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Distribution of elastase I and II messenger RNAs in calf tissue

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vation of significant population shifts. Due to its slower rate of hydrolysis, it is possible that resistant starch is degraded throughout the colon while lactulose hydrolysis and fermentation mainly occur in the proximal colon.

***In vitro* degradation of starch by *Bacteroides thetaiotaomicron* alone or in association with a human H₂-utilizing acetogenic bacterium.** V Rochet, A Bernalier, M Durand (*INRA, laboratoire de nutrition et sécurité alimentaire, 78350 Jouy-en-Josas, France*)

Starch that escapes digestion in the small intestine may constitute an important source of carbohydrates for colonic bacteria. Its degradation and fermentation by amylolytic bacteria produced short-chain fatty acids (SCFA) and the gases H₂ and CO₂. In methane-excreting subjects, H₂ can be reutilized *in situ* by methanogens. However it has recently been shown that when methanogenesis is low reductive acetogenesis ($4 \text{ H}_2 + 2 \text{ CO}_2 \rightarrow 1 \text{ acetate} + 2 \text{ H}_2\text{O}$) may become a significant pathway of H₂ disposal (Bernalier *et al* (1994) *In: Nouvelles tendances en microbiologie anaérobie* SFM, Paris, 110-118).

The objective of the present work was to investigate *in vitro* the interactions that occur during starch hydrolysis between *Bacteroides thetaiotaomicron*, a hydrolytic bacterium of the dominant colonic flora, and an H₂-utilizing acetogenic bacterium, strain M5a3, isolated in our laboratory from human feces (Decaudin *et al* (1994) *In: Nouvelles tendances en microbiologie anaérobie* SFM, Paris, 304-307).

The kinetics of the starch degradation was studied in monoculture (*B thetaiotaomicron*) and coculture (*B thetaiotaomicron* + M5a3) after 1–8 d of incubation at 37°C. The substrate consumption was assayed enzymatically (Boehringer assay).

The glucose liberated from the starch was measured by the GOP method. The fermentation products (gases and SCFA) were analysed by gas chromatography and the ethanol was determined enzymatically (Boehringer assay).

The addition of acetogenic bacterium induced an increase in the rate of starch hydrolysis by *B thetaiotaomicron*. The maximum extent of starch degradation was obtained after 3 d of coculture compared with 8 d in monoculture.

The hydrogen concentration in the coculture was always lower than in the monoculture (43 µmol/l vs 135 µmol/l respectively), where it accumulates, which indicates that the acetogenic bacterium was able to use the H₂ produced by the hydrolytic bacterium. Acetate, propionate and succinate were always the main products of the starch metabolism. However, a change in the fermentation pattern was observed in the presence of the acetogenic strain. The acetate concentration was higher in coculture (15.1 mmol/l vs 7.2 mmol/l in the monoculture). As the increase in acetate production was concomitant with a decrease in the H₂ concentration, it could be partly due to its formation by the strain M5a3 which mainly produces acetate from H₂/CO₂ metabolism. Nevertheless the existence of an interspecies H₂-transfer inducing a shift in the metabolism of *B thetaiotaomicron* to acetate is also likely.

This work demonstrates the existence of an interrelationship between *B thetaiotaomicron* and an acetogenic bacterium leading to a faster degradation of starch. It also suggests that in the colonic ecosystem, reductive acetogens have a significant effect in H₂-reutilization and acetate formation *in vivo*.

Distribution of elastase I and II messenger RNAs in calf tissue. I Le Huerou-Luron, M Gestin, R Toullec, P Guilloteau (*INRA,*

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Bovine lactoserum proteins, such as α -lactalbumin and β -lactoglobulin, have a high nutritional value and together with bovine caseins are the most commonly used proteins in infant formulae. Important allergenic characteristics in newborn infants are assigned to these proteins, especially β -lactoglobulin. According to Jakobsson *et al* (*J Pediatr Gastroenterol Nutr* (1983) 2, 613-616), the intolerance to these proteins may be partially due to deficient proteolytic equipment in the baby's digestive tract. Elastase II, on account of its important action on globular proteins, is one of the hydrolases implicated in this phenomenon, although no data in the literature report the presence of this form in the calf pancreas, which is one of the species best suited to hydrolyse cow's milk proteins.

The purpose of this study was to search for the presence of both elastases I and II in the calf pancreas and other tissues (antral gastric mucosa, fundic gastric mucosa, duodenum, liver, lung and kidney). The construction of a bovine pancreatic cDNA library allowed specific elastases I and II probes to be isolated. The former was selected by screening the bovine cDNA library with a rat elastase I probe, whereas the latter was hybridized with a probe synthesized by PCR using specific primers from 2 regions common to the human, rat and pig. The use of the elastase I-specific probe revealed an intense band with the pancreatic mRNAs in Northern-Blot analysis. There was weaker band with the mRNAs from the fundic gastric mucosa and no expression in the other tissues. In mice, Swift *et al* (1984, *Cell* 38, 639-646) found mRNA levels which were 10^3 - 10^5 higher in the pancreas than in the intestine, the liver, the spleen and the kidneys. Conversely, Han *et al* (1986, *Proc Natl Acad Sci USA* 83, 110-114) identified elastase I transcripts only in the rat pan-

creas. In the calf, the elastase II mRNA was only found in the calf pancreas, as is the case in humans (Kawashima *et al* (1987) *DNA* 6, 163-172). Han *et al* (1986; *Proc Natl Acad Sci USA* 83, 110-114) described their presence in various non-pancreatic tissues but with levels 10^3 lower than in the pancreas.

It would be of interest to measure the elastase I and II mRNA levels at different ages in different species in order to find out if any relationship exists between them and the appearance of allergenic response to cow's milk.

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METABOLISM

Resting energy expenditure in preadolescents with Duchenne muscular dystrophy. F Gottrand¹, R Hankard¹, M Robert¹, M Romon², D Turck¹, A Carpentier³, JP Farriaux¹ (¹ *Service de pédiatrie*; ² *Service de nutrition, CHRU de Lille*; ³ *CFR Marc-Sautelet, Villeneuve-d'Ascq, France*)

Obesity often occurs in preadolescents suffering from Duchenne muscular dystrophy (DMD), and impairs their quality of life. The mechanisms that lead to this obesity remain unknown and could involve low resting energy expenditure (REE), abnormal nutrient disposal, or overfeeding. A better understanding of these mechanisms will help us to prevent and treat obesity in DMD patients. Twenty-two boys, 9-13 years old (9 controls, 13 DMD patients) were studied. All of the subjects and their parents gave informed written consent. The protocol was approved by the Lille University Hospital Ethics Committee (CP 92/26). Their REE was measured by indirect calorimetry over a 3 h period (Sensor Medics, Yorba Linda, CA). Their fat-free mass (FFM) was estimated