

together to constitute a reference period. CCK perfusion led to an increase of 50% in the protein concentration and of 80% in the protein flow ($P < 0.05$), whereas the juice flow did not change significantly. Gastrin perfusion induced an increase of 90% in the protein flow and concentration and a decrease of 40% in the juice flow ($P < 0.05$). The protein concentration and flow remained high (+90%, $P < 0.05$) over the 30 min following gastrin perfusion but not CCK perfusion.

In conclusion, under our experimental conditions, gastrin was shown to stimulate pancreatic protein secretion in a more substantial and durable way than CCK. Since Le Meuth et al (1993) have described CCK-B/gastrin in calf from 1 month of age, it would be of a great interest to study pancreatic secretion by blocking these receptors with specific antagonists. It would be then possible to characterize the functional role of pancreatic CCK-B/gastrin receptors in the calf. The decrease in the juice flow might be due to the action of gastrin, unmediated by secretin. However, further investigations are required to precise the mechanism implicated. (This work was supported by the Region Bretagne).

Regulation of bovine pancreatic elastase II expression during ontogeny. M Gestin, I Le Huerou-Luron, G Le Drean, P Guilloateau (*Laboratoire du jeune ruminant, Inra, 65, rue de St Briec, 35042 Rennes cedex, France*).

In newborn infants, a deficiency in the proteolytic function in the digestive tract could partially account for intolerance to some milk proteins. Pancreatic elastase II may be responsible for this deficiency. In the calf, the specific activity of this hydrolase is high at birth but decreases sharply during post-natal development [Gestin et al (1996), *Reprod Nutr Dev*, n° 6]. This evolution has also been observed for other enzymes which

play an important role in milk digestion. In order to determine the regulation level of elastase II expression during ontogeny, we quantified the mRNAs of this enzyme using RT-PCR in six groups of milk-fed calves aged 0, 2, 7, 21, 28 and 119 days. On the basis of the cDNA sequences of several species, two oligonucleotides corresponding to identical regions were chosen. These two primers bind to the bovine cDNA regions +42 to +62 and +787 to +804, which enabled us to obtain a PCR product with a size of 763 pb. Preliminary studies were carried out to check the existence of a linear relation between the amounts of matrix-cDNA and the products of amplification. The mRNAs were quantified by extrapolating the intensity of the signal of the amplicon against a standard curve. This curve is drawn on the basis of successive dilutions of a determined quantity of specific elastase II cDNA. The expression rate of bovine elastase II specific mRNAs does not significantly change with age (*Kruskal-Wallis's test*) although it tends to increase. Indeed, the minimum and the maximum values are observed respectively in calves aged 0 day (85.8 ± 18 fg/ μ g of total RNA) and 119 day (197.6 ± 47 fg/ μ g of total RNA). This evolution differs considerably from that of elastase II activity, which decreases by 96% between these two stages. A mainly post-transcriptional regulation of bovine elastase II mRNAs could therefore account for the existence of these non parallel profiles. (This work has been supported by the Region Bretagne and the CNIEL).

Amylase in *Pecten maximus* (mollusca, bivalve): protein and cDNA characterization. S Le Moine¹, D Sellos², J Moal¹, JY Daniel¹, F San Juan³, JF Samain¹, A Van Wormhoudt² (¹*Ifremer, centre de Brest, BP 70, 29280 Plouzané;* ²*Collège de France, BP 225, 29182 Concarneau, France;* ³*Universidad de Vigo, Apto 874, 36200 Vigo, Spain*).

In the scallop *Pecten maximus*, the enzyme α -amylase is present in the digestive complex of the digestive gland where intra and extracellular processes occur. Its purification from the digestive gland has been performed using an affinity chromatography on modified starch (Minamiura et al, 1975). Two isoforms were present and the molecular weight was estimated at 60 000 on SDS gel electrophoresis.

In order to have information on its structure and regulation, a digestive gland cDNA library, constructed in lambda phage Zap II (Stratagene) was screened with a crustacean α -amylase cDNA probe (Van Wormhoudt and Sellos, 1996). Only 0.02% of the clones were positive and the longest clone, having a size of 1 700 bp and identical to that of the mRNA, was fully sequenced. It contains the complete cDNA coding frame for one of the amylase isoforms of *P maximus*. The deduced protein sequence is 508 amino-acids long, including the initial methionine, and corresponds to an 18 amino-acid highly hydrophobic signal peptide and a mature enzyme of 489 residues. The calculated molecular weight corresponds to 54 500 Da and the pHi to 6.76.

The consensus regions that characterize the α -amylase catalytic domains and the three amino-acids belonging to the active site are found in *Pecten*, confirming the high level of similarity with other amylases. A same amino-acid identity was determined with arthropod (57% homology with *Penaesus*, a marine crustacean, 55% homology with *Drosophila*) and vertebrate (60% homology with human) amylase sequences. The cDNA will be used to study mRNA expression in relation to nutritional and endocrine regulation.

Data obtained by both methods are in agreement. N-terminus serine, deduced from the nucleic sequence may be acetylated, preventing Edman degradation and direct knowledge of amino-acid sequence and explaining the lower molecular weight of

the molecule estimated from the nucleotide sequence.

References: Minamiura N, Kimura Y, Tsujino K, Yamamoto T (1975) *J Biochem* 77, 163-169; Van Wormhoudt A, Sellos D (1996) *J Mol Evol*.

Digestive effects of algal dietary fibres in humans. N Bentoumou, C Cherbut (*Centre de recherche en nutrition humaine, Inra, BP 1627, 44316 Nantes cedex 03, France*).

Dietary seaweeds are rich in fibre which could affect stool output and intestinal transit time in humans. We compared the digestive effects of three fibres isolated from seaweeds: xylans from *Palmaria palmata* (PP, 10 g/day), carragenans from *Eucheuma cottonii* (EC, 10 g/day), and alginates from *Laminaria digitata* (LD, 6 g/day) to those of cellulose (Cel, 10 or 6 g/day) in 18 healthy subjects. The study was designed in two experimental periods (21 days), separated by 15-day wash-out. During each period, the subjects consumed a basal diet, containing less than 10 g/day of dietary fibre, added with cellulose or algal fibres in a balanced randomised order. All the stools excreted during each period were collected then analysed (dry matter, water content, short chain fatty acids: SCFA). The orofaecal transit time (OFTT) was measured using radio-opaque markers. The data (mean \pm SE) were compared by a Student's *t* test for paired data.

EC doubled faecal wet weight (EC vs Cel: 293.7 \pm 24.6 g/day vs 152.1 \pm 19.1 g/day, $P = 0.0002$) by increasing water output (277.9 \pm 21.9 g/day vs 104.7 \pm 13.0 g/day, $P = 0.0006$). This effect is likely to be related to the high water-binding capacity of EC which resisted fermentation as suggested by the high value of stool pH (7.23 \pm 0.11 vs 6.47 \pm 0.15, $P < 0.05$) and the low faecal SCFA output rate (134.8 \pm 30.2 mmol/day vs 164.5 \pm 26.6 mmol/day, $P <$