Depletion of cellular glycogen during the early logarithmic growth phase of human fibroblasts

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Summary. Human diploid fibroblasts deplete 50% of their cellular glycogen by day 4 after subcultivation in 100 mg% glucose medium. The glycogen content increases again as the cells approach confluency. Growth of cells in low glucose medium results in rapid glycogen depletion and indicates that stored glycogen has a limited potential as an energy source.

Human skin fibroblast cells in culture synthesize glycogen when a physiological concentration of glucose is available in the medium[2-4]. The stored glycogen is mobilized for glucose formation when the extracellular glucose concentration is reduced[2,4,5]. However, the kinetics of synthesis and degradation of glycogen at different stages of the culture cycle have not been described. Human diploid fibroblasts grow in media lacking glucose if nucleosides are added to the medium[6]. Therefore, the possible role of glycogen in maintaining cell viability after glucose depletion has been raised. The present experiments demonstrate that the glycogen concentration in human skin fibroblasts decreased for 4 days during logarithmic growth and then increased with time as the cells approached confluency when cells are subcultured in medium with physiological concentrations of glucose. At low glucose concentrations, or in glucose-free medium supplemented with nucleosides, the glycogen was rapidly depleted in 8–24 h without impairing cell growth. These results indicate that glycogen is synthesized primarily after cells reach confluency and does not provide the energy required for the long-term growth of cells observed in medium supplemented with nucleosides.

Materials and methods. The cells used in this study were a strain of human diploid fibroblasts (JJ-71). All cell strains were between passage 10 and 15 and were negative for mycoplasma contamination (Microbiological Associates, Bethesda, Maryland). The media consisted of Eagle's minimum essential medium (MEM) with different amounts of glucose, amino acids, purines, pyrimidines, and 10% dialyzed fetal calf serum as previously described[6]. Medium consisting of 10% dialyzed fetal calf serum 100 µM hypoxanthine, 40 µM thymidine, 100 µM uridine, 100 µM glycine, and no added glucose is designated as HTU-MEM. The inoculum for all experiments was grown in 100 mg% glucose for 7 days. The monolayers were rinsed with physiological saline and the cells were dispersed with 0.25% trypsin. The detached cells were suspended in media which contained the same glucose concentration as the proposed test media. 0.5 ml of this inoculum, containing 0.6×10^5 to 1×10^5 cells, was added to 5 ml of medium with the appropriate glucose concentrations in a 60-mm diameter plastic petri dish. Cellular glycogen was determined by the method of Lust et al.[7]. The cell protein was determined by the method of Lowry et al.[8].

Results. The protein content of human fibroblast cells grown in MEM with different glucose concentrations or in HTU-MEM increased with time (figure 1). Cellular protein (µg per dish) increased at a normal rate for 5 days in HTU-MEM and 1 mg% glucose media. The same cell growth pattern was observed in the presence of 5 mg% glucose (data not shown). Cells grown at 20 and 100 mg% glucose showed a continual increase in protein for 7 days. The same results were obtained with 50 mg% glucose (data not shown).

The changes in the glycogen content with time of fibroblasts grown in media containing different glucose concentrations is shown in figure 2. The glycogen content of cells grown either in 1 mg% glucose or in HTU-MEM decreased 80–90% within 24 h after subcultivation and remained low for the remainder of the experiment. In media with 5 and 20 mg% glucose, the initial cellular glycogen decreased 70% after 24 h of growth and continued to decrease gradually thereafter. The glycogen content of cells grown in 50 and 100 mg% glucose decreased to 50% of its initial concentration by day 4, but in contrast to the effects in low glucose media, the glycogen increased after day 4 in both 50 and

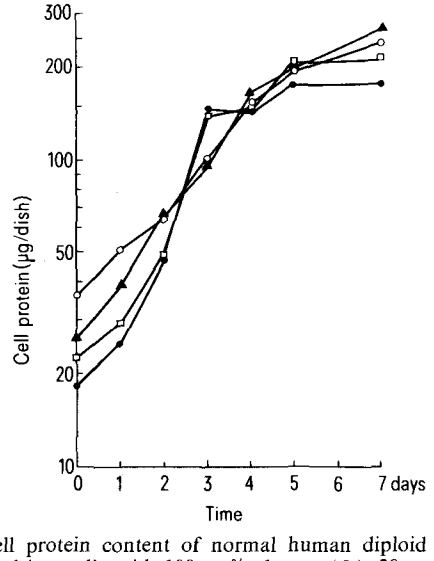

Fig. 1. Cell protein content of normal human diploid fibroblast subcultured in media with 100 mg% glucose (○), 20 mg% glucose (▲), 1 mg% glucose (●) or HTU-MEM (□).

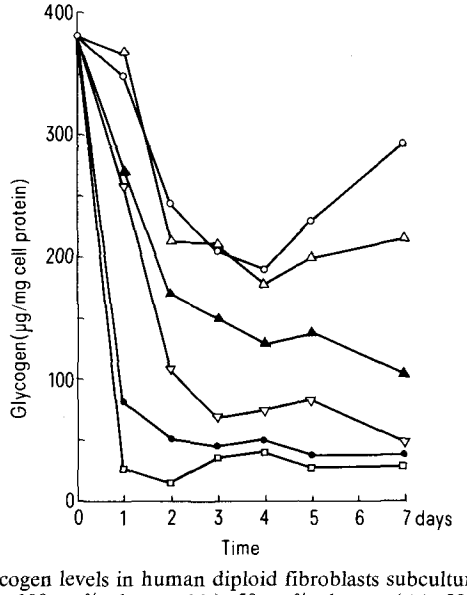

Fig. 2. Glycogen levels in human diploid fibroblasts subcultured in media with 100 mg% glucose (○), 50 mg% glucose (△), 20 mg% glucose (▲), 5 mg% glucose (▽), 1 mg% glucose (●) and HTU-MEM (□).

100 mg% glucose. The rate of increase was greater in medium with 100 mg% glucose than in 50 mg% glucose.

Trypsinization of confluent fibroblasts followed by the addition of 100 mg% glucose medium, without diluting the cell number per dish, did not change the cellular glycogen concentration (data not shown). The decrease in glycogen concentration during the first day after subcultivation was further investigated by refeeding confluent fibroblasts with media containing various concentrations of glucose in lieu of subcultivation (figure 3). 50% of the stored glycogen disappeared from the cells refed with HTU-MEM within 2 h and 97% within 8 h. From 1 to 20 mg% glucose, the rate of glycogen depletion was a function of the concentration of medium glucose. In 50 mg% glucose, the glycogen content did not change. The glycogen content of cells refed with 100 mg% glucose remained unchanged for the first 8 h and then increased 30% during the next 16 h.

Discussion. Glycogen storage and metabolism by human fibroblasts have been studied in glycogen storage diseases[2,3] and in normal controls[4]. The glycogen content for cultures in 50 and 100 mg% glucose increased as the cells approached confluency (figure 2). Seiter and Summer[3] and DiMauro et al.[2] also demonstrated that the glycogen content increased starting 3–5 days after subcultivation, but they did not provide data for early logarithmic growth. The present data showed that when confluent fibroblasts were dispersed and diluted in fresh medium with 100 mg% glucose the glycogen content per mg protein decreased during the early logarithmic phase of growth (figure 2). The rate of depletion of stored glycogen was a function of the glucose concentration (figures 2,3) although the cells continued to grow at a normal rate for 5 days in all media (figure 1). No decrease in glycogen concentration was observed when confluent cells were refed with 100 mg% glucose (figure 3). The trypsinization procedure itself had no effect on the glycogen content. Therefore, the decrease in glycogen observed after subcultivation was a reflection of the growth rate of the cells.

The glycogen content of cells grown in medium with low glucose, or in HTU-MEM which lacked glucose, was depleted within 8–24 h after refeeding. This indicates that glycogen has a very limited potential as an energy source. Under conditions in which the medium glucose and cellular glycogen are depleted, the cells utilize noncarbohydrate energy sources such as glutamine[6,9]. Therefore, the availability of glycogen is apparently not a requirement for cell growth or for maintaining cell viability but rather is a characteristic of in-vivo cells retained by fibroblasts.

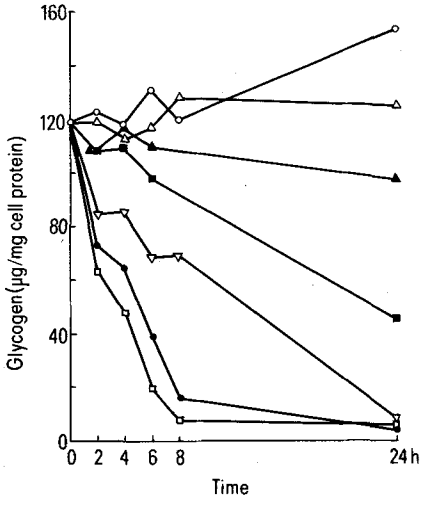

Fig. 3. Glycogen levels after refeeding of confluent cells grown in 100 mg% glucose with media containing different concentration of glucose. 100 mg% glucose (○), 50 mg% glucose (△), 20 mg% glucose (▲), 10 mg% glucose (■), 5 mg% glucose (▽), 1 mg% glucose (●) and HTU-MEM (□).

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Cardiac glycosides in *Danaus chrysippus* (L.) provide some protection against an insect parasitoid

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Summary. The larvae of *Danaus chrysippus* are less susceptible to attack by endoparasitic Diptera of the family Tachinidae if they feed on plants containing cardiac glycosides.

The Old World queen butterfly, *Danaus chrysippus* (L.) (Danaidae), is subject to severe attack by endoparasitic flies (Diptera) of the family Tachinidae[1,2]. In Sierra Leone nearly 100% of larvae were found to be infected at the start of the dry season[1] although in Ghana the peak frequency was only 8% and other parasites predominated[2].

In mid-April 1975 a wild population of *D. chrysippus* at Dar es Salaam, Tanzania, which had been under study since early 1972, suffered a major plague of tachinids which had scarcely abated by August when breeding was terminated. High mortality occurred even in broods reared indoors which was exceptional for in the previous 3 years only one