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# EFFECT OF DIET COMPOSITION ON THE PROTEIN SYNTHETIC PATTERN OF THE RAT PANCREAS AFTER A FEEDING PERIOD OF FIVE DAYS

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## Summary

Rats were fed for five days on a protein-rich and on a carbohydrate-rich diet, respectively. One half of the pancreas of these rats was incubated with [$^{3}$H]-leucine and the other half with [$^{14}$C]leucine and extracts from these pancreas halves were prepared. Mixtures of the differently labeled extracts were subjected to electrophoresis towards the anode as well as towards the cathode on a polyacrylamide gel containing urea at pH 8.5. Several secretory enzymes could be identified on the gels. Along the gels the $^{3}$H : $^{14}$C ratio was determined in 1 mm slices. The results show that after five days of feeding a diet there is some adaptation to diet composition. Generally rather small changes in synthetic rate occur. Only one component, the cathodic chymotrypsinogen shows an important difference in synthetic rate under the two circumstances.

## Introduction

The adaptation of the enzyme content of the pancreas to a predominant constituent of a diet has been reported in rat [1—21], dog [22] and chicken [23,24]. It is generally found that the relative enzymic composition of the exocrine pancreas is changed by a change in the composition of the diet.

Diets rich in carbohydrate, but low in protein, lead to an increase in the content of amylase and a decrease in that of the proteolytic enzymes in the

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pancreas and in the pancreatic juice. Diets rich in protein, but low in carbohydrate, result in a low amylase content along with a higher concentration of proteolytic enzymes [1—9,13,14,16—18,21]. When animals fed on a protein-rich diet are changed to a carbohydrate-rich diet and conversely, the adaptation process starts without delay and lasts 5—7 days [7,18].

Several authors extended these investigations to lipase, which recently was shown to be present in a higher concentration in the pancreas of animals fed a high-fat diet [10,15—20,22—24].

In all these studies the authors based their conclusions on measurements of enzyme specific activities. Without any evidence, some authors [6,8,10,11] assumed that a change in enzyme content was a consequence of a corresponding change in the rate of enzyme synthesis. However, the enzymatic content of the pancreas not only depends on its rate of synthesis, but also on its rate of secretion.

Desnuelle and his coworkers did very thorough work on this subject [25—28]. They studied the pancreatic content of enzymes in response to dietary modification, and also measured the specific radioactivity of the isolated enzymes amylase and chymotrypsinogen, using labeled valine as a precursor. A carbohydrate-rich diet greatly increased the rate of amino acid incorporation into amylase and decreased the incorporation into chymotrypsinogen. This was concomitant with an increase in the level of amylase and a reduction in the level of chymotrypsinogen in this pancreas. A protein-rich diet caused the reverse situation. The results of these experiments have to be interpreted carefully, because here too the incorporated activity measured in the isolated enzymes was related to enzymic activity [28], which can lead to erroneous conclusions. In this connection, it can be noted that Ben Abdeljlil reports, that in pancreata of rats fed a protein-rich diet the total amount of protein per $\mu$g DNA is higher than in pancreata of rats fed a carbohydrate-rich diet [9].

In order to avoid such pitfalls we have developed a double-label technique, in which the whole synthetic patterns of pancreas under different dietary conditions can be directly compared [29].

In this paper we apply this technique to a comparison between the protein synthetic pattern after feeding rats a protein-rich and a carbohydrate-rich diet for five days.

## Materials and Methods

### *Materials*

*Chemicals.* Acrylamide, amido black 10B, ammoniumpersulfate, *N*-acetyl-1-tyrosine-*p*-nitroanilide and elastine-orceïne were obtained from Merck A.G. (Darmstadt, F.R.G.); amino acids, $\alpha$-amylase, $\alpha$-*N*-benzoyl-($\pm$)-arginine-*p*-nitroanilide-HCl, bovine serum albumin, chymotrypsin, DNA, lipase, ribonucleosides, soybean trypsin inhibitor, trypsin and Coomassie brilliant blue R 250 were purchased from Sigma Chemical Corporation (St. Louis, U.S.A.); dioxane and toluene came from J.T. Baker Chemicals B.V. (Deventer, The Netherlands); enterokinase was obtained from Miles Laboratories, Inc. (Kankakee, IL, U.S.A.); indubiose A 37 (agarose) was purchased from l'Industrie Biologique Française s.a. (Gennevilliers, France); L-[4,5-$^3$H]leucine and L-[U-

$^{14}$C]leucine came from The Radiochemical Centre (Amersham, U.K.); Nuclear Chicago Solubilizer (N.C.S.) was purchased from Amersham/Searle Corporation (S. Clearbook, IL, U.S.A.); *N,N'*-methylenebisacrylamide was a product of Fluka A.G. (Bucks, Switzerland); *N,N,N',N'*-tetramethylenediamine and ribonuclease came from Koch-Light Laboratories Ltd. (Colnbrook, U.K.); Permablend TM III was purchased from Packard Inc. (Downers Grove, U.S.A.); Tris, urea and yeast RNA were products of B.D.H. Chemicals Ltd. (Poole, U.K.); all other, not specifically mentioned reagents were of analytical grade and were obtained from Merck A.G. (Darmstadt, F.R.G.) or from B.D.H. Chemicals Ltd. (Poole, U.K.).

*Animals and diets.* Male albino Wistar rats came from the Centraal Proefdierenbedrijf, TNO, Zeist, The Netherlands. The rats were kept at 23°C under artificial illumination with a light-dark cycle of 12 h and at about 50% humidity. They were supplied with a pelleted balanced diet B (from Hope Farms B.V., Woerden, The Netherlands, see BBA Data Bank) and water ad libitum, until they were 6 weeks of age. At this stage the animals were housed in groups of three in a wiremesh-bottomed cage and fasted for 24 h.

Thereupon the rats were fed either a protein-rich diet (P) or a carbohydrate-rich diet (G) ad libitum. The rats always had free access to drinking water. The diets were isocaloric (±19 000 kJ/kg) and in pelleted form (Hope Farms B.V., Woerden, The Netherlands). The composition of these experimental diets is taken from the work of the group of Desnuelle [9] and is given in detail in BBA Data Bank.

*Methods*

*Double-label technique.* Two groups of animals are given diets X and Y, respectively, for a certain period of time. At the end of this period, the animals are decapitated, the pancreas is taken out, sliced and divided in two. One portion is incubated with [$^{3}$H]leucine, the other with [$^{14}$C]leucine. After the incubation the pancreas halves are extracted by homogenization and centrifugation. A $^{3}$H-labeled extract from an X-fed animal is mixed with a $^{14}$C-labeled extract from a Y-fed animal ($X(^{3}H)/Y(^{14}C)$) and the same is done with the reverse combination ($Y(^{3}H)/X(^{14}C)$). For controls, differently labeled extracts derived from one pancreas, are mixed ($X(^{3}H)/X(^{14}C)$). The extracts are always mixed in such a way that in the trichloroacetic acid-insoluble material the ratio of dpm $^{3}$H : dpm $^{14}$C is about ten. The double-labeled extracts are subjected to polyacrylamide gel electrophoresis to separate the pancreatic proteins. The $^{3}H/^{14}C$ ratio in 1 mm slices is determined along the gel.

The double-label technique should meet two conditions. First, in control mixtures e.g. $X(^{3}H)/X(^{14}C)$, the $^{3}H/^{14}C$ ratio should be constant over the whole length of the gel. Secondly, it is required that the mixtures $X(^{3}H)/Y(^{14}C)$ and $Y(^{3}H)/X(^{14}C)$ are the reversed image of each other.

*Incubation.* The incubation procedure in all experiments started at 9.30 a.m. to eliminate possible diurnal variations in pancreas function. The rats were killed by decapitation, their pancreata were quickly removed, dissected free from connective tissue and fat and cut with a TC-2 Sorvall tissue sectioner in 1 mm$^{3}$ fragments. In order to remove broken cells, the pancreas fragments were preincubated at 37°C for 5 min in a conical flask with 2 ml Krebs-Ringer

bicarbonate buffer, pH 7.6, (24 mM $NaHCO_3$; 118 mM NaCl; 4.75 mM KCl; 2.5 mM $CaCl_2$; 1.2 mM $MgSO_4$; 1.2 mM $KH_2PO_4$; 12.9 mM glucose), supplemented with amino acids in rat blood concentration [30] and the four ribonucleosides (0.1 mM). The complete composition of the incubation medium has been given earlier [31], the only modification being that hydroxybutyrate was omitted.

After preincubation, the pancreas fragments were washed with fresh incubation medium at 37° C, thoroughly mixed and divided into two equal portions. Each portion was transferred to a vial with 2 ml incubation medium, one vial containing 200 μCi L-[4,5-$^3$H]leucine (specific radioactivity 46 Ci/mmol) the other 20 μCi L-[U-$^{14}$C]leucine (specific radioactivity 348 Ci/mol). The incubations were carried out under continuous gassing with $O_2/CO_2$ (19 : 1) and gentle shaking at 37° C. After 30 min the incorporation was stopped by washing the pancreas fragments twice with 5 ml of ice-cold incubation medium containing 8 mM unlabeled leucine. The major part of each pancreas was frozen in liquid nitrogen and stored at —20° C for later use.

*Determinations in the pancreas homogenate.* A small part of each portion of tissue was homogenized in 2 ml of 0.2 M $NaHCO_3$/NaOH buffer, pH 8.4 [32, 33] at 0° C with a Potter-Elvehjem homogenizer. After filtration through nylon cloth (meshwidth 140 μm), the trichloroacetic acid-insoluble radioactivity, DNA, amylase and trypsin were determined in aliquots taken from the filtrate. The incorporated radioactivity was measured by mixing an aliquot with an equal volume of a 20% trichloroacetic acid solution containing 8 mM unlabeled leucine. After standing for at least 2 h at 4° C, the precipitate was pelleted by centrifugation during 15 min at 5000 × *g*. The precipitate was washed three times with 5% trichloroacetic acid, dissolved in 0.3 ml formic acid and transferred to a counting vial using 10 ml of a dioxane-based scintillation solution. Radioactivity was measured in a Packard Tri-Carb-2002 liquid scintillation spectrometer (Packard Instruments Co., Downers Grove, IL). dpm were calculated from cpm using the external standard method for $^3$H counts and the channel ratio method for $^{14}$C counts. The incorporated radioactivity was related to μg DNA; the DNA concentration determined using the diphenylamine method of Burton [34].

Amylase was measured as described by Bernfeld [35] and also related to DNA content. For the determination of the total trypsin activity, the trypsinogen was activated by incubating 100 μl of homogenate for about 16 h at 4°C with 15 mg enterokinase in 9 vols. of 50 mM Tris-HCl buffer, pH 8.2, containing 50 μmol/ml $CaCl_2 \cdot 2H_2O$ [16]. The trypsin assay was performed according to the procedure of Kakade et al. [36] using α-*N*-benzoyl(±)-arginine-*p*-nitroanilide as a substrate, and also related to μg DNA.

All determinations were performed in duplicate.

*Extraction procedure.* The pancreas fragments, which had been stored at —20° C, from a number of rats fed the same diet and labeled with the same precursor, were combined and homogenized by means of ten strokes of a Potter-Elvehjem homogenizer at 0° C in 5 vols. 0.2 M $NaHCO_3$/NaOH, pH 8.4, containing 1 mg soybean trypsin inhibitor/ml. The soybean trypsin inhibitor was added to prevent activation of pro-enzymes due to catalysis by a small amount of possibly present trypsin [37]. After a first centrifugation of 30 min

at 5000 × $g$ and 4° C the supernatant was centrifuged for 17 h in a Spinco ultracentrifuge L-50 or L-65 at 105 000 × $g$ at 0—4° C. The final supernatants were stored at —20° C and thawed before use. The amount of radioactivity in the trichloroacetic acid-insoluble material was measured as described. This value was related to protein content.

Protein was determined by the method of Lowry et al. [38] with crystalline bovine serum albumin as a standard.

*Mixing procedure of the extracts.* For double-label experiments a $^3$H-labeled extract, derived from rats fed diet P, and a $^{14}$C-labeled extract, derived from rats fed diet G, were mixed ($P_H/G_C$). The reverse combination ($G_H/P_C$) was also prepared. For controls the mixtures $P_H/P_C$ and $G_H/G_C$ were used. The extracts were always mixed in such a way, that the ratio of $^3$H and $^{14}$C, both expressed in dpm, was about ten in the trichloroacetic acid-insoluble material.

*Electrophoresis.* The double-labeled extracts were subjected to polyacrylamide gel electrophoresis in a continuous buffer system consisting of 0.025 M Tris/0.19 M glycine, pH 8.5, containing 1.6 M urea. The gels were cast in perspex tubes of 9 mm diameter, using 7.5% (w/v) acrylamide, 0.375% (w/v) *N*,*N*′-methylenebisacrylamide, 0.03% (v/v) *N*,*N*,*N*′,*N*′-tetramethylenediamine and 0.02% (w/v) ammonium persulfate. Before adding the persulfate solution the monomer solution had been deaerated by suction. The polymerizing mixture was rapidly overlayed with water. After polymerization the water was sucked off and the gel was covered with 0.025 M Tris/0.19 M glycine buffer, pH 8.5, containing 1.6 M urea. Before sample application the gels were subjected to a pre-electrophoresis for 2 h towards the anode with a constant current of 2 mA per gel ($3.15 \cdot 10^{-2}$ mA/mm$^2$) to avoid artefacts by interaction of ammonium persulfate with the protein components [39—42].

Samples of 100 μl of a double-labeled extract, containing about 1 mg protein, made 6 M in urea and 30% in sucrose were put on the gels. A constant current of 4 mA per gel ($6.3 \cdot 10^{-2}$ mA/mm$^2$) was maintained during the electrophoretic runs (20—30 V/cm). Pre-electrophoresis and electrophoresis were performed in a cold room (4° C).

*Fixation, staining and radioactive measurements of the electropherograms.* After electrophoresis the gels were removed from the tubes and rinsed briefly in distilled water.

Gels, in which radioactivity was to be determined, were immersed in 12.5% trichloroacetic acid for at least three days for a complete removal of the trichloroacetic acid-soluble radioactivity. This was followed by staining the gels in a colloidal solution of 0.05% Coomassie brilliant blue in 12.5% trichloroacetic acid according to Chrambach et al. [43] and destaining with 12.5% trichloroacetic acid. The gels were sectioned into 1 mm slices with a gel slicer of our own design [44]. After sectioning the gel slices were eluted with NCS [45,46], to which a small amount of water (9 : 1, v/v) was added [47].

Radioactivity was counted adding 10 ml of toluene-based liquid scintillation fluid [44].

*Identification of secretory proteins on the electropherogram.* When possible, methods were used which led to a direct localization of enzyme activity in the gels.

Gels were split longitudinally. On ehalf of the gel was stained for 1 h at room

temperature in 0.5% Amido black 10B solution containing 5% $HgCl_2$ and destained by leaching in 5% acetic acid. The other half was incubated for 2 min in cold buffer solution, the composition of which guaranteed optimal enzymatic activity.

Amylase and ribonuclease were identified with the agarose substrate plate method according to Poort and van Venrooy [48]. Trypsinogen and chymotrypsinogen were localized by placing the gels on Whatman paper strips moistened with incubation buffer containing either α-*N*-benzoyl-(±)-arginine-*p*-nitroanilide or *N*-acetyl-1-tyrosine-*p*-nitroanilide respectively, according to Dijkhof and Poort [49]. Lipase localization was performed with the agarose-Tween plate method according to Mates [50].

For the visualization of proelastase the agarose-elastine-orceine plate method according to Dijkhof and Poort [51] was used.

After a long electrophoretic run some enzymes were irreversibly inactivated to a large measure [52] and in those cases the residual enzyme activity could only be detected in the solution obtained after extraction of the 1 mm gel slices.

## Results

### *Characterization of the pancreata*

In Table I the incorporation rate in total pancreatic protein is shown after feeding two groups of rats a P and a G diet, respectively, for five days. In the three experiments comparison of the rate of incorporation of pancreas fragments obtained from P-fed or G-fed rats, both with [$^3$H]leucine and [$^{14}$C]-leucine, showed a higher incorporation rate in the pancreas from G-fed rats than from P-fed rats. The amount of radioactive protein found in the medium after 30 min of incubation was negligible.

The amylase content in the pancreas from P-fed and G-fed rats is about the same.

TABLE I

INCORPORATION RATE OF [$^3$H]- AND [$^{14}$C]LEUCINE, AMYLASE CONTENT AND TRYPSINOGEN CONTENT OF PANCREAS FRAGMENTS AFTER FEEDING RATS A PROTEIN-RICH DIET AND A CARBOHYDRATE-RICH DIET, RESPECTIVELY, FOR FIVE DAYS

Diet P, protein-rich diet; G, carbohydrate-rich diet. Values given are means ± S.D.; the number of rats is given within parentheses. Amylase units: one unit of enzyme activity is defined as the amount of amylase liberating 1 μmol of maltose from soluble starch under the assay conditions. Trypsin units: one unit of activity is defined as an increase of 0.01 absorbance unit in 10 min under the conditions of the assay system.

| Experiment | Diet | dpm $^3$H × $10^3$/μg DNA | dpm $^{14}$C × $10^3$/μg DNA | Amylase units/μg DNA | Trypsin units/μg DNA |
|---|---|---|---|---|---|
| 1 | P | 225.7 ± 22.2 (4) | 25.8 ± 3.0 (4) | 8.2 ± 1.7 (4) | 8.1 ± 0.4 (4) * |
| | G | 265.9 ± 30.5 (4) | 31.7 ± 4.5 (4) | 9.1 ± 1.1 (4) | 4.3 ± 0.4 (4) |
| 2 | P | 150.4 ± 15.4 (5) * | 15.6 ± 2.4 (5) | 6.3 ± 1.2 (5) | 7.1 ± 0.6 (5) * |
| | G | 212.1 ± 21.2 (5) | 19.4 ± 3.3 (5) | 6.8 ± 1.8 (5) | 3.8 ± 0.2 (5) |
| 3 | P | 182.0 ± 16.2 (4) * | 17.4 ± 2.2 (4) * | 7.2 ± 1.4 (4) | 6.7 ± 0.4 (4) * |
| | G | 222.5 ± 19.0 (5) | 25.7 ± 3.8 (5) | 8.1 ± 1.8 (5) | 3.5 ± 0.3 (5) |

* Significantly different from diet group G (at $P < 0.05$ using the test of Wilcoxon).

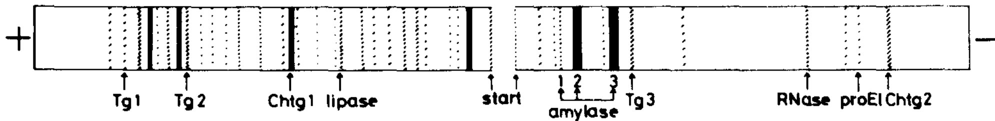


Fig. 1. Polyacrylamide gel analysis of a pancreas extract from a rat fed a balanced diet. The electrophoretic run at pH 8.5 towards the anode took 2.5—3 h, towards the cathode a 6—7 h run was made, in both cases with a constant current of 4 mA per gel (20—30 V/cm). The gel was stained with Amido black B. The measure of shading of the protein bands reflects the intensity of coloring. Tg 1, Tg 2 and Tg 3, trypsinogen 1, 2 and 3; Chtg 1, Chtg 2, chymotrypsinogen 1 and 2; ProEl = proelastase.

The trypsinogen content, related to $\mu$g DNA in the pancreas of P-fed rats is about twice that found in the pancreas of G-fed rats and the variability is lower than in the case of amylase.

*Separation and identification of the pancreatic proteins*

During electrophoresis we used the same pH as was used for the extraction of the pancreas and a good separation was obtained on 7.5% polyacrylamide gels in the presence of 6 M urea (results not shown). However, hardly any enzymatic activity of secretory protein could be detected after electrophoresis and therefore we carried out the electrophoretic separation in a 7.5% polyacrylamide gel in the presence of 1.6 M urea. This does not lead to any discernable change in the electrophoretic pattern. Towards the cathode, a run of 6—7 h was needed, whereas towards the anode a run of 2.5—3 h was sufficient for a good separation. As shown in Fig. 1, 12 cathodic and 29 anodic protein components could be distinguished. The pictures were quite reproducible. Most of the bands represent secretory proteins, since they correspond with components in rat pancreatic juice (a kind gift from Prof. H. Kern, Heidelberg, results not shown).

At present 11 secretory proteins have been identified: four anodic and seven cathodic bands. Three bands with amylase activity, all migrating towards the cathode could be detected. We found three trypsinogens, two anodic bands, marked as Tg 1 and Tg 2 and one cathodic band, Tg 3. Without activation no trypsin activity could be detected in the gels. Chymotrypsinogen was localized in one anodic and one cathodic band. In this case too, we made sure that the enzymatic activity was due only to activated proenzyme. Lipase activity was detected in one anodic band. Elastase activity could be found in one cathodic band, but only after activation with trypsin. Ribonuclease migrates into the cathodic gel.

*Double-labeled protein synthetic pattern*

In the first place we had to make sure, that it was permissible to work in an in vitro system. To this end, we injected a rat with [$^3$H]leucine. The animal was killed 20 min later and the pancreas was incubated for 30 min with [$^{14}$C]leucine in vitro. An extract was prepared from the pancreas and subjected to electrophoresis. The dpm $^3$H/dpm $^{14}$C ratio in the gel slice showed a variation that did not exceed the variation usually found in our control mixtures (not shown).

In Fig. 2 the dpm $^3$H/dpm $^{14}$C profiles in 1 mm gel slices are given and variations are observed in the $^3$H/$^{14}$C ratio, which indicates that the relative synthetic rate of several proteins is influenced by diet composition.

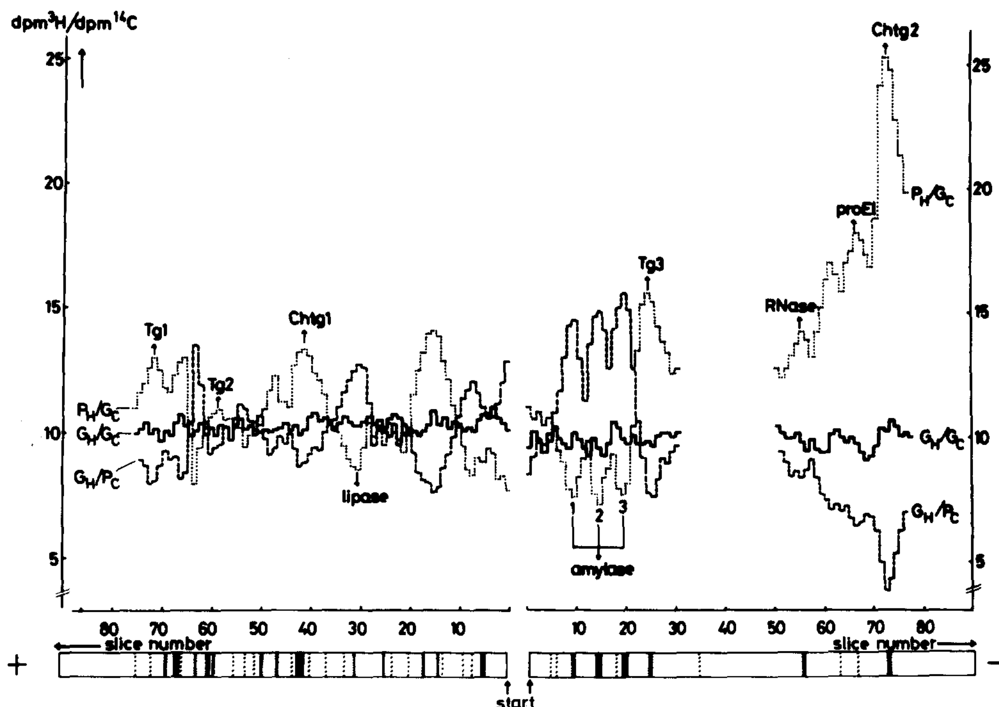


Fig. 2. Dpm ratio profiles obtained after gel electrophoresis of double-labeled mixtures of pancreas extracts derived from rats fed a protein-rich diet (P) and a carbohydrate-rich diet (G), respectively. The dpm $^{3}H$/dpm $^{14}C$ ratio of 1 mm slices is plotted along the length of the gel for a typical experiment. For abbreviations see legend to Fig. 1.

Confining ourselves to the identified components, it appears that the synthesis of the three amylases and lipase is somewhat increased in the case of feeding diet G, whereas the rate of synthesis of trypsinogen 1, trypsinogen 3, chymotrypsinogen 1, chymotrypsinogen 2, ribonuclease and proelastase is relatively lowered. All these changes are rather small, and only one component, chymotrypsinogen 2, shows an important difference in synthetic rate under the two dietary conditions.

The reliability of this double-label technique could be concluded from the outcome of the control mixtures $P_H/P_C$ and $G_H/G_C$. The label ratio, plotted against the distance in the gel, resulted in practically straight lines for both mixtures. Moreover, the ratio pattern of mixture $P_H/G_C$ was the mirror image of that of $G_H/P_C$ and this lends support to the validity of the changes found in the synthetic patterns.

## Discussion

From the earlier observations of Desnuelle and coworkers it was concluded that the synthetic rate of some pancreatic enzymes changed with dietary composition [25—28]: the relative synthetic rate of amylase was markedly increased by a diet containing a high percentage of carbohydrate, whereas the synthetic rate of chymotrypsinogen was found to be enhanced after feeding a

protein-rich diet. In their work amylase and chymotrypsinogen were isolated and purified, after being labeled with a radioactive precursor in vivo or in vitro. The radioactivity incorporated into an enzyme versus the radioactivity incorporated into the total proteins of the pancreas was determined. However, in this method the incorporated activity into the two enzymes had to be related to enzymatic activity in the homogenates, which is a serious drawback, as the amount of measurable enzymatic specific activity is dependent on many unknown factors.

Therefore we have repeated and extended the work of Desnuelle and coworkers using a method in which the determination of enzymatic activity could be avoided. In our study we have used a double-label technique in which an adaptive effect in the rate of synthesis of a certain protein would manifest itself in an altered $^{3}H/^{14}C$ ratio of that protein. Neither a change in the unlabeled protein concentration nor a change in enzymatic activity will influence this ratio. In a simple procedure we can directly compare the whole protein synthetic patterns under different conditions. The method implies that the number of precursor residues in an enzyme does not play a role. An advantage of working in vitro compared to in vivo is, that one can be certain that there is a total lack of secretory stimulus in vitro. This cannot be assumed a priori in vivo and this might lead to a false conclusion as a non-parallel secretion of newly synthesized proteins cannot be ruled out [31]. On the other hand it must be kept in mind, that by our method only relative differences in the rate of synthesis of proteins can be determined.

It is shown by our experiments that there is already some adaptation in protein synthesis after feeding a protein-rich diet for five days compared to a carbohydrate-rich diet. However, the differences in synthesis of most of the protein components are relatively small. The three electrophoretic forms of amylase decrease to about the same measure, lipase also decreases to some degree, while most of the proteolytic enzymes and ribonuclease increase to some extent. Only the cathodic chymotrypsinogen (chymotrypsinogen 2) shows a considerable difference in synthetic rate.

The results of the three experiments, performed on about five animals in each diet group, are highly similar in spite of the differences in incorporated activity in total protein and the degree of filling for amylase and trypsinogen in the pancreas homogenates.

In our electropherogram we could detect one anodic and one cathodic chymotrypsinogen. It is striking, that the anodic and cathodic chymotrypsinogen react in quite different ways to the dietary regime. This may be an indication that these forms are really distinct isoenzymes, synthesized on different messengers. The same goes for the three trypsinogens as these proenzymes also differ in their reaction to the dietary conditions. In the case of amylase we did indeed find three electrophoretic forms of amylase, but these bands showed about the same adaptational behavior. In this case it cannot be excluded, that a common precursor has been synthesized and that posttranslational modifications have taken place [53].

In this study adaptation in pancreatic protein synthesis by diet composition has been clearly proven.

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