FURTHER STUDIES ON AMYLASE BIOSYNTHESIS BY PANCREAS OF RATS FED ON A STARCH-RICH OR A CASEIN-RICH DIET.

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Previous investigations (Reboud et al, 1962. Ben Abdeljlil et al, 1963) have shown that the specific activities of amylase and proteolytic enzymes are markedly different in pancreas and pancreatic juices of rats fed on a starch-rich or a casein-rich diet (rats G or P). It is of interest to know whether these variations are associated with a change in the activity or in the amounts of the enzymes concerned and, in the latter case, whether the rate of biosynthesis of these enzymes has been altered by the diet. The following experiments indicate that different amounts of amylase are actually present in pancreas of the two groups of animals and that the relative rates of C^{14}-valine incorporation into pure amylase also are different.

1. Purification of Rat pancreatic Amylase.

The left part of Fig. 1 gives the chromatographic diagram of rat pancreatic juice on DEAE-cellulose at pH 8.0. Amylase emerges quantitatively in the break-through peak with ribonuclease and lipase. The second step of the purification procedure is founded upon the selective precipitation on a micro scale of the complex amylase-glycogen (Loyter and Schramm, 1962).

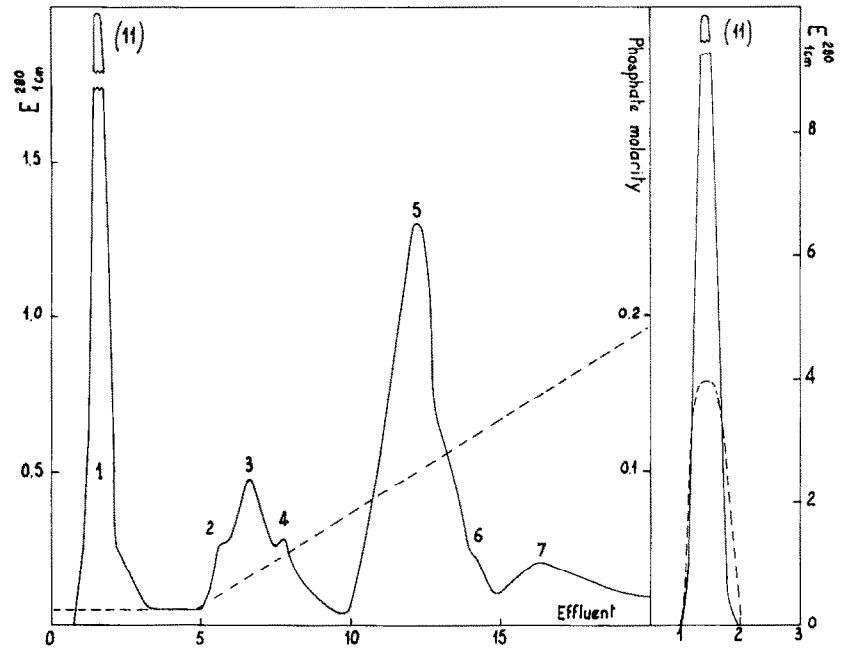

Fig. 1 : Chromatography of Rat pancreatic juice on DEAE-cellulose at pH 8.0.
The column is equilibrated with a 0.005 M phosphate buffer pH 8.0 containing DFP 10^{-4} M and eluted by a linear concentration gradient.On the left,complete diagram of juice G with one cationic peak (amylase,ribonuclease and lipase) and six anionic peaks ((2) unidentified, (3) carboxypeptidase B,(4) carboxypeptidase A,(5) chymotrypsinogen and trypsinogen, (6) procarboxypeptidase A,(7) procarboxypeptidase B).On the right,cationic peaks obtained with the same amount of proteins from juice G (solid line) and juice P (dotted line).The volume of the effluent is given in number of retention volumes of the column.

A pancreas G or P is homogenized in 9 volumes of 0.1 phosphate buffer pH 8.0 containing DFP 10^{-3} M. The clear supernatant (105,000 g during 20 min.) is dialyzed twice against 0.005 M phosphate pH 8.0 during 2 h. and passed through a 12 x 0.9 cm DEAE-cellulose column equilibrated with the same buffer. The break-through peak is dialyzed overnight against a 0.02 M phosphate buffer pH 6.9 containing NaCl 0.006 M. The concentration is adjusted to 1,000 amylase units/ml. Ethanol (10 %) and glycogen

(4 mg per ml) are added. The precipitate is spun down, washed twice with a small volume of the buffer containing 10 % ethanol and finally dissolved in the buffer. In spite of the presence of glycogen, the preparations thus obtained from pancreas G and P may for all practical purposes be considered as pure. Their specific activities (amylase units per mg protein) are similar to the maximal value found for porcine pancreatic amylase (Fisher and de Mantmollin, 1951 ; Loyter and Schramm, 1962). By hydrazinolysis, they exclusively give rise to lysine as COOH-terminal residue[M]. The yield is almost quantitative.

The amount of pure enzyme obtained from each pancreas can be evaluated by determining the number of amylase units in the final solution, or its protein content by colorimetry or spectrophotometry. On the average, this amount is found to be 3 times higher with pancreas G than with the same weight of pancreas P. Moreover, the right part of Fig. 1 shows that the area of the break-through peak obtained by chromatography is nearly twice as large with juice G as with juice P for the same weight of total proteins in the juice. Since the peak contains, as judged by its specific activity, about 95 % amylase in the first case and 75 % in the second, the amylase amounts are again found in the ratio 3 : 1.

2. Rate of Valine incorporation into pure Amylase by Pancreas G and P.

20 μC of DL-C^{14}-valine (5 mC/mmole) are injected intraperitoneally into rats G and P. 15 min. later, the pancreas

[M] These observations further suggest that pancreas G and P form the same amylase.

of each animal is excised and homogenized. Pure amylase (see above) and total proteins (Schneider, 1945) are prepared from the homogenate. Radioactivity (counts/min.) is determined in both preparations and calculated for the total amounts obtainable from 1 g fresh pancreas. The whole process is separately carried out on 10 pancreas G and 10 pancreas P. Average values and their ratios are given in Table I. Specific radioactivities (counts/min./mg) of amylase and total proteins are not indicated in the Table since, under the conditions used, they vary noticeably within the same group of animals. These variations may be due, at least partly, to dilution of newly synthesized molecules by different amounts of pre-existent material.

Table I

Incorporation of radioactive Valine into Amylase and total Proteins by Pancreas G or P during 15 min.

Diet	Radioactivity			Amylase specific activity (units per mg protein) in homogenates.
	Amylase	Total Proteins	Ratio	
Starch-rich (Pancreas G)	63,720	197,055	0.320 ± 0.006	150
Casein-rich (Pancreas P)	8,600	111,350	0.081 ± 0.007	51

Ratios similar to these of Table I are obtained when animals are killed 10, 15 or 25 min. after valine injection. Since

these ratios are independant from the respective size of the valine pool in pancreas G and P, they indicate that, for the same incorporation into total proteins, pancreas G incorporates about 4 times as much valine into amylase as pancreas P. If the assumption is made that the valine content is about the same in rat pancreatic amylase and total proteins, and that no amylase is being destroyed or exported during the assays, the ratios of Table I further suggest that, for 100 molecules of total proteins, pancreas G synthesizes 30 molecules of amylase and pancreas P, only 8. These values are in fair agreement with the specific activity and weight ratios determined above for amylase in pancreas G and P. They also agree with the fact that amylase represents about 25 % of the total proteins in pancreas G and 8 % in pancreas P.

Absolute rates of amylase biosynthesis in pancreas have not yet been determined. However, all results so far obtained are consistent with the view that a starch-rich diet induces in rat pancreas a more active biosynthesis of amylase than a casein-rich diet.

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