VARIATIONS IN THE BIOSYNTHESIS RATE OF PANCREATIC AMYLASE AND CHYMOTRYPSINOGEN IN RESPONSE TO A STARCH-RICH OR A PROTEIN-RICH DIET.

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Recent experiments (Marchis-Mouren et al., 1963 ; Reboud et al., 1964) using $\left[{}^{14}C\right]$ labelled valine have suggested that the adaptation of pancreatic amylase and chymotrypsinogen (ChTg) to a starch-rich or protein-rich diet (diets G or P) involves large variations in the biosynthesis rate of the enzymes. For the same incorporation into total proteins, pancreas G incorporate 5 times more radioactivity into amylase than pancreas P, and 2.5 times less radioactivity into ChTg. In order to eliminate the uncertainties associated with this relative expression of the biosynthesis rates, the rates themselves have now been evaluated according to eq. (1) :

$$dE^{*}/dt = nk_s.a - k_dE^{*} \qquad (1)$$

where E^{*} is the radioactivity of enzyme E at time t, k_s is the rate constant of the zero order biosynthesis reaction, n is the number of valine residues per mole of enzyme E, k_d is the rate of the first order reactions* leading to losses of radioactive

* In the special case of pancreas, losses of radioactive enzymes may occur by degradation (turnover) and exportation.

enzymes, and a is the variation as a function of time of the specific radioactivity of the precursor at the biosynthesis site. It will be shown later that $k_d E^M$ is negligible in our case, so that : $$E^M = nk_s \int_0^t a.dt \qquad (2)$$

It will also be demonstrated that $\int_0^t a.dt$ is the same in pancreas G and P. Thus : $$E^M_G / E^M_P = k_{sG} / k_{sP} \qquad (3)$$

In a first series of experiments in vivo, L-[^{14}C] valine (0.75 μ mole, 13 μC/μmole) was injected intraveneously into 30 rats G or P. After 2-15 min, the animals were bleeded and the pancreas were homogenized at once. The radioactivity of the TCA-soluble fractions, total proteins and pure samples of amylase and ChTg was measured in each case. The amount of valine in the TCA-soluble fractions was determined chromatographically with an automatic analyzer. The valine peaks obtained were well separated and contained nearly all the radioactivity of the fractions.

The upper curve of figure 1 indicates the shape of function (a) under the conditions used. It also shows that this function is nearly the same in pancreas G and P. Hence, according to eq.(3), the G/P ratios of the biosynthesis rates of amylase and ChTg can be readily derived from the curves of the lower diagramms of figure 1. It appears in this way that diet G, when compared to diet P, induces a 8-9 fold increase in the biosynthesis rate of amylase, and a 2 fold decrease for ChTg.

In order to use a simpler system and see whether the adaptation effect is also apparent in isolated pancreatic tissue, another series of experiments were performed with slices of pan-

creas G and P. The slices were incubated during 2,5 and 15 min in 15 ml Krebs medium N° III containing 90 µmoles [^{3}H] valine (405 µC/µmole). Radioactivity was measured in the TCA-soluble fractions, pure amylase and ChTg. Similar G/P ratios were obtained in spite of the fact that the radioactivity of the precursor varies quite differently in slices and in pancreas in vivo.

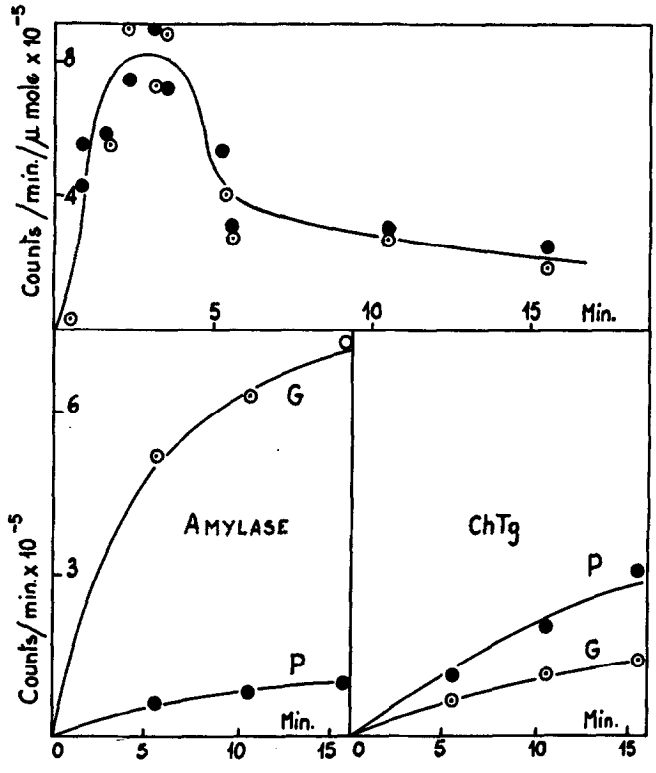

Fig.1 : Determination of the biosynthesis rate in vivo of pancreatic amylase and ChTg. Upper curve, specific radioactivity of the intrapancreatic precursor (function a of eq.(1) and (2)). Lower diagramms, radioactivity incorporated into pure amylase and ChTg by the same quantity (on a DNA basis) of pancreas G (white signs) or P (black signs).

Moreover, it has been checked that no losses of radioactive enzymes by exportation or degradation occur during the assays. [^{14}C] valine was injected into cannulated rats and the radioactivity of samples of pancreatic juice was measured. As shown in the left diagramm of figure 2, loss of radioactivity by exportation is still negligible after 20 min. On the other hand, the right diagramm of figure 2 gives the results of a typical chase experiment performed on pancreas slices labelled with [^{3}H] valine. Although the radioactivity of the intrapancreatic precursor is strongly depressed by incubation with non radioactive valine, there is no sign of an appreciable fall in the radioactivity of the proteins during 30 min. Because of the slight radioactivity remaining after chase and the very short period covered by the assays, this type of experiments cannot definitely exclude the possibility that pancreatic enzymes are partly degraded before exportation. But it shows that degradation is negligible during 15 min.

The G/P ratios of the biosynthesis rates given by eq.(3) clearly define the extent of the adaptation process. Alternatively, independant values for the rates in steady states G and P can be derived from eq.(2), in which $\int_0^t a.dt$ is the surface limited by the upper curve in figure 1, the time axis and the two vertical lines at times 0 and t. Theses values decrease rather sharply with time when slices are used. But they remain fairly constant <u>in vivo</u>. Then, taking into account the valine content of amylase and ChTg (31 and 23 residues/mole respective-

ly, (J.Christophe, personal communication)), the number of cells found by DNA determination and the fact that the exocrine part of pancreas corresponds to about 77 % of the total gland (Junqueira et al, 1957), it may be calculated that one acinar cell of pancreas G is able to synthesize in vivo 2.4×10^6 amylase molecules per min, and 5.2×10^5 ChTg molecules.

If, as it is the case in liver (Wilson and Hoagland,1965), only 1/3 of total pancreatic RNA is ribosomal RNA, this high production would imply that the time required for the complete

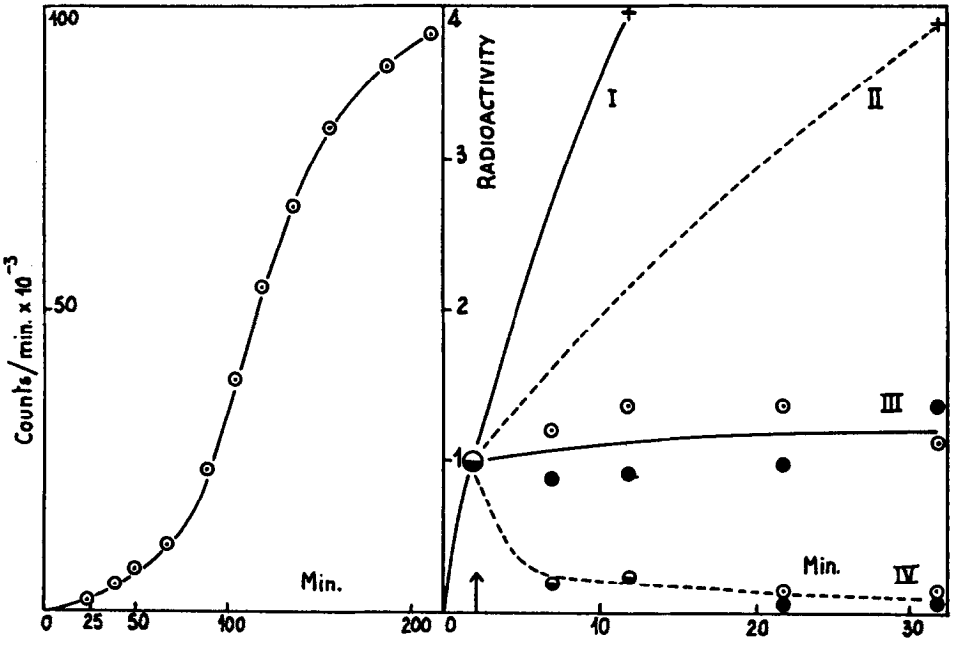

Fig.2 : Losses of radioactive enzymes by exportation and degradation. Left diagramm, radioactivity found in samples of pancreatic juice after injection of labelled precursor. Right diagramm, chase experiment performed on pancreas slices. The slices are labelled during 2 min (large sign and arrow) and then incubated with radioactive or non radioactive precursor. Curves I and III, radioactivity incorporated into proteins ; curves II and IV, radioactivity of the TCA-soluble fractions.

synthesis of one enzyme molecule by pancreas *in vivo* is much shorter than previously estimated for hemoglobin in isolated reticulocytes (Dintzis, 1961). A first explanation of this disagreement is that our own calculations are not valid because the specific radioactivity of the precursor measured in whole pancreas is different from the one existing at the biosynthesis site. But another, perhaps more likely, explanation is that biosynthesis is actually faster in pancreas *in vivo* than it appears to be in isolated systems.

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