Effects of dietary proteins on some pancreatic mRNAs encoding digestive enzymes in the pig

Evelyne F. Lhoste,* Michèle Fiszlewicz,* Anne-Marie Gueugneau,* Catherine Wicker-Planquart,† Antoine Puigserver,† and Tristan Corring*

**Laboratoire d'Ecologie et de Physiologie du Système Digestif, INRA, Domaine de Vilvert, Jouy-en-Josas France; and †Centre de Biochimie et de Biologie Moléculaire du CNRS, 31, Chemin Joseph-Aiguier, Marseille Cédex 9, France.*

The response of pancreatic protease synthesis to fish meal-enriched diets was investigated in the pig, which is generally held to be a suitable model for human digestive physiology. In three sets of experiments, pigs were fed either 7, 17, 48, or 68% protein diets for 8 days (1st set), or 17 or 48% protein diets for 3, 6, or 7 days (2nd set), or 7 or 48% protein diets for 4 days (3rd set). At the end of each experiment, the pancreata were removed for biochemical and gene expression assays. The specific activities of amylase, lipase, chymotrypsin, trypsin, and elastase decreased as the result of the 7% protein diet after an 8-day feeding period (1st set). In the same experiment, all the enzyme-specific activities (those of amylase, lipase, chymotrypsin, elastase as well as carboxypeptidases A and B) increased in response to the 48% protein diet, the most strongly affected enzyme being chymotrypsin. Only chymotrypsin and carboxypeptidase A-specific activities were further increased after feeding with the 68% protein diet for 8 days as compared with the 48% protein diet. The amylase, lipase, and trypsinogen mRNA levels remained unchanged throughout the experiments, but the mRNA encoding procarboxypeptidase A2 decreased, and that coding for chymotrypsinogen was enhanced after the animals had been fed the experimental diets for 3 days, but showed no change thereafter. Procarboxypeptidase B mRNA increased slightly only after a 6-day feeding period. When pigs were fed the 7 and 48% protein diets for 4 days, the enzymes synthesized in vitro *in pancreatic lobules were correlated with the relative levels of the corresponding mRNAs, as measured by means of an* in vitro *cell-free reticulocyte-lysate translation system: both amylase and carboxypeptidases specific activities and their mRNA levels decreased slightly, while those of serine proteases increased. It was concluded that the biosynthesis of each serine protease was regulated separately and transiently at the pre-translational level. On the other hand, it seems very likely that amylase may be regulated at the translational level, while a multiple-level control process may take place in the case of procarboxypeptidase A2.*

Keywords: pig; pancreas; gene expression; adaptation; dietary protein.

Introduction

Since the famous report by Pavlov[1] in the late 19th century, the adaptation of the exocrine pancreas to dietary changes in various species including the rat, chicken, dog, pig, and cow has been extensively studied.[2,3] A consensus has been reached that pancreatic protease and amylase secretion as well as their tissue contents are directly proportional to the amount of their respective dietary protein and carbohydrate substrates. Lipase secretion and activity depend on dietary lipid, but their increase is not proportional to the amount of this substrate. In the rat, the genetic mechanisms that mediate these changes have been investigated over the past few years.[4–10] By contrast, the information available about the molecular basis of pan-

This work was partly supported by research grant A.I.P. No. 89/4667 "Métabolisme des Protéines" from the Institut National de la Recherche Agronomique.
Address reprint requests to Dr. Evelyne F. Lhoste at the Laboratoire d'Ecologie et de Physiologie du Systeme Digestif, INRA, Domaine de Vilvert, F-78350 Jouy-en-Josas, France.
Received January 17, 1992; accepted October 9, 1992.

creatic adaptation in other species is rather scarce. On that account, it was first necessary to definitely establish that an increased protein intake leads to increased rates of proteolytic enzyme synthesis in the pig pancreas before elucidating the underlying mechanisms. Obviously, studies at molecular level on the response of pancreatic enzymes to nutritional substrates provided by the diet have not been numerous enough to be able to predict the mechanism involved in the regulation of a given enzyme under specific nutritional conditions in any animal species. Because pancreatic adaptation is particularly likely to involve different mechanisms in rodents and humans, we focused in particular on the pig, which is generally held to be a suitable model for studying both human nutrition and gastrointestinal function in view of its physiological characteristics.[11,12] The fact that the authors of previous studies have pointed out a number of discrepancies in the response of the pancreatic gland to changes in the composition of the diet between humans and rats[13–15] further encouraged us to carry out molecular studies on the pig.

The level of chymotrypsin activity in pig pancreatic juice has been found to be sensitive to the intake of dietary protein, whereas that of trypsin is not.[16] Moreover, preliminary experiments have suggested that, although pancreatic secretion and hydrolase tissue content are maximally increased after feeding high protein diet for 1 week, proteolytic enzyme synthesis was not modulated according to the same pattern. In the present study, the specific effects of dietary proteins on the other serine protease (elastase) and the two metalloproteases (carboxypeptidases A and B) were investigated by determining the responses of these pancreatic enzyme activities and those of their specific mRNAs to changes in the dietary protein levels in the pig.

Materials and methods

Diets and animal care

Seventy-two growing castrated male Large White pigs weighing 40.5 ± 0.5 kg, were housed in individual cages. The conventional diets were gradually replaced by experimental diets over a 3-day period before the experiment was started on day 0. The diets (see composition in *Table 1*) contained 7, 17, 48, and 68% protein in the form of a fish concentrate and were isocaloric at the expense of carbohydrate. The 17% protein diet was a standard growing pig diet that was used as the control diet. Food was given at 9 A.M. and 4 P.M., in two meals each consisting of 800 g dry food diluted in water (wt/vol: 1/2).

In the first experiment, 36 pigs were divided in four groups (n = 8–10) and were slaughtered after they had been fed a given experimental diet (7, 17, 48, or 68% protein) for 8 days. Protein, DNA, RNA, and enzyme activities were assayed. Gene expression was determined by performing dot-blot hybridization with specific cDNA probes using six pigs per group unless otherwise indicated in the results section. In the second experiment, 16 pigs were divided into two groups (n = 8) and were fed either the 17% or the 48% protein diet for 3, 6, or 7 days (n = 4) before slaughter.

Table 1 Composition of diets (% dry weight)

Fish concentrate[a]	8	20	56	80
Olive oil	9.2	7.2	0.8	0.2
Starch	55.2	50.4	27.7	10.0
Glucose	2.8	2.4	1.3	0.6
Cellulose	16.6	14.4	7.9	3.6
Vermiculite	4.6	2.4	4.4	4.6
Minerals[b]	2.7	2.4	1.3	0.6
Vitamins[b]	0.9	0.8	0.4	0.2
Water	0.4	3.0	2.3	4.4
Crude energy (J/ 100 g dry weight)[c]	418	428	418	418
Calories provided as:				
protein	7	17	48	68
lipid	19	18	11	12
carbohydrate	72	66	38	15

[a]Fish concentrate (CPSP 90) containing approximately 85% protein, 7% lipid, and 5% minerals, which were taken into account for balancing the diets.
[b]Minerals and vitamins complexes were as indicated in reference 17.
[c]Obtained by calculation.

Enzyme activities and gene expression were determined. In the third experiment, 12 pigs were divided into two groups (n = 6), one of which was fed on the 7% and the other on the 48% protein diet for 4 days before slaughter. Both enzyme activity and biosynthesis were determined and gene expression was assayed by carrying out mRNA translation assays in a cell-free reticulocyte-lysate system.

At the end of each experiment, pigs were sacrificed by exsanguination under light anesthesia. The pancreas was then carefully dissected and weighed. Six 1.5-g pieces were rapidly excised from the various parts of the gland and prepared for mRNA extraction. In the third experiment, *in vitro* protein biosynthesis was performed immediately. The remaining pancreatic tissue was stored at −70° C for further biochemical assays.

DNA, RNA, protein, and enzyme assays

After homogenizing each pancreas in ice-cold distilled water (1 g / 7 mL), its protein content was determined as described by Lowry et al.[17] using porcine serum albumin fraction V (Sigma, St. Louis, MO, USA) as a standard. DNA and RNA were extracted and assayed as previously described[18] using torula yeast RNA and salmon sperm DNA as standards. The amylase activity was measured using the procedure of Métais and Bieth[19] as modified by Corring and Saucier.[16] Lipase activity was assayed titrimetrically at pH 9 in the presence of bile salts and colipase.[20] Trypsin and chymotrypsin activities were assayed according to Reboud et al.[21] after 24-hr tryptic activation in a Tris-(hydroxymethyl)-aminomethane (Tris-Cl) buffer containing 5 mmol/L Tris, 40 mmol/L NaCl, 20 mmol/L $CaCl_2$ at pH 7.9 and 4° C (trypsin), and 2-hr activation in a 0.2 M phosphate buffer at pH 7.6 and 4° C (chymotrypsin). Carboxypeptidase A and B activities were determined as described by Yamasaki et al.[22] and Folk et al.,[23] respectively after tryptic activation at 37° C for 2 hr in either 5 mmol/L Tris-Cl, 40 mmol/L NaCl, 20 mmol/L $CaCl_2$, pH 7.9 for carboxypeptidase A, and 25 mmol/L Tris-Cl, 100 mmol/L NaCl, pH 7.9 for carboxypeptidase B. The elastase activity was assayed using the method of Gertler and Hofman[24] after tryptic activation (trypsin 1 mg/ml HCl 1 mmol/L) in 5 mmol/L Tris-Cl, 40 mmol/L NaCl, pH 7.9.

mRNA extraction

Total RNA was prepared using the procedure of Chirgwin et al.[25] with slight modifications. Immediately after excision, 1.5-g pancreas pieces were homogenized on ice with a Polytron (15 sec, medium speed) in 12 mL of a buffer containing 5 M guanidinium Tihiocyanate (Fluka AG, Basel, Switzerland), 50 mmol/L Tris-Cl (pH 7.5), 25 mmol/L EDTA (ethylenediamine tetraacetic acid, sodium salt), and 0.1 M 2-mercaptoethanol. After 10 min of centrifugation at 14° C (8,500 *g*, Sorval SS-34 rotor) to eliminate the cell debris, the supernatants were precipitated in 0.75 vol. ethanol and 25 mmol/L acetic acid at −70° C for at least one night. After an additional 10-min centrifugation at −10° C (5,000 *g*, Sorval SS-34 rotor), the pellet was resuspended in 0.5 volume 7.5 M guanidinium hydrochloride (Fluka AG), 200 mmol/L EDTA, pH 7.5, 5 mmol/L dithiothreitol. RNA was reprecipitated in ethanol as described above. This dissolution-precipitation step was repeated in a two-fold reduced volume of buffer. The resulting pellet was washed with ethanol to remove any remaining guanidinium hydrochloride, and finally dissolved in water (1 mL). To eliminate any remaining contaminant such as polysaccharides, RNA was precipitated with 1.25% trichloroacetic acid (TCA) on ice, and the pellet was successively washed with 66% ethanol, 150 mmol/L NaCl and 100% ethanol before being diluted in Tris EDTA buffer at pH 8. Total RNA was quantified spectrophotometrically at 260 nm. With each pancreas, aliquots from the six extracts were pooled, reprecipitated in 66% ethanol, 0.2 M potassium acetate, pH 5.0, and stored at −70° C until assayed.

Qualitative analysis of the total RNA

Quantitative analysis was routinely performed using 1.2% agarose gel electrophoresis (Northern-blot).[26] Total RNA samples (20 μg) and a RNA ladder (Boehringer-Mannheim, Mannheim, Germany) were denatured prior to electrophoresis on a 1.2% agarose gel for 4 hours at 80 V. In some cases, the gel was treated and the RNA transferred onto nitrocellulose (Schleicher und Schüell, Dassel, Germany) as described by the manufacturer. Nitrocellulose filters were baked for 30 minutes at 80° C.

Quantitative analysis of mRNA

In a few cases, individual mRNAs were quantified by their biological activity after translation of total RNA extracts in a rabbit reticulocyte lysate system in the presence of ^{35}S-methionine (100–400 μCi/mL).[6] In these cases, translated proteins were separated by a one-dimension sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12–17% polyacrylamide gel). Quantification of translation products was similar to that of radiolabeled secretory proteins from pancreatic lobules (see below). In most cases, however, quantification of individual mRNA was achieved by hybridization to specific cDNA probes (dot-blot) as described hereafter. mRNA or poly-A$^+$ mRNA samples were denatured in 7.4% formaldehyde, 10 × standard sodium phosphate EDTA (SSPE) by heating at 65° C for 15 minutes and dotted onto nitrocellulose in sequential dilutions (0.5–6 μg for mRNA and 0.5–2.5 μg for poly-A$^+$ mRNA) using a manifold apparatus (Minifold I, Schleicher und Schüell). The filters were then air dried and baked at 80° C for 30 minutes.

Hybridization of filters

Filter hybridization was carried out with cDNA probes labeled with (α-^{32}P)-dCTP (Amersham, Les Ulis, France) using a random primer kit (Boehringer-Mannheim). The specific activity of the probes was routinely set at 10^9 cpm/μg after an overnight incubation at room temperature. The filters were prehybridized for 4 hours at 42° C in 10 mL hybridization buffer (5 × SSPE, 50% formamide, 5 × Denhardt's reagent, 0.1% sodium dodecylsulfate (SDS), 100 μg/mL denatured salmon sperm DNA).[26] Hybridization was carried out at 42° C for 20 hours in the above buffer containing the denatured randomly primed probe (5 × 10^6 cpm/mL). After hybridization, the filters were washed in 2 × Standard Sodium Citrate (SSC), 0.5% SDS at 55° C for 3 × 45 minutes and then in 0.1 × SSC, 0.1% SDS at 55° C for another 45 minutes.

Filters were blotted dry and exposed to X-ray film. After film development, the filter-bound radioactivity was determined by Cerenkov counting in a LKB scintillation spectrometer. For each sample, slope of the dot intensity of each dilution was calculated on the linear portion of the curve by linear regression.

cDNA library construction and probe cloning

The total porcine RNA was prepared as described above (omitting the TCA precipitation step) and poly-A+ mRNA was subsequently isolated by chromatography on oligo(dT)-cellulose (Pharmacia-France, Guyancourt, France).[27] Double-stranded cDNAs were synthesized using a cDNA synthesis kit (Amersham, Les Ulis, France), and inserted into the PstI site of plasmid puC9 after G-C tailing. *Escherichia coli* JM 83 was then transformed by the recombinant plasmid mixture.[26] Screening of insert-containing colonies (2 × 10^3) was performed by hybridization with rat pancreatic cDNA probes previously ^{32}P-labeled using the nick-translation technique.[28] Identification of the clones was further checked by performing Southern hybridization[26] after agarose electrophoresis of PstI-digested plasmids and by partial sequencing using the Sequenase system (United States Biochemical Corp., Cleveland, OH USA). Rat amylase (1400 bp), lipase (930 bp), chymotrypsinogen (600 bp), trypsinogen (745 bp), and procarboxypeptidase A1 (1000 bp) probes were kindly provided by C. Wicker-Planquart (CNRS, Marseille, France). Rat procarboxypeptidase B (pcXp 48: 600 bp) was a gift from R. MacDonald.[29] The rat beta-actin clone was a 700 bp sequence of the 3′ end of the messenger. The lengths of the isolated porcine probes were as follows: a 700-bp lipase cDNA sequence, a 650-bp chymotrypsinogen cDNA sequence, a 600-bp trypsinogen cDNA sequence, a 750-bp procarboxypeptidase A2 cDNA sequence, and a 600-bp procarboxypeptidase B cDNA sequence. All of these sequences contained the 3′ end of the messengers. The amylase probe used in the porcine RNA hybridization assays was from a rat pancreas cDNA library.

In vitro protein synthesis using pancreatic lobules

Pancreatic lobules were prepared according to Scheele and Palade.[30] They were incubated in 1 mL Krebs-Ringer solution containing 3.7 MBq/mL ^{35}S-methionine at 37° C for 30 minutes. Incorporation of labeled amino acid has been checked to be linear for at least 2 hours when using rat lobules (data not shown). Following incubation, lobules were homogenized in 0.5 mL 0.1 M $NaHCO_3$. Pancreatic proteins (about 200,000 cpm) were then separated by one-dimension SDS-PAGE (12–17% polyacrylamide gel electrophoresis). Identification of pancreatic protein bands, as separated by one-dimension gel electrophoresis, has been achieved by Scheele[31] using antibodies against each protein. One-dimension gel electrophoresis clearly separates amylase, lipase, the group of procarboxypeptidases, and the group of serine proteases

(elastases, chymotrypsins, trypsins). Secretory proteins, which have been shown to represent more than 95% of total pancreatic proteins,[31] were quantitated by measuring radioactivity in each group of enzymes. Prior to counting in 10 mL scintillation liquid cocktail, each protein band was sliced and solubilized in 100 μL 30% hydrogen peroxide with overnight incubation at 60° C.

Statistical analysis

An analysis of variance followed by a Duncan test (multiple groups, i.e., first and second experiment) or a Bonferroni test (two groups, i.e., third experiment) was used on all biochemical parameters assayed.

A Mann-Whitney U test was used to analyze data from protein biosynthesis, mRNA translation, and dot-blot.

Results

Effects of changes in dietary composition on pig growth, pancreatic weight and total protein, RNA, and DNA contents

Pigs with the same starting body weight (40.5 ± 0.5 Kg) were used throughout this study, and all the diets were well accepted by the animals. Data from the first experiment are reported in *Table 2*. The other two experiments gave comparable results (data not shown). The weight gains observed were slightly, but significantly, smaller in the 7% protein-diet group than in the others ($P < 0.05$). The pancreas weight and its total protein, DNA, and RNA contents were therefore expressed as a function of body weight. The total weight of the pancreatic gland slightly decreased in animals fed the 7% protein diet but increased when feeding the 48% and 68% protein diets, as compared with the control (17%) diet. These effects were still more pronounced when expressed in terms of the total protein content of the pancreas (1.4-fold increase after feeding the 48% protein diet, $P < 0.01$, as compared with the control diet).The total RNA was slightly, but not significantly changed, while the total DNA decreased as much as 60% after feeding the 48% and 68% protein diets ($P < 0.05$ as compared with the 7% protein diet).

The total DNA, which reflects cell number, as well as the pancreatic weight:DNA, protein:DNA, and RNA:DNA ratios reflecting cell size, were expressed as a function of the values obtained with 17% protein-fed pigs. However, statistical analysis was performed on nonnormalized data. In addition, total DNA was expressed as a function of body weight. The decrease in the total DNA (mg/100 g BW) observed in the high-protein groups as compared with the control group (0.4-fold, $P < 0.05$), indicated that the total cell number decreased, because the amount of DNA in a cell is constant. In none of these parameters was any difference noted between the 48% and 68% protein diets. Administration of the 7% protein diet induced a decrease in cell size (hypotrophy), while high-protein diets induced cellular hypertrophy. This effect was less pronounced in the case of the 68% protein diet than in the 48% protein diet because the DNA levels were slightly higher in the latter than in the former group. In conclusion, the small increase in pancreatic size observed with high-protein diets may have been due to cellular hypertrophy (high protein content) along with some decrease in the DNA content (reflecting a decrease in cell number).

Effects of changes in dietary composition on the exocrine pancreatic enzyme levels

The specific activities (U/g protein) of seven pancreatic hydrolases derived from the first experiment are shown in *Figure 1*. The amylase specific activity decreased by 30% in pigs fed the 7% protein diet as compared with controls ($P < 0.05$). The lipase and, to a minor extent, serine protease specific activities also decreased (although not significantly) while the two carboxypeptidases A and B were not affected by the 7% protein diet. Amylase level increased moderately as the result of the 48% protein diet (1.2-fold, not significant) but

Table 2 Effect of feeding experimental diets containing 7, 17, 48, or 68% protein for 8 days on body weight gain, pancreatic weight, total protein, DNA and RNA contents, and parameters reflecting cell size and cell number

Diet (% protein)	7	17	48	68	*P* value
Body weight gain	1.08 ± 0.01^{a}	1.13 ± 0.02^{b}	1.16 ± 0.01^{b}	1.14 ± 0.01^{b}	0.05
Pancreatic weight (g)	45 ± 5	53 ± 7	62 ± 7	63 ± 6	N.S.
PW/BW (g/kg)	9.9 ± 1.0	11.3 ± 1.3	13.1 ± 1.5	13.0 ± 1.3	N.S.
Protein/BW (mg/kg)	180 ± 20^{a}	209 ± 26^{a}	297 ± 32^{b}	285 ± 28^{b}	0.01
RNA/BW (mg/kg)	17.5 ± 2.3	21.2 ± 3.1	23.3 ± 2.9	23.4 ± 2.5	N.S.
DNA/BW (μg/kg)	633 ± 118^{a}	550 ± 132^{a}	238 ± 69^{b}	253 ± 62^{b}	0.05
DNA	87^{a}	100^{ab}	43^{b}	46^{b}	0.05
PW/DNA	68^{a}	100^{a}	412^{b}	347^{b}	0.05
Protein/DNA	62^{a}	100^{ab}	343^{bc}	275^{c}	0.05
RNA/DNA	55^{a}	100^{ab}	241^{b}	220^{b}	0.05

PW, pancreatic weight; BW, body weight.
Body weight gain is the ratio: (BW0 − BWS) × 100/BW0.
BW0, body weight on day 0; BWS, body weight on slaughter day.
DNA, PW/DNA, Protein/DNA, and RNA/DNA ratios were calculated relative to control (17% protein).
Groups not sharing a common superscript are significantly different at the *P* value indicated.
N.S., not significant.

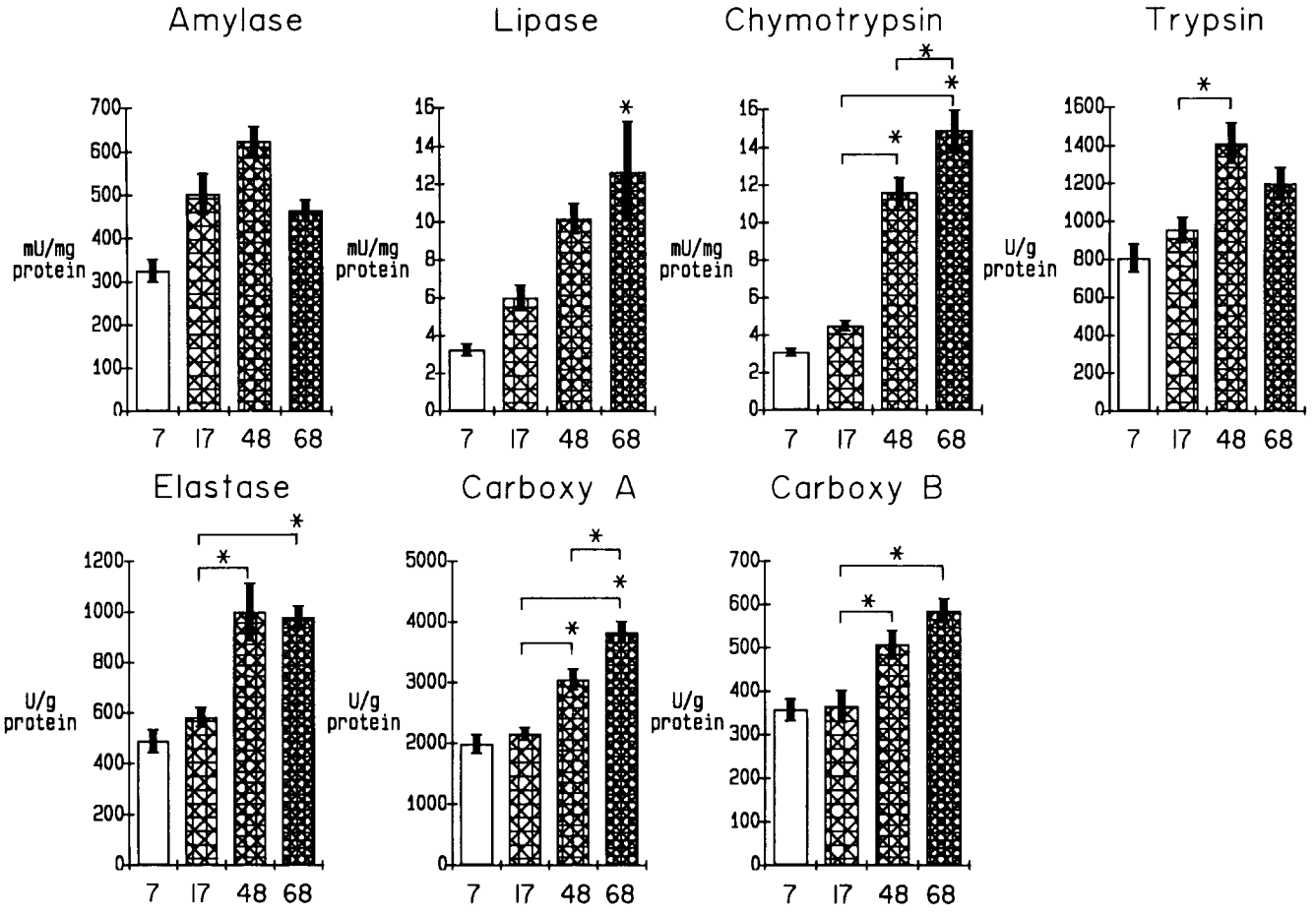

Figure 1 Changes in the specific activities of some pancreatic hydrolases in pigs fed experimental diets containing 7, 18, 48, or 68% protein for 8 days. Results are means ± S.E.M. of six animals. The *P* values are <0.05 (*) and <0.01 (**). Comparisons are indicated by horizontal bars. Carboxy, carboxypeptidase.

Table 3 Effect of feeding experimental diets containing 7, 48, and 68% protein for 8 days on total enzyme activities relative to the control diet (17% protein).

Diet (% protein)	7	17	48	68	*P* value
Amylase	51^{a}	100^{ab}	80^{c}	26^{bc}	0.01
Lipase	48^{a}	100^{a}	52^{b}	76^{b}	0.01
Chymotrypsin	56^{a}	100^{a}	368^{b}	457^{b}	0.01
Trypsin	68^{a}	100^{ab}	208^{c}	173^{bc}	0.01
	a	b	c	c	0.05
Elastase	67^{a}	100^{a}	247^{b}	225^{b}	0.01
Carb. A	79^{a}	100^{a}	203^{b}	246^{b}	0.01
Carb. B	45^{a}	100^{ab}	110^{bc}	122^{c}	0.05

Carb., Carboxypeptidase.
Groups not sharing a common superscript are significantly different at the *P* value indicated.

showed no increase in the animals fed the 68% protein diet. Lipase specific activity increased progressively in the groups fed 48% and 68% protein diets (1.7- and 2.1-fold, respectively, $P < 0.05$). The specific activities of the serine proteases increased in the case of the 48% protein diet, chymotrypsin being the most noticeably affected (2.6-fold, $P < 0.05$ as compared with control; while 1.7-fold for elastase, $P < 0.05$; 1.5-fold for trypsin, $P < 0.05$; and 1.4-fold for carboxypeptidases A and B, $P < 0.05$). The specific activities of chymotrypsin and carboxypeptidases further increased as the result of the 68% protein diet ($P < 0.05$ with chymotrypsin and carboxypeptidase A as compared with the 48% protein diet), but those of trypsin and elastase showed no change, while that of amylase remained below the control value. When total pancreatic enzyme activity is considered *(Table 3)*, the patterns of enzyme activity were not very greatly modified,

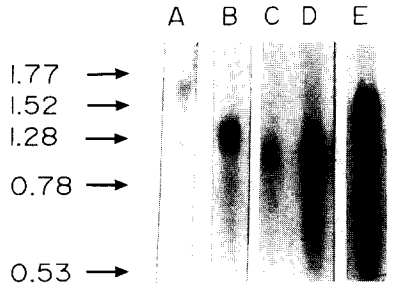

Figure 2 Northern blot of pancreatic RNA samples. After electrophoresis and transfer onto nitrocellulose, denartured mRNA samples were hybridized to amylase (A), lipase (B), chymotrypsinogen (C), trypsinogen (D), and procarboxypeptidase B (E) cDNAs. Radioactivity was detected by autoradiography for 1 night (amylase, chymotrypsinogen, trypsinogen) or 2 days (lipase, procarboxypeptidase B). Standard molecular weight markers are indicated in kb by arrows.

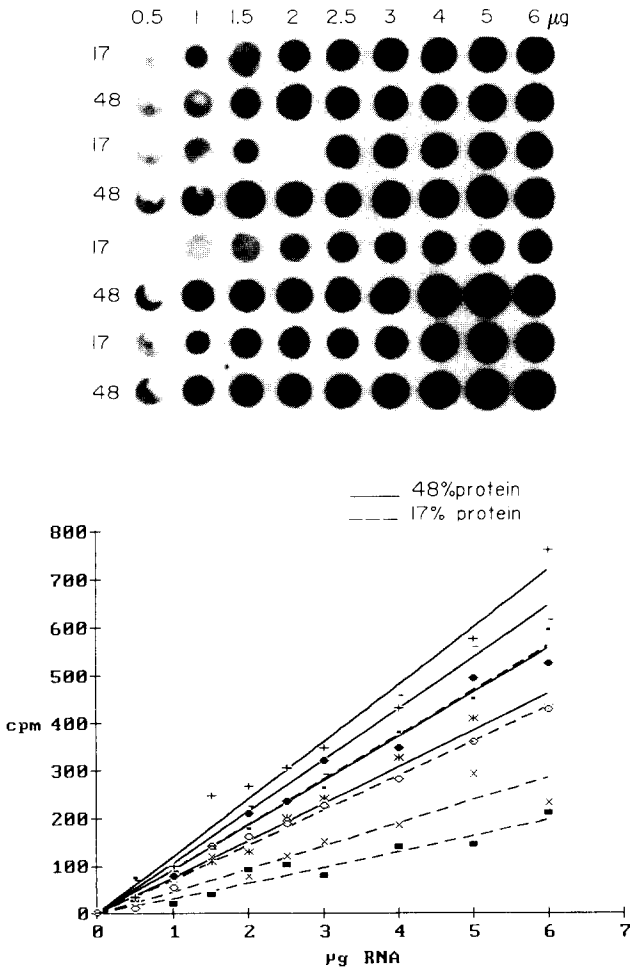

Figure 3 Autoradiography (top) and typical hybridization curves (bottom) obtained from dot-blot analyses of increasing amounts of pancreatic mRNA from pigs fed 17 or 48% protein diet for 3 days. For each pig, 0.5–6 µg of pancreatic mRNA was dotted onto nitrocellulose and hybridized to a chymotrypsinogen probe. After autoradiography, dots were punched out and radioactivity measured by Cerenkov counting. Lines were obtained by linear regression for each pig.

although quite striking differences could be observed between the groups. The same conclusion was drawn from enzyme activities per unit DNA (not represented). In the second and third experiments, a similar pattern of enzyme activity was observed, although both experiments were carried out over a shorter period than the first one (data not shown).

Effects of changes in dietary composition on the exocrine pancreatic protein mRNA levels

The hybridization specificity of the mRNAs with the corresponding cDNA probes was first assessed by performing Northern-blot analysis. The electrophoretically separated mRNAs were identified as single bands, the migration of which corresponded to those of amylase, lipase, chymotrypsinogen, trypsinogen, and procarboxypeptidase B *(Figure 2)*.

The specific mRNAs were quantified by carrying out dot-blot hybridization on all the pancreatic mRNA samples with the specific cDNA probes. One-half to 6 µg of mRNA from individual pigs were dotted onto nitrocellulose, hybridized to specific probes, and autoradiographed. Thereafter, the dots were cut out and counted using Cerenkov's method. *Figure 3* shows a representative audiograph of dot-blots hybridized with chymotrypsinogen cDNA and the corresponding regression lines. Correlation coefficient was regularly >0.97. For each pig, the radioactivity relative to amount of total mRNA (cpm/µg) was deduced from these lines and represents the amount of chymotrypsinogen relative to total mRNA.

In some cases, actin cDNA was hybridized to dot-blots as control. *Figure 4* shows autoradiographs of poly-A$^+$ mRNAs (0.5–2.5 µg) dotted onto nitrocellulose and hybridized to chymotrypsinogen and actin. Actin mRNA was not modified by diets, while chymotrypsinogen was increased after feeding pigs with 48% protein diet for 3 days. These data are consistent with those obtained with mRNA.

The effect of dietary protein on pancreatic mRNA expression is illustrated in *Tables 4 and 5*. In the first experiment *(Table 4)*, we studied the gene expression of pancreatic enzymes after an 8-day feeding period

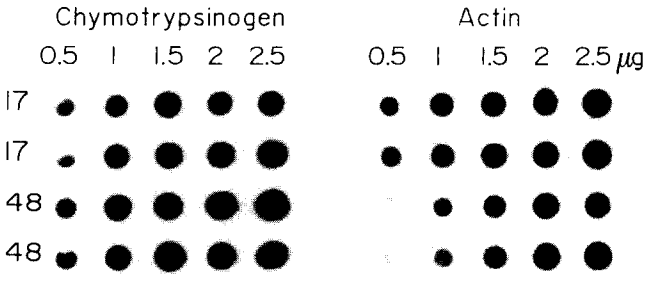

Figure 4 Autoradiography obtained from dot-blot analyses of increasing amounts of pancreatic poly-A$^+$mRNA from four pigs fed with 17 or 48% protein diet for 3 days. For each pig, 0.5–2.5 µg of poly-A$^+$ mRNA was dotted onto nitrocellulose and hybridized to actin or chymotrypsinogen probes.

Table 4 Effect of feeding experimental diets containing 7, 48, and 68% protein for 8 days on the mRNA levels expressed as a function of control diet (17% protein = 100)

Diet (% protein)	7	48	68
Chymotrypsinogen	95±9	123±16	129±15
Procarboxypeptidase B	112±30	169±64	171±71
Procarboxypeptidase A2	158±21	65±12	116±18

Table 5 Effect of feeding experimental diets containing 17 and 48% protein for 3 or 6 days on mRNA levels (cpm/μg)

Diet (% protein)	17	48	*P* value
3 days			
Amylase	52±9	55±11	N.S.
Lipase	14±2	19±4	N.S.
Trypsinogen	124±20	131±8	N.S.
Chymotrypsinogen	59±9	94±9	0.05
Procarboxypeptidase B	56±7	44±4	N.S.
Procarboxypeptidase A2	16±3	9±1	0.05
6 days			
Trypsinogen	76±7	49±9	N.S.
Chymotrypsinogen	87±11	93±16	N.S.
Procarboxypeptidase B	163±29	295±56	N.S.

with diets containing 7, 17, 48, or 68% protein. No significant difference in the levels of the specific mRNAs was observed among the four groups. In the second experiment, the gene expression of pancreatic enzymes was studied after a feeding period of 3, 6, or 7 days with diets containing 17 or 48% protein. Data for 3 and 6 days of treatment are represented in *Table 5*. When the 48% protein diet had been administered for 3 days, the amylase, lipase, trypsinogen, and procarboxypeptidase B mRNA levels remained unchanged, whereas chymotrypsinogen mRNA level increased ($P < 0.05$) and that of procarboxypeptidase A2 mRNA decreased significantly (40%, $P < 0.05$) as compared with 17% protein diet. Likewise, when the 17% and 48% protein diets had been fed for 6 or 7 days, all mRNA levels showed practically no change. The levels of mRNA after 7 days of treatment were equivalent to those after 8 days and were not represented.

Effects of changes in dietary composition on the levels of exocrine pancreatic proteins synthesized in vivo and in vitro

The protein biosynthesis levels in pancreatic lobules and the *in vitro* mRNA translation levels after a 4-day feeding period with 7% and 48% protein diets are shown in *Table 6* (third experiment). The amylase biosynthesis decreased moderately (20%, $P < 0.01$) in animals fed the 48% protein diet versus the 7% protein diet. The serine protease biosynthesis level increased (1.2-fold, $P < 0.05$), while that of procarboxypeptidases decreased (0.8-fold, $P < 0.01$) and that of lipase remained unchanged. *In vitro* translation assays yielded similar amylase and serine protease patterns, whereas the procarboxypeptidase translation products were significantly enhanced (1.4-fold, $P < 0.01$).

Discussion

In the present study we investigated the levels of accumulation of some specific proteins in pig pancreatic tissue under low and high conditions of dietary protein intake. We also examined the effects of in vitro enzyme biosynthesis after a 4-day feeding period with diets containing 7% and 48% protein, and measured the mRNA levels specific to these enzymes, in pigs fed diets containing 17% and 48% protein for 3, 6, and 7 days, on the one hand, and diets containing 7, 17, 48, and 68% protein for 8 days, on the other hand.

After an 8-day dietary intake, the body weight gain was slightly, but significantly, lower in the groups fed low protein diets and higher in those fed the high protein diets. This finding is consistent with previous data.[16] A low protein diet induced a slight decrease in the pancreatic weight, due to cell atrophy as shown by the decrease in total protein content, whereas high protein diets induced an increase in pancreatic weight due to cell hypertrophy and a decrease in the cell number as indicated by the enhanced protein content and decreased DNA content, respectively. This effect was more discrete than those previously observed in rats, where 30–80% increases in pancreatic mass were measured in response to a high protein diet for only a few days.[32,33] The same authors showed that high dietary protein diets induce pancreatic hypertrophy and hyperplasia in the rat. Moreover, Imondi and Bird[34] demonstrated that in the chick both proteolytic and mitotic activities increased as the result of high protein diets. Unfortunately, these authors did not measure the DNA content, which may have decreased due to extensive cell death. The phenomenon of pancreatic growth as an adaptive response seems to be peculiar to smaller species. Although very recently Buchler et

Table 6 Effect of feeding 7% and 48% protein diets for 6 days on the relative protein biosynthesis levels in lobules and translational activity of the corresponding mRNAs (expressed as the percentage of total incorporation of ^{35}S-methionine)

Diet (% protein)	7	48
Protein biosynthesis		
Amylase	37.5±0.5	31.2±1.7*
Serine proteases	26.1±1.4	31.1±1.8†
Procarboxypeptidases	20.3±0.5	16.1±1.5†
Lipase	8.6±0.9	9.6±1.1 (N.S.)
mRNA assayed by *in vitro* translation		
Amylase	33.2±2	26±3*
Serine proteases	20.7±1.4	22.8±1.1 (N.S.)
Procarboxypeptidases	25.4±3.3	34.8±2.3*

*$P < 0.01$.
†$P < 0.001$.
N.S., not significant.

al.[35] described an increase in pancreatic size in humans, it has not yet been established whether this effect was due to pancreatic growth or cell hypertrophy. Further information, such as the thymidine incorporation and cell death data, is certainly required before we can fully interpret the present findings on DNA content.

The main purpose of this study was to determine the molecular mechanisms underlying pancreatic adaptation to protein-rich diets in the pig. First we measured enzyme specific activities and total tissue contents. The enzyme contents and specific activities did not specifically decrease in response to a low protein diet and increased as the result of high protein diets. This is consistent with previous data on pig pancreatic secretion after several days of feeding high protein diets.[16] However, the lipase and serine protease specific activities increased more noticeably than those of other enzymes. In the rat, previous authors have reported that high protein diets mainly increased the protease (chymotrypsin, trypsin, elastase, and carboxypeptidase B but not carboxypeptidase A) activities and only slightly affected the lipase activity.[7,21,34,36–41] In the present study we demonstrated that serine proteases and carboxypeptidase A (but not carboxypeptidase B) increased in response to fish meal-enriched diets in pigs.

It is not yet clear whether the above discrepancies are due to differences between the species investigated or to differences in the composition and duration of the diets. In the present study we increased the dietary protein content at the expense of carbohydrate, while the lipid content was stable in quantity but not in quality because corn oil was gradually replaced by fish fat. In rats most authors have used casein as the protein source and have suggested that high quality proteins such as casein or fish, unlike low quality proteins such as zein or gluten, increase the chymotrypsin levels.[32,42] In rats, we recently observed that the effects of a high protein diet on the pancreatic enzyme activities and their corresponding mRNAs varied depending on the dietary protein used [unpublished data]. We therefore suggest that not only the nutritive value, but also the protein composition and its interaction with other nutritional components (casein is a pure protein as opposed to fish concentrate) may influence the pancreatic adaptation to dietary proteins. Another issue to be addressed is the importance of sex hormones on these differences. For practical purposes, we had to perform all of these experiments on castrated pigs. The effect of steroid hormones on pancreatic function is not known in the pig. However, we demonstrated previously that, in rats, orchiectomy had little effect on pancreatic growth and no effect on enzyme levels while testosterone administration to castrated rats induced an increase in amylase and chymotrypsin contents [Lhoste et al., unpublished].

To establish whether the specific increase in pancreatic protease activity observed after a high protein dietary intake was regulated at the level of gene expression in the pig, we measured the concentrations of the corresponding mRNAs in the pancreas of pigs fed diets containing 17% or 48% protein for 3–7 days, and 7, 17, 48, or 68% protein for 8 days. In pigs fed 7% or 48% protein diets for 4 days, the pancreatic enzyme biosynthesis was quantified by performing in vitro incubation of pancreatic lobules with ^{35}S-methionine, and the mRNA levels were determined by carrying out in vitro translation assays in cell-free reticulocyte lysate systems. When the in vitro translation products of equal amounts of pancreatic RNA from pigs fed 7% or 48% protein diets were separated on sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE), the ^{35}S-methionine incorporation increased with the amount of protein in the diet in the bands corresponding to serine proteases and decreased in those corresponding to amylase. A good correlation was found to exist between the amylase activity, its specific biosynthesis in lobules, and the mRNA translation level in vitro. In the case of the serine proteases, the biosynthesis in lobules (and mRNA translation level) was parallel to the enzyme specific activities although somewhat lower (1.2-fold with serine proteases versus 2.6-fold with chymotrypsin, 1.8-fold with elastase and 1.1-fold with trypsin). It was not possible to determine whether the enzymes chymotrypsin, trypsin, and elastase underwent equivalent changes because of the lack of resolution of the proteases in the biosynthetic studies using one-dimensional SDS-PAGE. Assuming that chymotrypsinogen is the major form of serine proteases in the pig, our data strongly suggested that its regulation is at least partly pre-translational. The control of serine protease synthesis may therefore involve similar pathways in both the pig and rat.[7,43–45] In the rat, it has been demonstrated however that the procarboxypeptidase levels were poorly affected by high dietary protein diets.[6,7] In the pig, the procarboxypeptidase biosynthesis decreased slightly but significantly by 20%, while the in vitro mRNA translation products and enzyme specific activities increased after feeding a diet containing 48% as compared with 7% protein. The differences observed in the enzyme biosynthesis and activities may be attributable to a difference in protein stability between the two groups.

Here we quantified pig pancreatic mRNAs by carrying out hybridization assays with their specific cDNA probes on animals fed the 17% and 48% protein diets for 3 days to differentiate between the mRNAs specific to serine proteases and procarboxypeptidases. An increase was observed in the chymotrypsinogen mRNA but not in the trypsinogen mRNA levels in pigs fed high protein diets. The chymotrypsinogen mRNA increased only 1.6 fold, whereas its specific activity increased 2.6 fold, suggesting that only part of the increase in biosynthesis was due to gene expression. Moreover, the increase in gene expression was only transient. As we know that chymotrypsin secretion is maximally stimulated after 2 days of feeding high protein diet,[16] we must suggest that the increase in chymotrypsin specific activity is regulated at the level of protein biosynthesis. Because the trypsinogen mRNA showed no change after feeding the 48% protein diet for 3 days, its biosynthesis may be controlled at the translational level. It is worth noting, however, that serine

proteases are present in the pancreas in several isoenzymic forms (three trypsinogens, three chymotrypsinogens, and two proelastases).[46] Moreover, upon the pancreatic stimulation resulting from a protein-rich diet[47] or from the decapeptide caerulein,[48] the synthesis of the anionic isoforms of trypsinogen increased while that of the cationic trypsinogen showed no change in the rat. Because it could not be determined whether our probe was anionic or cationic, it was difficult to compare the enzymatic assay data with those on the mRNA levels. The procarboxypeptidase A2 mRNA levels decreased, although the enzyme specific activity (corresponding to procarboxypeptidase A1 and A2 gene expression) was enhanced after feeding the high protein diet for 3 days. Similar effects have already been described in the calf pancreas[49] and may be attributable to a multiple-level regulation involving the simultaneous inhibition of transcription of one gene and stimulation of translation leading to an increase in the enzyme content.

When pigs were fed the high protein diets for 7 or 8 days, mRNA levels remained unchanged, although the protease specific activities were raised. The adaptation was therefore under translational control. The dramatic increase in the enzyme specific activities may have been due to some derepression of inhibited mRNA in the control pigs. This hypothesis might be tested by measuring the translationally active mRNAs (i.e., polysomal RNAs). It is also possible that in the control pigs the amount of preproteins synthesized may not have been entirely activated, whereas this pool was activated in pigs fed high protein diets. The development of a specific protein assay for each enzyme instead of measuring their activities would enable us to check the activation step directly.

It seems likely that the transcriptional control of enzyme adaptation to dietary protein is a transient phenomenon in the pig. In fact, studies performed on rats have demonstrated that the mRNAs were modulated after 8–15 days of high protein diet,[6,7] but no kinetic studies have been performed in this connection. However, Lahaie and Dagorn[41] have shown that the increase in protease synthesis induced by a protein-rich diet was complete within 24 hr of feeding and persisted for up to 10 days. We are presently performing a careful kinetic study with a view to drawing up a detailed picture of the molecular pancreatic adaptation to a 48% protein diet in the pig.

The aim of this study was to determine the regulatory steps involved in pancreatic adaptation to dietary protein in the pig. Our conclusions can be summarized as follows: (1) the molecular regulation of adaption appears to be highly species-dependent, and it is consequently very important to further investigate this phenomena in the pig, which is a suitable model for human digestive physiology; (2) pancreatic adaptation in the pig results in the conjunction of complex phenomena and must therefore be carefully analyzed: the present data suggest that transcriptional control is rapid and transient; (3) each enzyme is regulated via different pathways because the specific mRNAs are not affected to the same extent and in the same way. Chymotrypsinogen, for example, is mostly regulated via transcription, while amylase is probably regulated at a posttranscriptional level. Other proteolytic enzymes such as procarboxypeptidases, elastases, and trypsinogens show different patterns, but more data on isoenzymes are necessary before the importance of each level of regulation can be established.

Acknowledgments

The authors wish to thank Dominique Besnard, Francis Cointepas, and Gérard Lebonnois for their careful technical assistance and Joël Gallé for his help in realizing figures.

References

1 Pavlov, I.P. (1879). *The work of the digestive gland.* Translated by W.H. Thompson, 1910. Griffin and Co., London, U. K.
2 Corring, T., Juste, C., and Lhoste, E.F. (1989). Nutritional regulation of pancreatic and biliary secretions. *Nutr. Res. Rev.* **2,** 161–180
3 Brannon, P.M. (1990). Adaptation of the exocrine pancreas to diet. *Annu. Rev. Nutr.* **10,** 85–103
4 Wicker, C., Scheele, G., and Puigserver, A. (1983). Dietary adaptation of levels of mRNA coding for pancreatic amylase and serine proteases in the rat. *C.R. Acad. Sci. Paris* **297,** 281–284
5 Giorgi, D., Bernard, J.P., Lapointe, R., and Dagorn, J.C. (1984). Regulation of amylase messenger RNA concentration in rat pancreas by food content. *EMBO J.* **3,** 1521–1524
6 Wicker, C., Puigserver, A., and Scheele, G. (1984). Dietary regulation of levels of active mRNA coding for amylase and serine protease zymogens in the rat pancreas. *Eur. J. Biochem.* **139,** 381–387
7 Giorgi, D., Renaud, W., Bernard, J.P., and Dagorn, J.C. (1985). Regulation of proteolytic enzyme activities and mRNA concentrations in rat pancreas by food content. *Biochem. Biophys. Res. Commun.* **127,** 937–942
8 Wicker, C. and Puigserver, A. (1987). Effects of inverse changes in dietary lipid and carbohydrate on the synthesis of some pancreatic secretory proteins. *Eur. J. Biochem.* **162,** 25–30
9 Wicker, C., Scheele, G.A., and Puigserver, A. (1988). Pancreatic adaptation to dietary lipids is mediated by changes in lipase mRNA. *Biochimie* **70,** 1277–1283
10 Wicker, C. and Puigserver, A. (1989). Changes in mRNA levels of rat pancreatic lipase in the early days of consumption of a high-lipid diet. *Eur. J. Biochem.* **180,** 563–567
11 Dodds, W.J. (1982). The pig model for biomedical research. *Fed. Proc.* **41,** 247–256
12 Miller, E.R. and Ullrey, D.E. (1987). The pig as a model for human nutrition. *Ann. Rev. Nutr.* **7,** 361–382
13 Bucko, A., Kopec, Z., Ovecka, M., and Grunt, J. (1982). Adaptability of pancreatic enzymes activity to various food nutritive values in man. Influence of high protein diet. *Die Nahrung* **26,** 59–64
14 Emde, C., Liehr, R.M., Gregor, M., Pleul, O., Riecken, E.O., and Menge, H. (1985). Lack of adaptative changes in human pancreatic amylase and lipase secretion in response to high-carbohydrate, low-fat diet applied by a 10-day continuous intraduodenal infusion. *Dig. Dis. Sci.* **30,** 204–210
15 Boivin, M., Lanspa, S.J., Zinmeister, A.R., Go, V.L.W., and Di Magno, E.P. (1990). Are diets associated with different rates of human interdigestive and postprandial pancreatic enzyme secretion? *Gastroenterology* **99,** 1763–1771
16 Corring, T. and Saucier, R. (1972). Sécrétion pancréatique sur porc fistulé adaptation à la teneur en protéines du régime. *Ann. Biol. Anim. Bioch. Biophys.* **12,** 233–241
17 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall,

R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275
18 Lhoste, E.F., Aprahamian, M., Pousse, A., Hoeltzel, A., and Stock-Damgé, C. (1985). Trophic effect of bombesin on the rat pancreas: is it mediated by the release of gastrin or cholecystokinin? *Peptides* **6,** 89–97
19 Metais, P. and Bieth, J. (1968). Détermination de l'α-amylase par une microtechnique. *Ann. Biol. Clin.* **26,** 133–142
20 Rathelot, J., Julien, R., Canioni, P., Coeroli, C., and Sarda, L. (1975). Studies on the effect of bile salt and colipase on enzymatic lipolysis. Improved method for the determination of pancreatic lipase and colipase. *Biochimie* **57,** 1117–1122
21 Reboud, J.P., Ben Abdeljlil, A., and Desnuelle, P. (1962). Variations de la teneur en enzymes du pancréas de rat en fonction de la composition des régimes. *Biochim. Biophys. Acta* **58,** 326–337
22 Yamasaki, M., Brown, J.R., Cox, D.J., Greenshields, R.N., Wade, R.D., and Neurath, H. (1963). Procarboxypeptidase A-S6. Further studies of its isolation and properties. *Biochemistry* **2,** 859–866
23 Folk, J.E., Piez, K.A., Carroll, W.R., and Gladner, J.A. (1960). Carboxypeptidase B IV. Purification and characterization of the porcine enzyme. *J. Biol. Chem.* **235,** 2272–2277
24 Gertler, A. and Hofmann, T. (1970). Acetyl-L-alanyl-L-alanyl-L-alanine methyl ester: a new highly specific elastase substrate. *Can. J. Biochem.* **48,** 384–386
25 Chrigwin, J.M., Przybyla, A.E., MacDonald, R.J., and Rutter, W.J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18,** 5294–5299
26 Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982). *Molecular cloning. A laboratory manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY USA
27 Aviv, H. and Leder, P. (1972). Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412
28 Rigby, P.W.J., Dieckman, M., Rhodes, C., Berg, P. (1977). Labelling deoxyribonucleic acid to high specific activity in vitro by nick-translation with DNA polymerase *J. Mol. Biol.* **113,** 237–251
29 Clauser, E., Gardell, S.J., Craik, C.S., MacDonald, R.J., and Rutter, W.J. (1988). Structural characterization of the rat carboxypeptidase A1 and B genes. Comparative analysis of the rat carboxypeptidase gene family. *J. Biol. Chem.* **263,** 17837–17845
30 Scheele, G. and Palade, G. (1975). Studies on the guinea pig pancreas. Parallel discharge of exocrine enzyme activities. *J. Biol. Chem.* **250,** 2660–2670
31 Scheele, G.A., (1980). Biosynthesis, segregation and secretion of exportable proteins by the exocrine pancreas. *Am. J. Physiol.* **238,** G467–G477
32 Ikegami, S., Takai, Y., and Iwao, H. (1975). Effect of dietary protein on proteolytic activities in the pancreatic tissue and contents of the small intestine in rats. *J. Nutr. Sci. Vitaminol.* **21,** 287–295
33 Snook, J.T. (1971). Dietary regulation of pancreatic enzymes in the rat with emphasis on carbohydrate. *Amer. J. Physiol.* **221,** 1383–1387
34 Imondi, A.R., and Bird, F.H. (1967). Effects of dietary protein level on growth and proteolytic activity of the avian pancreas. *J. Nutr.* **91,** 421–428
35 Büchler, M., Malfertheiner, P., Friess, H., Wanjura, D., Seitz, J., and Beger, H.G. (1989). Human pancreatic adaptation following high-dose camostate treatment. *Digestion* **43,** 133–134
36 Grossman, M.I., Greengard, H., and Ivy, A.C. (1943). The effect of dietary composition on pancreatic enzymes. *Am. J. Physiol.* **138,** 676–682
37 Ben Abdeljli, A., Visani, A.M. and Desnuelle, P. (1963). Adaptation of the exocrine secretion of rat pancreas to the composition of the diet. *Biochem. Biophys. Res. Commun.* **10,** 112–116
38 Reboud, J.P., Marchis-Mouren, G., Pasero, L., Cozzone, A., and Desnuelle, P. (1966). Adaptation à la vitesse de biosynthèse de l'amylase pancréatique et du chymotrypsinogène à des régimes riches en amidon et en protéines. *Biochim. Biophys. Acta* **117,** 351–367
39 Robberecht, P., Deschodt-Lanckman, M., Camus, J., Bruylands, J., and Christophe, J. (1971). Rat pancreatic hydrolases from birth to weaning and dietary adaptation after weaning. *Am. J. Physiol.* **221,** 376–381
40 Bourdel, G. (1983). Effect of separate feeding of proteins and lipids on pancreatic adaptation in the rat. *Am. J. Physiol.* **244,** G125–G130
41 Lahaie, R.G. and Dagorn, J.C. (1981). Dietary regulation of pancreatic protein synthesis. II. Kinetics of adaptation of protein synthesis and its effect on enzyme content. *Biochim. Biophys. Acta* **654,** 119–123
42 Johnson, A., Hurwitz, R., and Kretchmer, N. (1977). Adaptation of rat pancreatic amylase and chymotrypsinogen to changes in diet. *J. Nutr.* **107,** 87–96
43 Bell, G.I., Quinto, C., Quiroga, M., Valenzuela, P., Craik, C.S., and Rutter, W.J. (1984). Isolation and sequence of a rat chymotrypsin B gene. *J. Biol. Chem.* **259,** 14265–14270
44 Craik, C.S., Choo, Q.L., Swift, G.H., Quinto, C., MacDonald, R.J., and Rutter, W.J. (1984). Structure of two related rat pancreatic trypsin genes. *J. Biol. Chem.* **259,** 14255–14264
45 Swift, G.H., Hammer, R.E., MacDonald, R.J., and Brinster, R.L. (1984). Tissue-specific expression of the rat pancreatic elastase I gene in transgenic mice. *Cell* **38,** 639–646
46 Scheele, G.A. (1986). Regulation of gene expression in the exocrine pancreas. *The exocrine pancreas: biology, pathobiology and diseases,* (VLW Go et al., eds), p. 55–67, Raven Press, New York, NY USA
47 Schick, J., Verspohl, R., Kern, H., and Scheele, G. (1984). Two distinct adaptative responses in the synthesis of exocrine pancreatic enzymes to inverse changes in protein and carbohydrate in the diet. *Am. J. Physiol.* **247,** G611–G616
48 Steinhilber, W., Poensgen, J., Rausch, U., Kern, H.F., and Scheele, G.A. (1988). Translational control of anionic trypsinogen and amylase synthesis in rat pancreas in response to caerulein stimulation. *Proc. Natl. Acad. Sci. USA* **85,** 6597–6601
49 Le Huérou, I., Wicker, C., Guilloteau, P., Toullec, R., and Puigserver, A. (1990). Specific regulation of the gene expression of some pancreatic enzymes during postnatal development and weaning in the calf. *Biochim. Biophys. Acta* **1048,** 257–264