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GLYCOGEN IN RAT ADIPOSE TISSUE : SEQUENTIAL SYNTHESIS AND RANDOM DEGRADATION

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SUMMARY : In the adipose tissue like in the liver, glycogen molecules are synthesized in a molecular order such that some molecules are completed before others start to grow. In the adipose tissue, the degradation of glycogen occurs at random, whereas, in the liver, molecules that were synthesized last are degraded first and vice versa. This difference in the catabolism of glycogen between the two tissues may be related to the fact that glycogen is in the form of monomeric beta particles in the adipose tissue and of polymeric alpha particles in the liver.

Labeling of glycogen by pulse administration of a radioactive precursor in a period of sustained synthesis has shown that in both the foetal (1) and the adult (2) livers, the synthesis of glycogen is sequential, with the result that some molecules are completely synthesized before others start to grow. Indeed, labeled glucosyl units incorporated at an early stage of synthesis remain equally distributed in the internal and the external chains of the polysaccharide after a several fold increase in the amount of glycogen synthesized. A similar observation has also been done in the case of the "de novo" synthesis of glycogen catalyzed by a partially purified glycogen synthase of *Aerobacter aerogenes* (3). Furthermore, these pulse-labeling experiments have revealed that the phosphorolytic degradation of glycogen in the adult liver is also ordered; thus the molecules that were synthesized last are degraded first and *vice versa*. This ordered degradation was observed *in vivo*, in isolated hepatocytes and in a cell-free system (2).

In the liver, glycogen is present in the form of large alpha particles, made up of subunits called beta particles (4,5). The beta particle seems to

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correspond to the tree-like structure which is usually considered the glycogen molecule. One could therefore hypothesize that the association of these molecules into an alpha particle may be an important factor in determining the order of synthesis and degradation of glycogen in the liver. It thus appears of interest to check if a similar order would be found in tissues in which glycogen is present in the form of beta particles. The adipose tissue was chosen as an appropriate model for this type of experimentation. Indeed, ultrastructural studies have revealed that glycogen is present in the form of beta particles both in the white (6) and the brown (7,8) adipose tissue. Furthermore, glycogen concentration is very low in the adipose tissue under normal nutritional conditions but increases enormously when starved animals are refed. After one or two days of refeeding, however, this glycogen is degraded at about the same rate at which it was formed (9). This experimental model is therefore appropriate for pulse labeling of glycogen in the course of synthesis in order to detect an ordered or random release of radioactivity during the degradation. Preliminary experiments showed, however, that the incorporation of radioactive glucose into glycogen was extremely slow in the white adipose tissue but could be easily measured in the brown adipose tissue which was therefore chosen.

METHODS

Male Wistar rats weighing about 100 g, were fasted for three days and then refed. They received, by intraperitoneal injection, 2 μg (6.10^6 cpm) of [U-^{14}C]glucose dissolved in 0.5 ml of 0.15 M NaCl, either 10 min (early labeling) or 21 hrs (late labeling) after initiation of refeeding. Preliminary experiments had revealed that the content of glycogen in the brown adipose tissue as well as in the liver is maximal at approximately 24 hrs after initation of refeeding. The experimental schedule was set up accordingly. At each time indicated in fig. 1, four rats of each series were killed by decapitation and glycogen was isolated from the liver and the interscapular brown adipose tissue. The methods used for the isolation and the analysis of glycogen as well as the source of materials were as previously described (2).

RESULTS AND DISCUSSION

After one day of refeeding, the interscapular brown adipose tissue of rats previously fasted for 3 days, weighed approximately 300 mg and contained

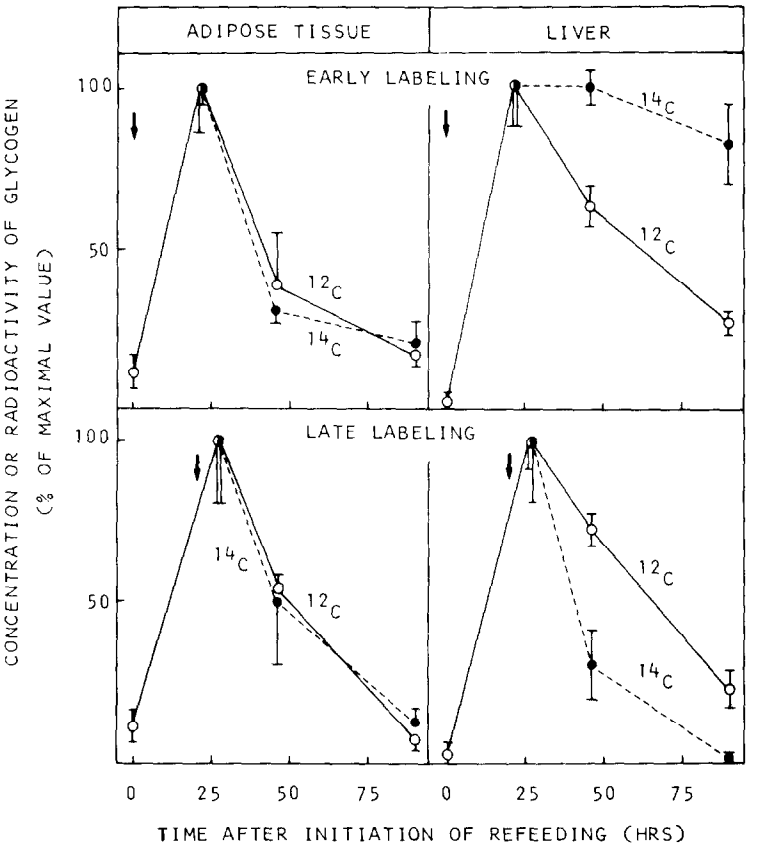

Figure 1. Glycogen synthesis and degradation in the brown adipose tissue and in the liver upon refeeding of fasted rats. The highest concentrations of glycogen that were registered (100 % values) were 5 % (w/w) of the wet weight in the liver and 1 % in the adipose tissue. Arrows indicate the time at which [U-^{14}C]glucose was administered. The radioactivity incorporated in glycogen was, for the early and late labeling respectively, 1200 and 150 cmp per mg of liver glycogen and 2300 and 2100 cpm per mg of adipose tissue glycogen. Beta amylolysis of the latter glycogen revealed no significant difference between the percentage of ^{12}C (mass of polysaccharide) and ^{14}C remaining in the beta dextrin both in the case of early labeling and at times 21, 26 and 46 hrs.

about 3 mg of glycogen and as much as 1 % of the radioactivity that had been administered in the form of [U-^{14}C]glucose, either at the beginning of the refeeding period or 21 hrs later (left part of Fig. 1). The fact that the same amount of radioactivity was incorporated in the two experimental conditions indicates that the rate of synthesis of glycogen was approximately the same in the two experimental periods. Beta amylolysis was used as a tool to analyze the distribution of radioactivity on the inner and the outer

chains of the polysaccharide. It revealed that the specific radioactivity of the beta dextrin was the same as that of the glycogen from which it originated. This observation is in agreement with the results of our previous study of the synthesis of glycogen in the rat liver (1,2) and also with the works of Gahan and Conrad on the *de novo* synthesis of glycogen by a partially purified enzymatic system from *Aerobacter aerogenes* (3). This indicates that glycogen synthase has a high affinity for its product, glycogen, and remains attached to the same glycogen molecule until its synthesis is terminated. A more extensive discussion of this mechanism can be found in a previous publication (2).

The degradation of glycogen in the brown adipose tissue occured during the second and third days after initiation of refeeding. As shown in the left part of Fig. 1, there was no preferential degradation of the glycogen synthesized last nor any delayed degradation of the glycogen synthesized first. These results are in great contrast with those observed in the liver of the same animals and illustrated in the right part of Fig. 1. In this tissue, the preferential removal of the glucose units incorporated last and the delayed degradation of the units incorportated first are in agreement with our previous published data (2). It is also noticeable that much more radioactivity was incorporated into liver glycogen soon after the initiation of refeeding than 21 hours later. This was to be expected since the high rate of synthesis (1 % per hr) known to occur during the first 4 to 6 hrs of refeeding (2), can obviously not be sustained during a much longer period.

There is therefore a remarkable difference between the patterns of degradation of glycogen in the liver and the adipose tissue : indeed, in the liver, glycogen molecules are orderly degraded whereas in the adipose tissue their destruction occurs at random. This difference is presumably related to the fact that the polysaccharide is in a monoparticulate form in the adipose tissue whereas it is in the form of the more complex alpha particles in the liver; it suggests that the ordered degradation is dependent on the alpha

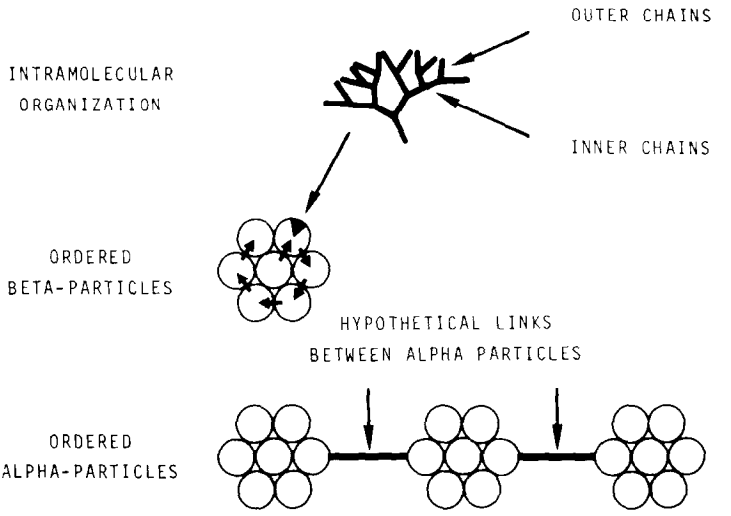

Figure 2. The glycogen molecule, corresponding to a beta particle, is made up of inner and outer chains; the major part of the outer chains corresponding to approximately 50 % of the mass of glycogen can be removed by beta-amylolysis. The alpha particle is made up of a large number (as a mean, approximately 30) of beta sub-units; it is not known at the present time if these subunits are linked covalently or not. One may hypothetize that these beta units are synthetized in a defined order (arrows) inside of an alpha particle and degraded in the reverse order. There may also be an order in the synthesis and degradation of the alpha particles. This second hypothesis requires that there exists a link either physical or dynamic, between several alpha particles.

structure. As more extensively discussed previously (2), this observation may be explained in two ways : 1) the beta particles are synthesized inside of the alpha particles in a defined order and degraded in the reverse direction; 2) several alpha particles are attached to each other by a link which would indicate the way for synthesis and degradation (see Fig. 2). In favor of the second interpretation is the fact that, as revealed by ulstrastructural examination, the difference between a glycogen-rich and a glycogen-poor liver is in the number of alpha particles and not in their size (10).

Another interpretation which cannot be completely discarded is that the random degradation of glycogen in the adipose tissue would be hydrolytic and lysosomal through the process of autophagy. There is, however, to the best of our knowledge, no morphological evidence for such an autophagic process In contrast, the combination of biochemical and morphological data indicate

that autophagy may be responsible for the glycogenolysis which occurs in the neonatal liver and also in the liver of adult rats treated with phlorizin (11).

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