamble of remnants of plant components. Starch polysaccharides consist of amylase and amylopectin, they are the energy reserves of the plants (like glycogen in mammals) and can be found in internal structures of plants. Non-starch polysaccharides are derived from the skeletal structures of plants.

The American Association of Cereal Chemists made the following definition: “Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine...”.

Biochemically it is polysaccharides with the molecular formula (C6H10O5)n, each monosaccharide is composed of an alpha-D-glucose molecule. The two main components of starch are amylase and amylopectin. Amylose consists of straight chains with alpha-1,4-glycosidic bond, amylopectin
Persorption Of Plant Microparticles After Oral Plant Food Intake

consists of branched sugar chains with alpha-1,6-glycosidic and alpha-1,4-glycosidic bond. The mass proportion between amyllose and amylopectin is different from plant to plant, the bigger portion comes from the amylopectin. Size and morphology of the granules is strong depending on the herbal origin. Big granules can reach up to 100 µm in diameter, very small ones can be smaller than 0.3 µm and can thus escape light microscopy. Rice, wheat and oat have very small granules.

**Figure 1**

Table 1. Granule sizes of starch from different origins

<table>
<thead>
<tr>
<th>Source</th>
<th>Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blimodal starch</td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>2-3 &amp; 12-22</td>
</tr>
<tr>
<td>Immature sweet corn</td>
<td>1.5 &amp; 10-20</td>
</tr>
<tr>
<td>Rye</td>
<td>2-3 &amp; 22-36</td>
</tr>
<tr>
<td>Triticale</td>
<td>22-36</td>
</tr>
<tr>
<td>Wheat</td>
<td>&lt;10 &amp; 10-35</td>
</tr>
<tr>
<td>Small granule starch</td>
<td></td>
</tr>
<tr>
<td>Buckwheat</td>
<td>2-14</td>
</tr>
<tr>
<td>Cattail</td>
<td>2-15</td>
</tr>
<tr>
<td>Drop word</td>
<td>0.5-10</td>
</tr>
<tr>
<td>Durian</td>
<td>3-14</td>
</tr>
<tr>
<td>Grain tel</td>
<td>2-6</td>
</tr>
<tr>
<td>Oat</td>
<td>2-14</td>
</tr>
<tr>
<td>Parsnip</td>
<td>1-6</td>
</tr>
<tr>
<td>Rice</td>
<td>2-10</td>
</tr>
<tr>
<td>Small millet</td>
<td>0.8-10</td>
</tr>
<tr>
<td>Wildly Rice</td>
<td>2-8</td>
</tr>
<tr>
<td>Very small granule starch</td>
<td></td>
</tr>
<tr>
<td>Amaranth</td>
<td>1-2</td>
</tr>
<tr>
<td>Canary Grass</td>
<td>1.5-3.5</td>
</tr>
<tr>
<td>Cow cockle</td>
<td>0.3-1.5</td>
</tr>
<tr>
<td>Daishen</td>
<td>0.5-3</td>
</tr>
<tr>
<td>Pinwead</td>
<td>1.5-3</td>
</tr>
<tr>
<td>Quinoa</td>
<td>0.5-3</td>
</tr>
<tr>
<td>Taro</td>
<td>2-3</td>
</tr>
</tbody>
</table>

Three morphological main forms are distinguishable (depending on the botanical origin):

1.) polygonal shapes: oat, rice, maize, wheat, sorghum. Typically there is an excentric hilus.

2.) elliptical or oval shapes: arrowroot, potato, sago, canna. The hilus has typically eccentrically, concentric rings or stripes. Legumes have oval granules, the hilus is located centrally.

3.) Cap-shaped forms: tapioca (manioc).

Past electron microscope studies of wheat have shown that the granules appear in three size classes. The smaller B-granules have an average diameter of 5 µm, the bigger A-granules are 12-24 µm, the smallest ones are the C-granules (0.3-3 µm). So it is evident, that smaller granules are existent and that they cannot be made visible by means of light microscopy. Most other starch granules show only one dimension class.

**SUBJECTS, MATERIALS AND METHODS**

In the period from January to March 2012 we examined the ingestion from plant food in adults.

In our study we want to confirm that starch granules and plant derived fiber structures can be observed in blood samples as a physiological phenomenon. Our mission is defined by the distinction between human cellular microparticles, human blood cells and plant derived microparticles.

In the studies of the past centuries, starch was detected by light microscopy (staining with Lugol’s solution) or polarization light microscopy. Due to the improved resolution and possibility of better identification of particles, we expected a larger number of particles in the human blood samples (especially of smaller particles).

30 samples from 30 subjects were collected in a private general medical practice. Two groups where randomized: sober and not sober volunteer subjects (in each group 15 persons). The volunteers who were not sober received a starchy meal.

5 people could choose their breakfast to their liking (breakfast group). 5 persons had 150g muesli with 200ml milk (muesli group). 5 persons had 500g of mandarins (mandarin group).

All the persons provided written, informed consent before entering the study. Taking of blood was performed according to the Helsinki-Tokyo Declaration following ethical principles.

Exclusion criteria: feverish infections and illnesses during the previous 2 weeks, patients post-hospitalisation during the previous 6 weeks. Age from 22 to 79. Female and male subjects were selected in equal parts. Subjects were excluded if they were on medication for any chronic disease, pregnant or lactating women were also excluded.

Sober group: The subjects were asked to eat nothing 12 hours before planned examination. Drinking water was permitted.

The 15 persons were given a full blood analysis from 08:00 to 10:00 am. Blood was collected before and 100mins after ingestion of the meal. We used 22 gauge needles and 3ml plastic syringes (Greiner disposable plastic tubes with
K3EDTA, Vacuette®). Prolonged placement of a tourniquet was avoided, the first 5ml of blood where discarded (vascular damage, endothelial derived microparticles). All procedures were performed at room temperature and with powder-free gloves (Latex, soft-hand®).

8 plastic syringes (24ml) were used and 10ml of blood sample was examined in each subject.

Preliminary investigations with Triton X-100 and sodium hydroxide solution resulted in unsatisfactory results. Finally we chose a solution of 8.29g ammonium chloride and 1.0g potassium hydrogen carbonate in 1000ml destilled water as an ideal cell lysis medium. The particle amount was the best with this method, although Triton X solution has much better lysis capacity. One milliliter of blood was collected in a 15ml centrifuge tube with screw cap (Carl Roth®, Germany) and dissolved in 14ml of the cell lysing agent (10 tubes per subject). After 10min the centrifuge tubes (10x) were centrifuged at 5300g for 30min (Fa. Hettich®, Heba 8S). The supernatant was discarded and the centrifugate was resuspended in 14ml cell lysis agent. The centrifugation was repeated at 5300min for 30min. After repeating the same procedure three times, the centrifugate of one centrifuge tube was placed on one microscope slide with a cover slip (for light microscopy examination) and at a 13mm polycarbonate filter with 0.2µm or 0.6µm pores (Millipore®, Isopore GTTP, pretreatment with poly-L-lysine) or Thermanox® Plastic Coverslip, 13mm diameter. After air drying for 16 hours (drying oven at 38°C) the Millipore or Thermanox® filters were placed on 13mm aluminum stubs (pretreated with double sided sticky carbon plates) and sputtered with platinum (Agar® Auto Sputter Coater, argon gas quality: 99.98%, Linde Gas®, 12 x 15 sec at 20mA and 0,025mbar).

The samples (10 stubs per subject) were examined with a field emission scanning electron microscope - working distance: 2-8mm, 5-10kV (Zeiss®, DSM 982, Everhart Thornley SE detector, Se inlens detector and BSE detector). The electron microscope was always operated by the same researcher and he was not given any information about the subject (sober, not sober, kind of meal). Each subject sample was first screened with a magnification of 500x. This was facilitated by the automated scanning function of the microscope. In cases of suspected plant derived particles (starch granules and non-starch polysacharide complexes/fragments of plant cells) we increased the magnification factor (5000x - 50000x). Length and width of particles were measured. The particles were classified into starch granules, plant cell fragments and not identifiable particles. The latter were classified in most cases as plastic fragments from the centrifugal tubes due to the microcentrifugal force.

To assess the morphology of the particles, we made preliminary investigations. First we investigated possible contamination by particles from the used plastic tubes. The full preparation procedure was carried out only with destilled water. The particles found in the electron microscope hat to originate from the plastic tubes or environmental dust particles.

Following this, we examined the raw materials of food (muesli, rice, various cereal grains, fruit juices, vegetable juices, mandarines and white grapes) under the electron microscope. We built an image database and where able to compare each detected particle with our database. So we had practice with the morphology of the particles founds and confusion could be kept to a minimum.

Polarization light microscopy was performed with an Olympus® BH-2 microscope. The whole sample was scanned with an Aplan 10x objective (100x). Starch granules could be identified by their maltese cross formation in polarized light. Only glass particles or micro-bubbles made the distinction difficult. In these cases we used the 40x Aplan objective (400x) to be beyond doubt.

**RESULTS**

Sober subjects did not present any starch particles in their blood samples. Contrary to our expectations we could not detect any starch particles by means of scanning electron microscopy in sober subjects. The number of starch particles was equal in the light microscopy and the electron microscopy line (paired student t: p<0.05). It seems that smaller starch granules (0.2 - 5µm) cannot be persorbed better than bigger ones. Most of the starch particles detected had a size from 5-30µm.
Figure 2
Figure 1. Field emission scanning electron microscope view of a corn starch granule after oral intake of muesli (subject from the “muesli group”). Magnification: 11.500x, 5 kV, working distance 3mm

The highest starch granule content could be found in the muesli group. There was no sex difference concerning particle number and particle size. There was a significant difference of particle number between several individuals. Younger subjects had better persorption capacity than older. Neither light microscopy nor scanning electron microscopy was able to detect any plant derived cell fragments.

Figure 3
Table 2. Counted particles by means of scanning electron microscope and polarization light microscope, sample volume 10ml EDTA blood

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean particle size (µm)</th>
<th>Max particle size (µm)</th>
<th>Min particle size (µm)</th>
<th>Median particle size (µm)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.3</td>
<td>6.1</td>
<td>0.5</td>
<td>1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>6.2</td>
<td>0.6</td>
<td>1.8</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>2.2</td>
<td>6.0</td>
<td>0.4</td>
<td>1.6</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>2.4</td>
<td>6.1</td>
<td>0.5</td>
<td>1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>2.6</td>
<td>6.3</td>
<td>0.6</td>
<td>1.8</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>2.7</td>
<td>6.4</td>
<td>0.7</td>
<td>1.9</td>
<td>0.6</td>
</tr>
<tr>
<td>7</td>
<td>2.8</td>
<td>6.5</td>
<td>0.8</td>
<td>2.0</td>
<td>0.7</td>
</tr>
<tr>
<td>8</td>
<td>2.9</td>
<td>6.6</td>
<td>0.9</td>
<td>2.1</td>
<td>0.8</td>
</tr>
<tr>
<td>9</td>
<td>3.0</td>
<td>6.7</td>
<td>1.0</td>
<td>2.2</td>
<td>0.9</td>
</tr>
<tr>
<td>10</td>
<td>3.1</td>
<td>6.8</td>
<td>1.1</td>
<td>2.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Starch granules measured by means of light microscopy are bigger compared to those in the electron microscope. This is a result of shrinking process due to air drying in the drying oven. With our results we can confirm the data of past decades. We were able to count 10-30% more starch granules with our new particle separation method (ammonium chloride/potassium hydrogen carbonate) compared with the previous method using acetic acid.

DISCUSSION
After extrapolation to the total amount of circulation blood in human adults we estimated a total number of 55,000 starch granules after oral ingestion of plant food. Previous studies counted starch particles to the extent of 10-80 particles per 10ml of blood ($10^2$ - $10^3$). This amount can be compared with our results, although it seems that our preparation method produces more particles. Contrary to our expectations, we did not find more starch particles by means of electron microscope. According to the laws of persorption, we expected more small starch particles, that would escape detection by means of light microscopy. One explanation may be the late examination of blood after 100mins following plant food intake. Early and repeated blood examinations could contribute to an increase of detection of small starch granules.

We were not able to find any one particle with secured plant origin (except starch granules). We would like to point out, that no plant particles could be found with our current preparation technology. The current preparation technique is time-consuming and associated with potential sources of error. Future preparation techniques should count starch particles selectively (fluorescence microscopy, flow cytometry, immuno gold staining) and rapidly.

Currently we can not explain why the inter-individual variability of particle numbers are so large.

The question of potential benefits or harm of starch granules in human serum is obvious. Answers remain purely speculative. The existence of starch granules in human blood could change our knowledge concerning the function of plant food. It is possible that these particles have beneficial health effects not only in the intestinal lumen but directly in the blood stream and on the endothelial surface of vessels. According to recent studies, small particles are better persorbed than large particles. This suggests that plant food with small starch granules could have better health promoting effects than plant food with large granules. Of course this allegation has no actual scientific explanation. Another interesting question is whether malabsorption syndromes like celiac disease, ulcerative colitis or Crohn’s disease have a higher extent of persorbability and this may influence the disease activity.
A potential for sensitization of immune system must be considered. In a study using rats, a positive correlation between the amount of oral wheat intake and autoimmune diabetes could be demonstrated.

Many future studies will be necessary to get a better understanding of the phenomenon of persorption. We are particularly interested in potential benefits, possible damages and the association between persorption and several diseases. In the future, particular attention should be paid to the sample preparation and particle separation technology. At this point there are major hurdles to overcome.

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