Rat pancreatic hydrolases from birth to weaning and dietary adaptation after weaning

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KROBERECHT, P., M. DESCHODT-LANCKMAN, J. CAMUS, J. BRUYLANDS, AND J. CHRISTOPHE. Rat pancreatic hydrolases from birth to weaning and dietary adaptation after weaning. Am. J. Physiol. 221(1): 376-381. 1971. —Within 2-4 days after birth, there was a marked depletion of α-amylase and chymotrypsinogen in the rat pancreas. During the period 14-21 days, α-amylase, lipase, trypsin, and chymotrypsin increased in the small intestine, the three first corresponding levels of (pro)enzymes in the pancreas doing the same, but less evidently. After weaning (21 days) enzyme activities in the pancreas were modified by the nature of the diet. Amylase was markedly augmented by increasing the dietary intake of starch, lipase by lard, and trypsinogenes by cascin. The most important modifications, already manifested after 2 days, were a twofold increase in the specific activity of amylase on starch diet and a fourfold increase in the specific activity of lipase and trypsinogens, on lard and cascin diets, respectively. Comparison of enzyme activities in pancreas and in small and large intestines indicates that rates of biosynthesis and secretion were not the sole factors determining intestinal activities in weanling rats. This was confirmed by a study of the in vitro stability of hydrolases in the washings of the small intestine, indicating that the stability of trypsins and amylase was reduced by 4 days of high-casem or high-lard diets.

exocrine pancreas; small and large intestine; α-amylase; lipase; trypsinogens; trypsins; chymotrypsinogen; chymotrypsin; procarboxypeptidase B

The development of enzymatic activities in the pancreas of rats from birth to weaning has only been documented for amylase (22). The first purpose of the present investigation was therefore to examine the enzymatic development of the rat pancreas from birth to weaning. The second purpose was to study the short-term adaptation of weanling rats on five different solid diets. The adult rat pancreas possesses a great power of adaptation to individual nutrients (12-14, 23, 30). It was of interest to observe in much younger animals to what extent the diet influenced the development of pancreatic enzymes, provided the genetic material was mature enough to be regulated.

The hydrolase content in the small and large intestine was determined in parallel with that of the pancreas. We were interested to find out whether the variations in the activities in the intestine of young rats could result from variable labilities, since it has been found that the half-life of pancreatic enzymes in intestinal washings of adult rats varies markedly with the nature of the enzyme (15). A preliminary report has been published (26).

Materials and Methods

All animals were raised in air-conditioned quarters and fed ad libitum up to the time of sacrifice. The lights were kept on from 7 AM to 7 PM each day. The mother rats weighing about 200-250 g were maintained on commercial rat pellets (Protector, Brussels, Belgium). Birth dates were recorded after two daily inspections. The number of pups per litter was reduced to 10 on the 2nd day after birth.

In the first experiment, pups were randomly selected from the litters on two different occasions at each of the stages of the postnatal growth and sacrificed by decapitation around 10 AM. No attempt was made to prevent cuproplagia.

In the second experiment, the 10 rats, 21 days old and weighing 25-28 g, were taken away from their mother and divided into five pairs with no attention to their sex. Each pair was housed in a screen bottomed metallic cage and was given ad libitum one of five solid diets. The composition of these diets is given in Table 1. The standard diet was finely crumbled. The first animal of each pair was decapitated after 2 days and the second animal after 4 days, between 8 and 10 AM.

The pancreas was quickly removed, freed of fat and connective tissue, weighed, and stored in the deep freezer before assays. Pancreases from two to five animals were pooled when provided by pups under 14 days of age. Individual pancreases were analyzed in older animals.

The small intestine and the entire large intestine (with cecum) were excised separately and weighed. The ileocecal valve was closed with a suture to prevent the loss of ileal contents to the cecum. The small intestine was severed between this suture and a second suture at the entrance of the common bile duct. Taking into consideration their reported properties, the intrinsic enzymes of the brush border, such as the intestinal amylase (1), disaccharidas (7, 27), lipase, and dipeptidase could not cause significant hydrolysis of the substrates used for measurement of the pancreatic enzymes extracted from the intestine. The contamination by these intestinal enzymes was therefore much less critical for proper assays than the considerable binding of pancreatic enzymes to the intestinal mucosa and to intestinal debris (10, 15). In order to extract the pancreatic enzymes as completely as possible, a detergent
was used in conjunction with ionic strength (15). Each intestinal segment was cut in small pieces and placed immediately at 0 °C in 2 volumes (w/v) of 0.04 M Tris-HCl buffer (pH 7.4) enriched with 0.1 % Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) and 0.1 M NaCl. Small intestines from two to five animals were pooled when provided by rats under 14 days of age. The intestinal suspension was shaken magnetically for 10 min. Assays were performed on the supernatant, after centrifugation at 900 X g for 10 min at 0 °C.

When the in vitro half-life (T½) of inactivation of pancreatic hydrolases was studied in the washings of the small intestine, the 0.04 M Tris-HCl buffer was replaced by 0.1 M imidazole in the suspension medium described in the preceding paragraph, the purpose being to inhibit bacterial growth (15). The pooled 900 X g supernatant from the fragments of three small intestines was stirred magnetically for 6 hr in 20-ml vials, in aerobiosis at 37 °C. Aliquots were removed and analyzed hourly (technical details and methods of calculation in ref. 15).

Measurement of Enzymatic Activities and Proteins of Pancreas and Intestinal Contents

The pancreas tissue was defrosted and homogenates were prepared in water (2 %, w/v under 23 days of age, 5 % for 23- and 25-day-old rats). Bovine trypsin (2 X tryst, Worthington Biochemical Corp.), in a proportion corresponding to 1 % of the proteins in the homogenates, was added in order to activate the proteolytic zymogens at 0 °C. The activation of trypsinogens and chymotrypsinogen was prolonged for 24 hr, and that of procarboxypeptidase B for 4 hr.

All enzymatic measurements of hydrolases were expressed in units, i.e., in micromoles of products liberated per minute at 25 °C. The substrates were the same as those previously used in our laboratory (15, 31) for lipase (emulsified olive oil (17)), chymotrypsin (N-acetyl-L-tyrosine ethyl ester), trypsins (N-α-tosyl-L-arginine-methylester, which does not distinguish between trypsins 1 and 2 (31)), and carboxypeptidase B (hippuryl-L-arginine).

The constant pH titration methods for lipase, chymotrypsin, and trypsins were automated by an alternating stop and flow system and were never substrate limited. Six reactions were recorded per hour; each kinetics was monitored for 6 min (32). Aliquots containing 3-5 units of protease or lipase were taken whenever possible for the assays.

The saccharogenic method for α-amylase (18, 23) was also automated. Aliquots containing 1-2 units were incubated with starch for 3 min. The sampling plate was alternately loaded with samples diluted in water, and equivalent aliquots were diluted and denatured in 0.05 M NaOH acting as blanks. The rate of analysis was 20/hr (unpublished data).

Protein concentrations were determined according to Lowry et al. (16), using bovine serum albumin as a standard.

Enzyme activities were related to 1 mg of proteins in the pancreas (specific activity) or to the intestinal content per 100 g body wt.

RESULTS

A. Preweaning Development

Figures 1 and 2 show the pattern of activity of enzymes in the pancreas (specific activity) and small intestine (activity/100 g body wt). The activity of α-amylase in the
pancreas and small intestine decreased rapidly to a minimum at age 2–4 days. The intestinal activity increased somewhat during the 3rd week. Lipase activity in the pancreas and in the small intestine was found to be low at birth and to increase markedly after 2 weeks. Pancreatic trypsinogens 1 and 2 were low in younger pups. There was a large and relatively flat peak at 4–14 days of age (the mean value at 10 days being significantly higher (P < 0.05) than that observed at 1–2 days) and a second smaller peak at 21 days of age. Intestinal trypsins were found to increase slowly, their activities at 6–10 days being already significantly higher (P < 0.05) than those recorded at 1–2 days. For chymotrypsinogen, a great decrease occurred in the pancreas within 3 days of birth. The intestinal activity of chymotrypsin was low in the neonate but increased markedly after 14 days.

B. Dietary Regulation of Exocrine Enzymes in Postweaning Period

Body weight. As shown in Fig. 3, all animals gained weight steadily after weaning. The body weight gain of animals given the chow diet or a casein-rich diet was not significantly lower than that of animals given a diet rich in carbohydrate or fat.

Pancreas. Enzyme adaptations in the pancreas were rapid and the adjustments observed after only 2 days were often almost as great as those obtained after 4 days (Fig. 4). On the other hand, total protein concentrations were not significantly affected.

Amylase exhibited the highest activity in the starch-fed group of animals, and a marked depression after withholding of carbohydrate, i.e., after ingestion of the high-lard diet (Table 1). An intermediate adaptation was observed with the high-glucose diet and when the amount of dietary carbohydrate ranged between 48% (chow) and 14% (high casein).

The pancreases of rats kept on a lard diet exhibited the highest lipase activity, i.e., a sixfold increase in specific activity after 4 days, as compared with the standard diet (chow). High-carbohydrate diets tended to increase pancreatic lipase somewhat. The highest specific activities of trypsinogens and chymotrypsin were obtained with chow. The highest specific activities of trypsinogens 1 and 2 were low in younger pups. There was no difference with control group maintained on chow (Fig. 5). These variations are attributable to the weight of gut contents and may be due to the relatively high bran content of the chow diet, compared to the other diets (Table 1).

In general, the comparison of the activities in the pancreas with the intestinal contents indicated in Tables 2 and 3 and Fig. 6 shows that trypsins were relatively stable and lipase very labile in the small intestine. A further consideration is that the lipase inactivation of pancreatic enzymes in the washings of the small intestine under conditions of bacterial inhibition (MATERIALS AND METHODS). In the main, the half-life of inactivation of lipase and chymotrypsin was much shorter than that of a-amylase and trypsins.

The lowest T½ values for amylase were obtained after...
were maintained on chow for 63 days and were 12 weeks of age weighing 180 g.

Two indices were used. A: The ratio of the average activity (in units) of each (pro)enzyme/g pancreas wet weight (i.e., the specific activity X protein concentration) to the corresponding average activity in the small intestine (in units related to 1 g body wt). B: the ratio of the average activity (in units) in the small intestine to the average activity in the large intestine. * After weaning, these rats were maintained on chow for 63 days and were 12 weeks of age weighing 180 g.

### TABLE 1. Type of diet and its composition

<table>
<thead>
<tr>
<th>Composition</th>
<th>Rat Milk*</th>
<th>Semisynthetic Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard chow</td>
<td>High glucose</td>
</tr>
<tr>
<td>Protein mix</td>
<td>36.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Cow casein (NB Corp.)</td>
<td></td>
<td>18.0</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Starch</td>
<td>-8.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>12.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Vegetable oil*</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Animal fat*</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Choline</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Salt mixture†</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamin mixture†</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Cellulose*</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Composition in grams per 100 g of solids. * According to Dymza et al. (8). † 88% of which was digestible. ‡ Contaminated (14%) with other carbohydrates in the standard chow and offered as corn starch in the other diets. Diet composition in grams per 100 g of solids. a According to Dymza et al. (8). b 88% of which was digestible. c Contaminated (14%) with other carbohydrates in the standard chow and offered as corn starch in the other diets. d Vegetable oil mix in the standard chow, corn oil in the other diets. e Butter in the rat milk, lard in the high-lard diet. f Salt mixture USP XIV and vitamins diet fortification mixture from Nutritional Biochemicals Corp. (Cleveland, U.S.A.). g Crude cellulose in the standard chow; alphacel, Nutritional Biochemicals Corp., in the other diets.

casein and lard and the highest value was observed after glucose. The average T+ obtained for trypsins after casein and lard was markedly lower than after chow, glucose, and starch. Chymotrypsin and lipase, the two most labile hydrolases, were not rendered more labile by casein and lard and the highest value was observed after glucose. It is well known that the fetus receives glucose and amino acids via the placenta. At birth the maternal source of nutrients is milk, a high-fat and high-protein but low-carbohydrate (lactose) diet (Table 1). Pups begin to suckle regularly 20 hr after birth and thereafter eat frequently, behaving as nibblers during the neonatal period. The fall in amylase and chymotrypsinogen (Figs. 1 and 2) that occurred immediately after birth in the pancreas might be caused by the stimulation of the secretory process by sucking, enteric hormones, or a vagal reaction to the postnatal hypoglycemia. (The average protein concentration was 14.8 mg/100 mg tissue wet weight at 1 day of age and as low as 9.3 mg/100 mg at 3 days of age.)

The enzymic differentiation and the hyperplasia (28) of acinar cells seen when the rat pup was more than 14 days old might be due to hormonal variations (11). Another factor of probably great importance is the gradual change from an exclusively milk diet to a mixed diet richer in carbohydrates. Pups 15 days old were observed to reach the chow cubes offered to the mother and to chew on the edges of this standard diet. It would be desirable to design cages where pups have no access to maternal food, in order to see if these pups show the same changes at 14–21 days.

The development of α-amylase activity was very obvious in the small intestine and less evident in the pancreas itself. Procházka et al. (22) also observed amylase induction after 2 weeks. The dependance of α-amylase synthesis on dietary glucose and insulin in the adult rats (3, 4, 19) probably applies to its development before and during the

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**TABLE 2. Effect of 2 and 4 days of dietary induction in weanling rats on distribution of activities of pancreatic enzymes (or proenzymes) among pancreas and intestine tract**

<table>
<thead>
<tr>
<th>Distribution Ratio</th>
<th>Composition</th>
<th>Chow</th>
<th>High glucose</th>
<th>High starch</th>
<th>High casein</th>
<th>High lard</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A) Pancreas:small intestine</strong></td>
<td>Amylase</td>
<td>268</td>
<td>254</td>
<td>318</td>
<td>359</td>
<td>709</td>
</tr>
<tr>
<td></td>
<td>Lipase</td>
<td>399</td>
<td>488</td>
<td>915</td>
<td>914</td>
<td>1,329</td>
</tr>
<tr>
<td></td>
<td>Trypsins (ogen) 1 and 2</td>
<td>83</td>
<td>103</td>
<td>292</td>
<td>273</td>
<td>255</td>
</tr>
<tr>
<td></td>
<td>Chymotrypsin (ogen)</td>
<td>103</td>
<td>203</td>
<td>592</td>
<td>661</td>
<td>437</td>
</tr>
<tr>
<td><strong>B) Small intestine:large intestine</strong></td>
<td>Amylase</td>
<td>2.7</td>
<td>2.7</td>
<td>2.1</td>
<td>2.0</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>Lipase</td>
<td>6.6</td>
<td>4.4</td>
<td>5.3</td>
<td>5.8</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>Trypsins 1 and 2</td>
<td>1.9</td>
<td>1.2</td>
<td>2.0</td>
<td>4.1</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Chymotrypsin</td>
<td>4.0</td>
<td>3.1</td>
<td>7.3</td>
<td>7.9</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Two indices were used. A: The ratio of the average activity (in units) of each (pro)enzyme/g pancreas wet weight (i.e., the specific activity X protein concentration) to the corresponding average activity in the small intestine (in units related to 1 g body wt). B: the ratio of the average activity (in units) in the small intestine to the average activity in the large intestine. * After weaning, these rats were maintained on chow for 63 days and were 12 weeks of age weighing 180 g.

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### FIG. 5. Effect of diet composition on intestinal weight of weanling rats. Wet weight includes intestinal contents and are related to 100 g body wt. Same experimental conditions and representation as in Fig. 4.
and that all (pro)enzymes are agglomerated at proportional rates before secretion. In the pancreas are proportional to their rates of synthesis increased as the intake of carbohydrate in the diet increased in response to similar dietary changes. I-period and the neonatal period also occur in the adult rat ingredients varied from one diet to another. Some caution is therefore necessary in the interpretation. Many of the enzymatic adaptations that occurred during the weaning period, and that the adaptation of amylase and lipase activity in the pancreas during the first 4 days of the weaning period, and the neonatal period also occur in the adult rat except when this weaning is on to high-fat or high-protein diets (21, 29, 33, 34).

In weanling rats, there was perhaps an overshoot of protease synthesis during the first 2 days on the high-casein diet. Procarboxypeptidase B, at variance with the other proenzymes, seemed nonadaptive since its pancreatic level was not significantly affected by an increase in dietary casein from 18 to 70 g/100 g of solids (Table 1 and Fig. 4).

Our results indicate that lipase was directly related to the intake of lard. This adaptation differs from the results obtained by Grossman et al. (12, 13) and Reboud et al. (23), but corroborates those of Bučko and Kopeć (5) in adult rats receiving 20% or more of corn oil.

Intestinal tract. The divergent variations in enzymatic activities in the small intestine and in the gland, observed after dietary manipulation (Table 3), is likely to be due to different problems in the small intestine rather than to selective changes in rates of secretion. In general, our results indicate that the rate of these enzymes was not the same when they passed through the entire intestinal tract (Fig. 6 and Table 2). Inactivation was much more rapid for

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**TABLE 3. Effect of diet in weanling rats on in vitro lability of pancreatic hydrolases in fluids of small intestine**

<table>
<thead>
<tr>
<th>Composition</th>
<th>Chow</th>
<th>High glucose</th>
<th>High starch</th>
<th>High casein</th>
<th>High lard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>6.1</td>
<td>8.2</td>
<td>4.4</td>
<td>2.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Lipase</td>
<td>1.3</td>
<td>1.7</td>
<td>1.2</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Trypsins 1 and 2</td>
<td>38.0</td>
<td>40.0</td>
<td>35.0</td>
<td>5.2</td>
<td>5.3</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>1.5</td>
<td>1.2</td>
<td>1.1</td>
<td>1.0</td>
<td>2.2</td>
</tr>
</tbody>
</table>

After 4 days of dietary induction, the pooled washings of three small intestines were incubated at 37°C for 6 hr in 0.1 M imidazole buffer (pH 7.4)-NaCl 0.1 M-Triton X-100 0.1%. The half-life ($T_1/2$) of in vitro inactivation of the pancreatic enzymes is expressed in hours and is the mean of three experiments.

Weaning period. In the liver also, glucose and insulin are important regulators. At birth, extrahepatic tissues can no longer depend on the maternal supply of glucose or on much carbohydrate in the diet. Fortunately, gluconeogenesis, absent in fetal liver, appears within 2 days after birth while hepatic lipogenesis decreases 10-fold. A new decrease in activity in the glucogenetic pathway and an increase in hepatic enzymes involved in glucose utilization occur during weaning, except when this weaning is on high-fat or high-protein diets (21, 29, 33, 34).

The low activity of lipase during the first 2 weeks is intriguing, considering the high fat content of rat colostrum and rat milk (2). This might be due to the immaturity of the corresponding genetic material or to the low inducing effect of the short-chain saturated fatty acids of rat milk (2), as compared to the fatty acids of vegetable origin present in our low-fat standard chow.

**B. Dietary Regulation in Postweaning Period**

Pancreas. The comparison with values observed in adult rats on the same solid commercial diet rich in carbohydrate (4 in Fig. 1) suggests that hydrolases did not reach a steady activity in the pancreas during the first 4 days of the weaning period, and that the adaptation of amylase and lipase occurred over a long period of time.

Our data provides the comparison of 5 diets per se, but they do not show the relations between the individual dietary factors and enzyme activities, because many ingredients varied from one diet to another. Some caution is therefore necessary in the interpretation. Many of the enzymatic adaptations that occurred during the weaning period and the neonatal period also occur in the adult rat in response to similar dietary changes.

It is thought that the various changes in the enzymatic contents of the pancreas observed in the present study represent variations in rates of biosynthesis. Indeed, the near identity not only of the in vivo turnover times of five pancreatic hydrolases (30), but also of the in vitro secretion of two hydrolases (23) and of enzyme distribution in pancreatic tissue and pancreatic juice (3) are good arguments in favor of a metabolic homogeneity of the secretory process. It appears that the relative contents of hydrolases in the pancreas are proportional to their rates of synthesis and that all (pro)enzymes are agglomerated at proportional rates before secretion.

Figure 4 indicates that the specific activity of amylase increased as the intake of carbohydrate in the diet increased (Table 1). This relationship was more gradual than that reported in adult rats when large increases occurred only if starch or glucose represented more than 60% (4, 6, 12). It has been suggested that glucose, the major product of carbohydrate digestion, is an inducer for pancreatic amylase (3), while insulin (4, 19) and dietary proteins (30) play a permissive role.

It is known that chymotrypsin (23, 24) and trypsins (12–14) follow the protein content of the diet in adult rats. In weanling rats, there was perhaps an overshoot of protease synthesis during the first 2 days on the high-casein diet. Procarboxypeptidase B, at variance with the other proteases, seemed nonadaptive since its pancreatic level was not significantly affected by an increase in dietary casein from 18 to 70 g/100 g of solids (Table 1 and Fig. 4).

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Intestinal tract. The divergent variations in enzymatic activities in the small intestine and in the gland, observed after dietary manipulation (Table 3), is likely to be due to different problems in the small intestine rather than to selective changes in rates of secretion. In general, our results indicate that the rate of these enzymes was not the same when they passed through the entire intestinal tract (Fig. 6 and Table 2). Inactivation was much more rapid for...
lipase and chymotrypsin than for trypsins and \( \alpha \)-amylase. This same conclusion has already been made for adult rats (15, 20).

Our data on the in vitro half-life \( (T_h) \) of pancreatic enzymes was obtained when bacterial inactivation was minimized by the imidazole buffer (15). Other in vivo events might still influence the \( T_h \) of in vitro inactivation. For example, factors such as the net fluid movement, the microbial status (15) as influenced by diet, the protective effect of dietary protein on proteolytic enzymes, the formation of enzyme-substrate complexes protecting lipase and amylase from proteolysis, the levels of pancreatic hydrolases and trypsin inhibitor, and the reversible binding of hydrolases to intestinal debris, fibrous material of vegetal origin (lignin, cellulose), and epithelial mucosa (10, 15), may also play an important role in the stability and inactivation of these enzymes in vivo.

Trypsins being the main factors responsible for their own inactivation (15), their increased in vitro lability (Table 3) on a casein diet increasing their intestinal concentration could be expected. The high trypsins levels observed on this casein diet were also speeding up the in vitro inactivation of amylase. On the other hand, the in vitro lability of trypsins on the high-lard diet, contrasting with the relative stability of chymotrypsin on the same diet, cannot be readily explained. The possible role of dietary lard as a stabilizer of lipase in vivo could not be clearly substantiated from the data in Tables 2 and 3.

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REFERENCES