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Glycogen Accumulation in Cultured Skin Fibroblasts

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The use of skin fibroblasts in the study of inborn errors of metabolism is now a well-established technique (Hsia, 1970); coupled with histological and chemical investigations, it has provided an additional insight into a number of lysosomal storage disorders (Matalon & Dorfman, 1969). Hitherto, however, application of such methods to the glycogen-storage diseases has been limited to enzyme assays (Dancis *et al.*, 1969). Hug *et al.* (1971) were able to demonstrate a Best's Carmine-positive substance in fibroblasts from a case with α -glucosidase deficiency (type II glycogenosis). Other workers (Nitowsky & Grunfeld, 1967) have been unable to demonstrate glycogen accumulation in fibroblasts from cases of glycogen-storage disease. Recently it has proved possible to promote glycogen accumulation in skin fibroblasts by blocking the lysosomal α -glucosidase with the disaccharide trehalose (Brown & Brown, 1972).

Using a histochemical technique developed in this laboratory (Lake, 1970), we have been able to demonstrate glycogen accumulation in skin fibroblasts and to show the dependence of such accumulation on the glucose concentration of the culture medium of the cells.

Skin fibroblasts were obtained by standard procedures (Paul, 1970) and maintained on medium 199 supplemented with foetal calf serum (20%, v/v). Glycogen accumulation was noted in normal skin fibroblasts within 24 h of renewal of the culture medium. As the glucose concentration of the culture medium became exhausted (2–3 days in our experiments), so the glycogen content of the fibroblasts diminished. Virtually complete glycogen depletion of normal skin fibroblasts was noted within 24 h of cells, initially replete glycogen, being transferred to medium 199 without glucose (with or without supplementation with foetal calf serum). Glycogen reaccumulated when such cells were returned to the normal medium containing glucose,

Skin fibroblasts were grown from a case of glycogen-storage disease of unknown type (liver glycogen content 8.5%, w/w). The depletion of glycogen from these fibroblasts maintained in glucose-free medium was much slower than in control cultures maintained similarly. Some glycogen was still evident after 48 h under these conditions.

From our experiments we conclude that glycogen accumulation is a normal function of skin fibroblasts; such accumulation may to some extent cushion these cells against the effects of glucose deprivation. Such histochemical observations may serve to complement other techniques used in the study of glycogen-storage diseases, and the fact that, in cell cultures, glycogen metabolism bears an apparently simple relationship to glucose availability may be of value in studying the control of such metabolism and pathological situations.

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Activation and Phosphorylation of the Subunits of Phosphorylase Kinase

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Phosphorylase kinase is a key enzyme in the nervous and hormonal regulation of glycogenolysis in skeletal muscle. Its activity is totally dependent on Ca^{2+} (Heilmeyer *et al.*, 1970; Brostrom *et al.*, 1971) released from the sarcoplasmic reticulum after electrical excitation, and it is also linked to adrenaline via adenylate cyclase and cyclic-AMP (adenosine 3':5'-cyclic monophosphate)-dependent protein kinase, the latter enhancing the activity a further 20–30-fold at physiological pH (Walsh *et al.*, 1971).

Purified rabbit muscle phosphorylase kinase was homogeneous by sedimentation velocity (22.4S) but showed four bands staining for protein on gel electrophoresis in sodium dodecyl sulphate. Proteolytic enzyme inhibitors added during the preparation, or denaturation of the final product in various ways designed to eliminate proteolysis and ensure complete reduction and denaturation, did not alter the gel pattern. The subunit molecular weights were: α , 132000;