(Castell et al., 1997)

Intestinal absorption of undegraded proteins in men: presence of bromelain in plasma after oral intake

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Unidad de Hepatología Experimental, Centro de Investigación, Hospital Universitario La Fe, E-46009 Valencia, Spain; PharmBiodym, Research Services, D-79211 Denzlingen, Germany; and MUCOS Pharma G.m.b.H. & Co., D-82254 Geretsried, Germany

Castell, José V., Gerhard Friedrich, Cai-Steffen Kuhn, and Georg E. Poppe. Intestinal absorption of undegraded proteins in men: presence of bromelain in plasma after oral intake. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G138–G146, 1997.—The human adult intestinal epithelium has traditionally been described as nonpermeable to proteins. However, indirect evidence suggests that reduced absorption of undegraded proteins might take place under physiological conditions. Using bromelain (an enzyme obtained from pineapple stems) as a model protein, we studied the extent of this mucosal permeation in 19 healthy men. The protein was detected in plasma by immunossay and by its proteolytic activity after oral administration. The estimated plasma half-life was 6–9 h. After oral multidosing (3 g/day), plasma concentration reached as much as 5,000 μg/ml by 48 h. From the plasma concentration curve, it could be estimated that an average of 10.8 μg of bromelain was present in plasma in the 3- to 51-h period. The presence of undegraded bromelain in plasma was shown unequivocally by immunoprecipitation of plasma samples with antibranelain antibodies, followed by gel electrophoresis and immunodetection. Moreover, the enzyme retained its biological activity, at least in part. Circulating bromelain was found associated with α-macroglobulin and α1-antichymotrypsin. The results of this work confirm the existence of a small but significant intestinal transport of undegraded proteins in healthy men.

intestinal protein absorption; intestinal permeability; food intolerance; allergy; oral delivery of peptides

The intestinal absorption of intact macromolecules has been a matter of scientific controversy for many years. Despite early evidence showing a certain permeability of the intestine for particulated substances (32, 35), the healthy adult intestinal epithelium was generally considered to be absolutely nonpermeable to large macromolecules. More recent evidence has suggested that absorption of macromolecules (among them undegraded proteins) could occur under certain circumstances (8, 20, 28) and that this phenomenon could be involved in the pathophysiology of certain intestinal diseases (28, 34).

The experimental evidence of intact protein transport through the intestinal epithelium of adult is weak (20, 28). It is generally assumed that during digestion, proteins are fully hydrolyzed by intestinal proteinases and lysosomal enzymes of enterocytes to amino acids (17) before they can reach the portal system. The intestinal wall in mammals is only open in preterm babies (2) and for a few days after birth, when immunoglobulins from the colostrum can easily reach the blood of the newborn (18, 21, 36). After then, an intestinal closure takes place that is characterized by the replacement of fetal absorptive cells by adult cells lacking this transport ability and the formation of tight junctions between neighboring epithelial cells (7). This picture can be altered in young and adult individuals under pathological circumstances [i.e., enterocolitis (25), viral enteritis (11, 14), gastroenteritis (12), and severe burns (9)]. Transcellular uptake of macromolecules has often been considered the mechanism behind the induced immune response to dietary components in intestinal allergic diseases (6, 13, 15, 23–25, 28, 29, 33, 34), despite the lack of convincing evidence.

Some studies have lent support to the idea that there is a small but significant transport of biologically active peptides and proteins through the epithelial cell wall in animals [i.e., factor VIII (10), thyroid stimulating hormone and adrenocorticotropic hormone (31), insulin (27), and calcitonin (5)]. However, the occurrence and extent of undegraded protein absorption in adult humans remains to be quantified. Research on this transport phenomenon would be of interest not only for a better understanding of pathophysiological events in the intestinal epithelium but also for a future oral delivery of peptides and proteins with pharmacological action.

In the present study, we have investigated the extent of mucosal permeation to undegraded proteins in men. For this purpose, bromelain, a biologically active plant protein present in pineapple stems and fruits (26), was chosen as a model protein. After bromelain was administered orally to human volunteers, the plasma kinetics and the nature of the protein present in blood were investigated. Our study demonstrates the presence of intact, biologically active bromelain in the plasma of individuals who received the protein orally and thus lends support to the existence of a small but significant transmural transport of nondegraded proteins in healthy adult humans.

Materials and Methods

Materiales. Bromelain, isolated from the stem of the pineapple plant (mol mass 24–26 kDa, pharmaceutical grade), was obtained from MUCOS Pharma (Geretsried, Germany) and was dissolved in double-distilled water, filtered, and further purified by gel-permeation chromatography through G-25M and PD-10 columns (Pharmacia, Uppsala, Sweden). Iodobeads and N-hydroxy-sulfosuccinimide (C-5 long chain)-biotin (NHS-LC-biotin) were purchased from Pierce (catalog nos. 28665 and 21335). [3I]-I-Na (specific activity, 535 MBq/μg) was obtained from Amersham–Buchler (Braunschweig, Germany). Z-Arg-Arg-N-hydroxy-methylcoumarin was supplied by Bachem (Basel, Switzerland). Alkaline phosphatase bound to avidin (A-7294), alkaline phosphatase bound to extravidin (E-2636), nitroblue tetrazolium chloride (N-6639), and 5-bromo-4-chloro-3-indolyl phosphate (B-1026) were obtained.

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Intestinal Absorption of Undergraded Proteins

from Sigma Chemical (St. Louis, MO). The fluorogenic substrate 4-methylumbelliferyl-O-phosphate was obtained from Fluka (cat. no. 69605). Sepharose-bound anti-rabbit immunoglobulin G (IgG) (Immunobeads; cat. no. 170-5602) was obtained from Bio-Rad (Munich, Germany). Maxisorp microtiter plates from NUNC (Roskilde, Denmark) were used for the assays. Fluorescence measurements were made in opaque microtiter plates (Microtuf; Dynatech) by using a fluorescent microplate reader (Titertec Fluorocan II, ICM; excitation, 355 nm; emission, 406 nm). Agarose M was from LKB (2206-101).

Antibody production. Antibodies against bromelain were produced in rabbits after multimodal injection of both poly- and monoclonal bromelain and Freud's complete adjuvant emulsified bromelain at 2-wk intervals. Rabbits were bled after 3 mo, and the specificity of the immunoglobulin fraction against bromelain and its lack of response against human plasma proteins were examined by immunoelectrophoresis and Western blot. The IgG fraction was purified by ammonium sulfate precipitation and diethylaminoethyl (DEAE) chromatography and stored as described previously (22).

Biosynthesis of immunoglobulins. About 0.3 ml of DEAE-affine (rabbit IgG) (300 μg/ml at pH 8.5) was added, and the reaction mixture was incubated for 1 h at 0°C. After addition of 20 μl of 0.1 M glycine and further incubation for 20 min at room temperature, the reaction mixture was extensively dialyzed against 50 mM phosphate buffer for 5 h. Bovine serum albumin (BSA) was added to the labeled immunoglobulins at a final concentration of 10 mg/ml and they were stored at +4°C until use (22).

Study design with human volunteers. The study was performed with 19 healthy white men, aged 18–45 yr. The study was randomized, controlled, and double-blind; it was conducted at Biodesign facilities (Freiburg), in accordance with good clinical practice standards, and conformed to the "Declaration of Helsinki" as adopted by the World Health Organization guidelines and the German Drug Law. Informed consent was obtained before starting the clinical study. Volunteers were nonhabitual ethanol consumers, and they had not been administered any medication in the days before the trial, in particular nonsteroidal anti-inflammatory drugs or compound-containing medication. After the period of the villous mucosa. None of the selected individuals had first-degree relatives with Crohn's disease.

Fifteen volunteers were given enteric-coated film tablets, with each tablet containing 200 mg of bromelain. Four individuals were given placebo tablets. During the first 2 days of the trial, three tablets were administered at 0800, 1100, 1400, and 1700, and 2000, followed by five tablets at 2300. On the third day, only three tablets were administered at 0800. The volunteers received standard meals at 0900, 1200, 1530, and 1830. A sample of 12 ml of blood was drawn by direct venipuncture and collected in sodium citrate tubes before each bromelain administration. Blood was also collected on days 5, 4, and 5, following the same schedule as described above. After the blood was centrifuged (15 min at 2,000 g; +4°C), plasma was separated and assayed for bromelain-specific proteolytic activity and for the presence of immunoreactive bromelain.

Immunological quantification of bromelain in plasma. Bromelain was determined in human plasma using a capture enzyme-linked immunosorbent assay. By selecting rabbit antibodies that recognize different bromelain epitopes in a one-species immunoglobulin sandwich enzyme-linked immunosorbent assay was developed. The capture of bromelain by the antibodies was visualized by using avidin-bound phosphatase and a fluorogenic phosphatase substrate. Microtiter plates (NUNC) were coated overnight with 200 μl of a 1,400 dilution of rabbit IgG antihemoglobin (coating buffer: 0.1 M Na2CO3/NaHCO3 and 0.02% NaN3, pH 9.6). Plates were rinsed, and plasma samples, calibration probes, and controls (20–200 pg protein in 200 μl) were incubated for 12–24 h at +4°C. After extensive washing (10 mM Tris buffer, 15 mM NaCl, and 0.05% Tween 20, pH 7.5), 200 μl of biotinylated IgG (1,500 dilution) was added to each well, and the plate was incubated for 2.5 h at 37°C. After repeated washing, 200 μl of avidin-bound alkaline phosphatase (1:12,500 dilution in 10 mM Tris buffer, 150 mM NaCl, and 0.05% Tween 20, pH 7.5) was added to each well and incubated for 1 h at 37°C. Wells were thoroughly washed and incubated with 100 μl of the fluorogenic substrate methylumbelliferyl-O-phosphate (0.33 mM in 0.1 M diethanolamine hydrochloride and 0.5 mM MgCl2, pH 9.8). The incubation was stopped by transferring the probes to opaque microtiter plates for fluorescence measuring. A concentration-dependent increase in fluorescence was observed in the 20–2,000 pg/well range. The variability was small (<5%) when different plasma samples were used to prepare the standards. This variability was reproducible quantification of bromelain in human plasma down to 100 pg/ml. The interassay variation was 95–103%.

Measurement of proteolytic activity of bromelain present in plasma samples. Microtiter plates were incubated overnight (+4°C) by placing 200 μl of the coating solution (1:400 dilution of purified rabbit IgG antihemoglobin in 0.1 M sodium phosphate buffer containing 0.05% NaCl) into each well. The next morning, calibration standards and controls were prepared. The coated plates were washed, and 200 μl of plasma, samples, controls, or calibration standards were added and incubated for 12 h at +4°C. Dilution and sampling of probes were performed automatically (Bioek, Beckman). Plates were washed, and 200 μl of substrate solution (0.05 mg/ml Z-Arg-Arg-N-hydroxy-methylcoumarin in 0.1 M phosphate buffer, 4 mM EDTA, and 8 mM cysteine) was added per well and incubated for 4 h at 25°C. The fact that bromelain was attached to the surface of the microwell by an adsorbed antibody apparently did not impair the catalytic activity of the enzyme toward small, easily diffusible substrates. The reaction was stopped by transferring the incubation mixture and the associated fluorescent material into the microwell by centrifugation. The fluorescence was measured at 355 nm excitation and 460 nm emission. The assay showed a linear increase in fluorescence during a 3-h period, which was proportional to the bromelain present in the samples. A practical limit of detection for 1 ng bromelain/ml plasma was recorded.

Identification of circulating bromelain in plasma of volunteers. One milliliter of plasma was diluted with 0.5 ml TNE buffer (20 mM Tris, pH 7.5, 140 mM NaCl, 5 μM EDTA, 2 mM methionine, 1% Triton X-100, and 0.02% NaN3), and 0.5 ml DEAE chromatography-purified IgG rabbit antihemoglobin (1,500 dilution) was mixed and incubated overnight at +4°C. One hundred microliters of Sepharose-bound goat IgG anti-rabbit IgG suspension (Immunobeads; 50 mg/ml in TNE buffer) was added subsequently, and the mixture was incubated for 2 h at 37°C. After centrifugation (100 g for 5 min), the supernatant was discarded, and the sediment was washed two times with 1 ml of TNE buffer and two times with 50 mM phosphate buffer, pH 7.5. Thirty microliters of dissociation buffer (1% SDS and 0.1% diethiothreitol (DTT)) was added to the pellet and incubated for 5 min at 100°C. After centrifugation (100 g for 5 min), the supernatant was analyzed by SDS-polyacrylamide gel electrophoresis (PAGE; under denaturing conditions) and blotted to polyvinylidene difluoride.
**RESULTS**

**Time profile of plasma concentration of bromelain after oral administration.** Plasma samples of individuals who were given bromelain and placebo were assayed at regular time intervals after oral intake of compounds. In subjects who were given the enzyme, an increase of immunoreactive bromelain in blood was evident. In all cases, a characteristic time-dependent concentration curve was observed, reaching a maximum after the night intake of the protein (Fig. 1A). Thereafter, the concentration decreased after a pseudo-first-order kinetics. The individual peak blood concentration of bromelain varied within the experimental group between 2,000 and 10,000 pg/ml (Table 1), with average mean values of 2,500-4,000 pg/ml. Individuals who received placebo tablets showed no detectable bromelain in their plasma at any time during the course of the clinical experiment (Fig. 1B).

The subjects in the study showed significant variability in their plasma concentration profiles. Consequently, the pharmacokinetic parameters peak plasma concentration (C_{max}), timing of peak plasma concentration (T_{max}), half-life (t_{1/2}), and area under the curve (AUC) were calculated independently for each experiment (Table 1), assuming a monocompartmental model of distribution. For most volunteers, C_{max} was reached at ~48 h, with an average mean value close to 5,000 pg/ml. To estimate the t_{1/2} of intestinally absorbed bromelain, we used the data of the decay phase of the second peak of the plasma concentration curve. Results obtained from each volunteer are tabulated in Table 1 and show quite similar t_{1/2} values of bromelain in blood (average value, 6.07 h).

The AUC for the period of 3–51 h was calculated from the available experimental data. The average blood concentration of bromelain in the period of 3–51 h, in individuals administered the protein orally, was 10.28 μg (Table 1).

**Presence of intact bromelain in plasma of human volunteers after oral administration.** To elucidate whether the immunoreactive bromelain present in plasma corresponded to the full-size protein, an experiment was performed in which the plasma of volunteers, drawn at the time of maximal concentration, was
Table 1. Pharmacokinetic data on orally administered bromelain

<table>
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<tr>
<th>Subject</th>
<th>C_{max}, µg/ml</th>
<th>T_{max}, h</th>
<th>t_{1/2}, h</th>
<th>Area Under the Curve, pg·h·mL^{-1}</th>
<th>Mean Amount in Plasma, µg</th>
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<td>6.22</td>
<td>78,364</td>
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C_{max}, peak plasma concentration; T_{max}, timing of peak plasma concentration; t_{1/2}, half-life. Fifteen volunteers received enteric-coated film tablets, each containing 200 mg bromelain at the times indicated in MATERIALS AND METHODS. Four individuals (1, 7, 12, and 17) received placebo tablets. Bromelain was immunologically measured in blood samples drawn before bromelain administration. t_{1/2} could not be determined with enough accuracy in volunteers 5 and 15.

immunoprecipitated with specific antibodies. The immunocomplexes were affinity-isolated with Sepharose-bound goat anti-rabbit IgG. The complexes were dissociated with SDS and DTP and subjected to SDS gel electrophoresis followed by protein transfer of the gel to PVDF membranes. After blots were incubated, first with biotinylated antibromelain antibodies and then with extravidin-bound alkaline phosphatase, the presence of a 24-kDa protein band (Fig. 2, lanes 3-5) became evident. This value is coincident with the full-size bromelain molecule (lane 6). No staining was observed in the plasma of control individuals (lane 2).

Biological activity of circulating bromelain. Having demonstrated that a small amount of orally administered bromelain can be found in plasma as a full-size protein, we considered it relevant to determine whether the protein would still retain its characteristic proteolytic activity after passage through the intestinal cell wall.

The sensitivity of the proteolytic assay could be greatly improved by using a combination of an antibody-mediated capture of bromelain and a specific fluorogenic substrate (Z-Arg-Arg-Val-hydroxy-methylcoumarin). Plasma samples were added to IgG antibromelain-coated wells, and the activity of the retained enzyme was fluorimetrically monitored. The hydrolytic activity of bromelain toward synthetic small-sized substrates was not blocked by the presence of plasma antiproteinases (0.1 mg/ml α2-macroglobulin) or by antibromelain antibodies, as found in control experiments with pure bromelain, and the assay showed a linear increase in fluorescence during a 3-h period, which was proportional to the bromelain present in the samples.

When the plasma of volunteers was examined by this method, a time-dependent variation in enzyme activity, overlaying that of the immunoreactive bromelain, was found (Fig. 3). The plasma concentration, as well as the hydrolytic activity of bromelain in individuals who received the protein orally, showed two peaks: one at 24–28 h and another at 48–52 h. No proteolytic activity was found in individuals who received placebo tablets.

Binding of bromelain to human plasma proteins. Because of the existence of antiproteinases in human plasma that are able to form complexes with a wide array of proteinases, it was of interest to investigate whether absorbed bromelain would be present in blood in the free form or bound to plasma antiproteinases. To elucidate this point, 125I-bromelain was incubated with human citrated blood. After 3 h of incubation at 37°C, cells were separated by centrifugation, and the radioactivity was measured. Most of the radioactivity (>97%) was found in the plasma. After subsequent high-performance gel-permeation chromatography of the radioactive plasma, samples of the eluate were examined by fused-rocket immunoelectrophoresis on agar-containing antibodies against α2-macroglobulin, α1-antitrypsin, and α1-antichymotrypsin. After protein staining, plates were scanned for radioactivity. As shown in Fig. 4, most of the radioactivity was associated with the α2-macroglobulin precipitation line of the rocket immunoelectrophoresis, and, to a lesser extent, with that of α1-antichymotrypsin. From the high-performance liquid chromatography radioactivity profiles, it became evident that >50% of bromelain was associated with plasma proteins.

DISCUSSION

In the adult mammal, the intestinal mucosa has been described as a barrier to the passage of proteins (20). Because the intestinal proteinases rapidly hydrolyze proteins, only peptides resistant to hydrolysis by the digestive enzymes of the gastrointestinal tract might have the chance of being absorbed into the body when administered orally.

In newborn animals, the ability of the intestine to absorb whole proteins is great. This seems to be of immunologic importance in many species, including humans (2). Nevertheless, gastric and pancreatic enzymes present at birth and rapidly increasing in the perinatal period, together with the formation of tight junctions in intestinal enterocytes (7), greatly reduce the capability for intact transport of large peptides. Under normal circumstances this “closure” occurs a few days after birth (1, 21) and remains for life.

The present study was undertaken to ascertain whether a transmural transport of proteins could take place in the healthy adult male and, if so, to estimate its magnitude. To this end, we selected a medium-size (24 kDa), biologically active protein, bromelain, that was present in pineapples (26) and for which no evidence of oral toxicity at doses up to 12 g/day existed (26). Zbinden, Institut für Toxikologie der Universität, Zurich, Switzerland.
Fig. 2. Presence of intact bromelain in plasma after oral administration. One-milliliter sample of plasma taken at the times of maximal concentration of bromelain (lanes 3–5) was incubated with antibromelain immunoglobulin G (IgG), and the immunocomplexes were isolated with Sepharose-bound goat anti-rabbit IgG. Proteins were dissociated from the agarose with 1% SDS, 0.1% dithiothreitol (DTT), analyzed by SDS-polyacrylamide gel electrophoresis, and blotted to polyvinylidene difluoride membranes. After incubation with biotinylated antibromelain IgG and extravidin-bound alkaline phosphatase, the presence of bromelain in plasma was revealed with 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium chloride. Lane 1, predosage human plasma of volunteer 6; lane 2, no plasma sample; lane 3, plasma taken on day 2 at 0800; lane 4, plasma sample taken on day 3 at 0800; lane 5, sample taken on day 3 at 1100; and lane 6, predosage plasma of volunteer 6 containing 2.5 ng bromelain/ml plasma.

land, personal communication April, 1988). A study was designed in which bromelain was administered orally to human subjects, and its presence in the blood was investigated. A crucial point in this study was the development of suitable methods for a precise and unequivocal demonstration of bromelain in the blood of volunteers. Skepticism about former experimental evidence of transmural protein absorption has been common among scientists, probably because of the limitations of the methodology used (30). For this reason, the development of sensitive, specific, and reproducible assays for bromelain determination in plasma was of the utmost importance. This was possible with the use of high-affinity specific antibodies and fluorescent probes.

The results of the pharmacokinetic experiments (Fig. 1, A and B) clearly showed an increase in the plasma concentration of immunoreactive bromelain after its oral administration to human volunteers. This greatly contrasted with the values found in control subjects, in whom no measurable bromelain was detected (Fig. 1B).

Despite the fact that the immunoassay used was unaffected by the presence of other plasma proteins, the measured plasma values could simply reflect the existence of circulating immunoreactive bromelain fragments. Therefore, the experiment depicted in Fig. 2 was crucial to show whether full-size bromelain was present in blood. It clearly demonstrates that, in healthy

Fig. 3. Time course of bromelain proteolytic activity in plasma after oral intake of the enzyme. Bromelain was administered to volunteers in a randomized, controlled, double-blind study as described in detail in MATERIALS AND METHODS. Blood samples were drawn before each bromelain administration. Plasma was assayed for the presence of bromelain-specific proteolytic activity.
adult men, a small fraction of bromelain can cross the intestinal epithelium as whole, nondegraded protein.

By making use of the catalytic properties of bromelain, we were able to address the question of whether the transcellular passage of bromelain might result in the denaturation of the protein. Consequently, we investigated the presence of bromelain-associated proteolytic activity in the plasma of the subjects in the study. To accurately measure this activity, we developed an antibody-mediated capture assay that combined the immunologic approach with the use of a specific fluorogenic substrate. In the plasma of individuals who received the placebo, the proteolytic activity was undetectable, whereas in volunteers receiving the enzyme orally (Fig. 3), the time course of the measurable proteolytic activity in plasma overlapped with that of the immunoreactive bromelain, thus showing that the transcellular passage of bromelain did not fully denature the protein.

Because of its proteolytic nature, circulating bromelain was found associated in part with the plasma antiproteinase \( \alpha \)-macroglobulin. Although this protein is known to bind and inhibit serine proteinases, it can bind essentially to all proteinases (3). This explains why a significant amount of the absorbed bromelain appears associated with this antiproteinase in the immunoelectrophoresis experiments shown in Fig. 4.

The magnitude of this transcytotic protein transport is quantitatively small. The peak plasma values of bromelain ranged between 2,000 and 10,000 pg/ml. A similar value was reported recently by Lovegrove et al. (19), who in a pilot study with eight human subjects found an average maximal concentration of 4,000 pg/ml of cow milk \( \alpha \)-lactoglobulin after a milk load. Our study, with a much larger sample size, revealed a certain variability in plasma bromelain concentration among volunteers. From the AUC, it was estimated that the total amount of bromelain present in the blood was close to 10 \( \mu \)g on average. The significance of this transport phenomenon in humans is intriguing. Because of its reduced extent, this is clearly not a mechanism of nutritional significance. However, the consis-
tency of the results presented here indicate that it is probably a common phenomenon in healthy adults.

An interesting question arises as to how a protein can reach blood circulation in an entire, nondegraded, and biologically active form. Mechanisms to explain intestinal protein transport have been postulated (8, 28). The existence of specific receptors on enterocytes and protein transport has been demonstrated for some growth factors (28), but it could hardly be extended to the transport of proteins such as bromelin. The possibility that the intestinal transport of proteins could involve pinocytosis was suggested early on, on the basis of electron micrographs. Pinocytic vesicles formed by the brush border membrane normally fuse with lysosomes to form phagolysosomes. Only if the protein escapes hydrolysis could it conceivably enter the extracellular space and reach the blood stream. Finally, the follicle-associated epithelium cells (M cells) of Peyer’s patches could also be involved. There is evidence of the participation of these cells in the transmural transport of particulated material (15), and it has been speculated that they might convey macromolecular compounds for the uptake of antigens, a process that seems specifically concerned with immunologic intestinal defense (28). Another possible explanation is that the time course of bromelin absorption could be associated with enhanced paracellular permeability secondary to nutrient absorption during meals.

In conclusion, the evidence presented in this report clearly shows that the intestinal transport of undergraded, nondenatured proteins (here exemplified by bromelin) can take place to a small but significant extent in the healthy adult male. The implications of this phenomenon are not yet clear but could be of relevance to achieve a better understanding of the etiology of some allergic intestinal diseases (6, 13, 16, 23, 24, 29, 33). Furthermore, a better knowledge of this transport system might be helpful for future oral administration of biologically active peptides and proteins in humans.

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