GLUCOSE POLYMERISATION AND CORTISOL

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Experiments concerning the influence of glucocorticoids on the polysaccharide metabolism in connective tissue (cf. Hilz, 1960, 1962) lead us to study some aspects of the problem on a simpler model of polysaccharide synthesis. We choose the glycogen formation in rat liver, which is strongly stimulated by these hormones. Inspite of the fact that glycogen formation is routinely used as a biological test for glucocorticoid action, and although a number of enzyme induction processes habe been made probable on the way from amino acid transformation into glucose units (cf. Feigelson, 1961; Hübener, 1960; Weber, 1961; Matzelt, 1962, and others), practically nothing is known about the influence of the hormones in question on the glucose polymerisation itself (cf. Matschinski et al.,1961). We determined therefore the enzyme activities and the corresponding substrate concentrations in the system glucose-6-phosphate (G-6-P) $\rightleftharpoons$ glycogen and their behavior after cortisol administration.

Injection of a suspension of cortisol acetate into adrenalectomized rats in a single dose leads to an increase in G-6-P-concentration already after 30 min. which after 6 hrs. amounts to a 40 fold increase (fig. 1). A (less drastic) increase of G-1-P and finally of glycogen follows, whereas the concentration

of the immediate glycogen precursor uridine diphosphoglucose (UDPG) is strongly decreased. Blood glucose rises only when the glycogen content of the liver also starts to increase.

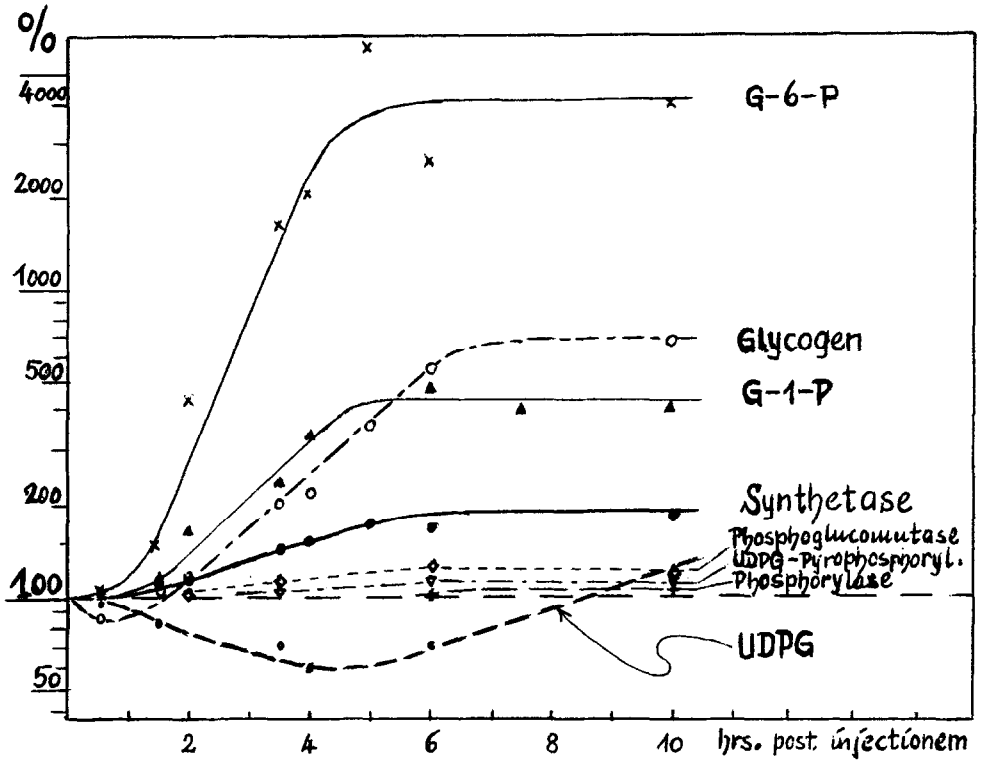

Fig. 1: The effect of cortisol on the system G-6-P $\rightleftharpoons$ glycogen.

Male rats ($\sim$200 gm) were adrenalectomized and injected i.p. with cortisol acetate suspension (2,5 mg/100 gm), on the 4th day, after a 14 hrs. fast. At the time indicated, they were decapitated after a blow on the head, the livers rapidly removed, and three homogenates from weighted pieces were prepared: One for enzyme detns. with 0.25 m sucrose-0.001 m EDTA, one for substrate tests with 4 % $HClO_4$, and one for glycogen phosphorylase with a solution containing 0.1 m NaF-0.005 m K_2HPO_4. A detailed account of the methods employed will appear elsewhere (Biochem.Z.). The changes are expressed in % of the starting point (adrenalect., without cortisol), and all values are based on DNA content. Each point represents 4-16 animals.

In contrast to the marked changes at the substrate levels, the activities of the enzymes phosphoglucomutase, UDPG-pyro-

phosphorylase, glycogen phosphorylase, and G-6-P'ase show only little, if any, increase except glycogen synthetase (UDPG-glycogen-1,4-transglucosylase), which is significantly elevated at the onset of glycogen synthesis. This enzyme activity, measured with saturating levels of G-6-P (cf. Leloir, 1959), increased nearly twofold after 6 hrs. Nevertheless, such an increase apparently is not sufficient to explain the early, efficient, and rapid formation of glycogen. Here, one has to consider the stimulating role of G-6-P. As Leloir et al.(1959) have shown, G-6-P is an activator of the synthetase. With our (different) method of assay (incorporation of C-14-labelled "active glucose" (UDPG) into glycogen), we could demonstrate that G-6-P has the properties of a cofactor for the liver enzyme. Without G-6-P, the homogenate shows a definite, but very small activity which amounts to less than 1/30 of the fully activated preparation[1]. Since in our system the action of cortisol is visible first as a rapid rise in G-6-P concentration from very low levels, this increase of the activator should primarily act by increasing the activity of the synthetase present. If one takes the values determined in the homogenate as a basis, the stimulation of the synthetase by the elevated G-6-P niveau at 6 hrs. would be about 10-fold. But in addition, one observes an increase of the enzyme itself, which is nearly doubled at this point. The overall activity may be increased therefore by a factor of 20. The effectivity of this combined stimulation is clearly demonstrated by the behavior of the substrate UDPG: Soon after the increase in G-6-P, the system partially is

1) Purified synthetase behaves in a similar way. These results indicate at the same time that in rat liver, practically no "G-6-P independent" transglucosylase is present (cf.Friedman, 1962).

deprived from its "active glucose" inspite of the fact that increasing levels of G-1-P are formed. Apparently, the polymerisation runs faster than the supply is made[1]. Our results therefore indicate that cortisol induces primarily the formation of G-6-P (by an yet unknown mechanism). The increased polymerisation proper is a secondary process which is achieved by an increased activity of the enzyme synthetase coming about in two ways: 1) by an elevated concentration of the activator G-6-P, and 2) by an (inductive?) augmentation of the enzyme protein[2].

The latter point leads to the question by which factor the augmentation of the synthetase is caused. At first sight, one is inclined to consider it as the result of a direct hormonal action, since the cortisol-induced rise in glycogen and the augmentation of the synthetase can be suppressed by ethionine. This fact, however, is not necessarily in favor of the explanation above. Since, after ethionine administration, the increase in G-6-P is also suppressed, the decisive action of cortisol not only must be sought at an earlier stage of glycogen formation from protein: the failure of the different enzyme levels to rise (esp. synthetase, later on phosphoglucomutase, G-6-P'ase etc.) could also be interpreted as an <u>indirect</u> consequence of the hormone action, then namely, when one considers the G-6-P concentration as the deciding cybernetic factor for the glycogen formation system. In fact, we could show that an increase in G-6-P which was <u>not</u> hormonally induced, but by injection (or feeding) of glucose to adrenalectomized rats,

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1) It is not clear yet if there is also an additional need for UTP.

2) A last proof for a true induction process is still lacking, although a number of tests make it very probable.

produced alterations in the system G-6-P $\rightleftharpoons$ glycogen very similar to the cortisol-induced ones. Here, too, a primary rapid increase in G-6-P concentration is followed by a true increase in synthetase (measured at saturating levels of G-6-P). And here, too, the glycogen synthesis runs so effectively that the substrate UDPG becomes partially exhausted.

At present, these findings may be described best by the terms "precursor activation" and "precursor induction": (G-6-P, a metabolite in an early stage of the glucose polymerisation chain, not only is an activator of the irreversibly acting key enzyme synthetase; it probably also acts as an inducer of the same enzyme[1]).

How far these phenomena bear connections to the action of cortisol on mucopolysaccharide and glycoprotein synthesis, remains to be determined.

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1) A definite decision on the existence of a true "precursor induction" mechanism is only possible with an isolated system. The increase in the (fully activated) synthetase could also be due, for instance, to an increased insulin level provocated by the rise in blood glucose.

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