EFFECT OF INSULIN ON PANCREATIC AMYLASE AND CHYMOTRYPSINOGEN

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The level and rate of biosynthesis of amylase and chymotrypsinogen are markedly different in pancreas of rats fed on a starch-rich or a casein-rich diet (Reboud, Ben Abdeljlil and Desnuelle, 1962 ; Ben Abdeljlil, Visani and Desnuelle, 1963 ; Ben Abdeljlil and Desnuelle, 1964 ; Marchis-Mouren, Paséro and Desnuelle, 1963 ; Reboud, Paséro and Desnuelle, 1964). The major constituant of the diet appears in both cases to enhance the production of the enzyme, or group of enzymes, required for its digestion. Since an excess of glucose (Ben Abdeljlil et al, 1964) or amino-acids (Ben Abdeljlil, unpublished experiments) in the diet exerts the same effect as an excess of starch or casein, it may be assumed at least provisionally that the biosynthesis of pancreatic enzymes is directly or indirectly regulated by the digestion products reaching pancreas after intestinal absorption rather than by the dietary products themselves. The main purpose of this note is to show that insulin is probably involved in the regulation of amylase biosynthesis. The role of the hormone with respect to the biosynthesis of chymotrypsinogen is still dubious.

Male Wistar rats (200-250 g) fed ad libitum on a balanced

diet and fasted overnight receive a single intraperitoneal injection of 180 mg alloxan per kg. After 7 days, the diabetic animals (3.5 - 6.0 mg glucose per ml blood ; normal content, about 1 mg) are selected and separated into three groups. Group 1 is used at once. Group 2 is used after an additionnal week in order to study the action of alloxan during a 14 days period. Each animal in group 3 receive daily for 3 or 7 days 5 or 10 in-

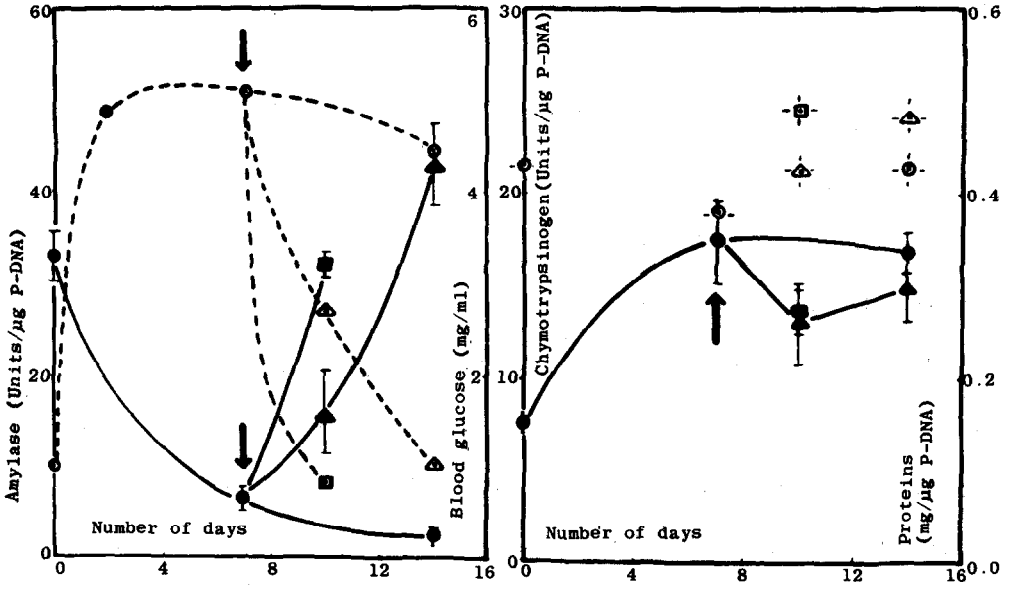

Figure 1 : Effect of alloxan and insulin injections on pancreatic amylase and chymotrypsinogen. Experiments begin when alloxan is injected. After 7 days (vertical arrows), some of the diabetic animals are treated with insulin. On the left, pancreatic amylase (black signs and solid lines) and blood glucose (white signs and interrupted lines). On the right, chymotrypsinogen (black signs and solid lines) and total pancreatic proteins (white isolated signs). Circles, alloxan alone. Triangles and squares, daily injections of 5 or 10 insulin units, respectively. Standard deviations from the mean are indicated for amylase and chymotrypsinogen. 4-8 animals are used in each experiments.

sulin[x] units by subcutaneous injection. Pancreas are excised and individually homogenized. Amylase, chymotrypsinogen, total proteins and DNA are determined in each homogenate as previously described. Enzyme units and total proteins (mg) are referred to 1 µg P-DNA in order to obtain an expression of the amounts per cell. Fig. 1 gives the variations of these amounts after alloxan and insulin injection.

Figure 1 shows that alloxan injection induces a very strong decrease of amylase level and that this decrease is fully reversed by insulin. After 7 days, amylase is nearly 20 times higher in diabetic animals treated daily with 5 insulin units than in untreated animals. It is already 15 times higher after 3 days with 10 units. This effect on amylase is not likely to be caused by a non specific change in the ability of pancreas to synthesize proteins, since : (a) chymotrypsinogen calculated on a DNA basis varies in the opposite direction during alloxan treatment and (b) the protein content of pancreas, also calculated on a DNA basis, remains fairly constant in all cases. Thus, insulin seems to be specificially involved by a still unknown mechanism in the biosynthesis of pancreatic amylase.

A first possibility to be considered here is that the regulation is actually brought about by glucose. Then, amylase would be low in diabetic animals because pancreas acinar cells cannot absorb or/and metabolize glucose. Glucose, and consequently

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[x] On the first day, the dosage is half insulin and half protamine zinc insulin. On the other days, it is only protamine zinc insulin. We are indebted to Organon, Oss, Holland for a gift of these compounds.

amylase, would also be low in normal animals ingesting little starch. This interpretation is not in agreement with the assumption that the "protein anabolic" and glucose effects of insulin are distinct in isolated rat diaphragm (Manchester and Young, 1958). A second possibility is that insulin is acting in a more direct way and that an excess of starch or glucose in the diet promotes amylase biosynthesis through an accelerated discharge of the hormone. Insulin has been recently shown to influence the level of other enzymes involved in carbohydrate metabolism, such as hepatic glucokinase (Salas et al, 1963 ; Sharma et al, 1963) and to modify the labelling of a specific fraction of RNA in isolated rat diaphragm. (Wool, 1963 ; Wool and Munro, 1963).

Figure 1 also suggests that chymotrypsinogen is about twice as high in alloxan-diabetic as in control animals and that insulin is not able to reverse the effect. In another assay with a different sample of insulin, chymotrypsinogen was observed to return to normal after 3 days and again to increase between the 3^{rd} and the 7^{th} day. Thus, the influence of insulin on chymotrypsinogen is still dubious and, in any case, relatively slight.

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