ON THE DIFFERING APPEARANCE OF INTRANUCLEAR AND CYTOPLASMIC GLYCOGEN IN LIVER CELLS IN GLYCOGEN STORAGE DISEASE

H. SHELDON, M. SILVERBERG, and I. KERNER. From the Pathological Institute, McGill University, and The Montreal Children's Hospital, Canada

With new methods of preparing thin sections for electron microscopy, glycogen has been identified in the cytoplasm of liver cells as dense particles of various sizes (3, 4, 7, 9). Glycogen does not usually occur within the nucleus, but it has been described there in a variety of pathological conditions (1). This report concerns the appearance of glycogen in the nucleus of liver cells from a patient with glycogen storage disease, type I[1] (von Gierke's disease).

MATERIALS AND METHODS

Specimens of liver were obtained from a wedge biopsy of a 6-year-old male patient (MCH-135536) with hepatomegaly, retarded physical development, a flat adrenalin tolerance curve, and abnormal liver function tests. Markedly decreased glucose-6-phosphatase and normal debrancher enzyme activity was found in the specimen of liver. For electron microscopy, the tissue was fixed for 1 hour in either a cold 1 per cent potassium permanganate or osmium tetroxide solution buffered to pH 7.4 in sodium Veronal acetate which contained a mixture of salts (13). Dehydration in graded alcohols was followed by embedding in Epon 812 (5). Sections were cut with a glass knife on either an LKB or Porter-Blum microtome, mounted on carbon-coated Formvar films, stained with lead hydroxide according to either Watson (12) or Millonig (6), and examined in an RCA EMU-3E electron microscope.

For light microscopy, specimens were fixed in alcohol or cobalt formalin, embedded in paraffin and stained with Best's carmine for glycogen, or haematoxylin and eosin. Frozen sections were made for fat stains.

The stain for glycogen was controlled by diastase digestion which removed the nuclear and cytoplasmic material which stained with Best's carmine.

OBSERVATIONS

The large liver cells contain much glycogen in their cytoplasm when seen in the light microscope. Many liver cells contain a large centrally placed intranuclear glycogen body which stains in the same way as the cytoplasmic glycogen (Fig. 1). Because of the staining qualities of this body, it can be differentiated from the nucleolus or other intranuclear inclusions; Pollister and Himes have recently discussed the identification of intranuclear glycogen (8). Fat stains show small lipid droplets in the cytoplasm of most of the liver cells.

Electron microscope observations show that the cytoplasm of these liver cells contains closely packed, large, dense rosettes which are fairly

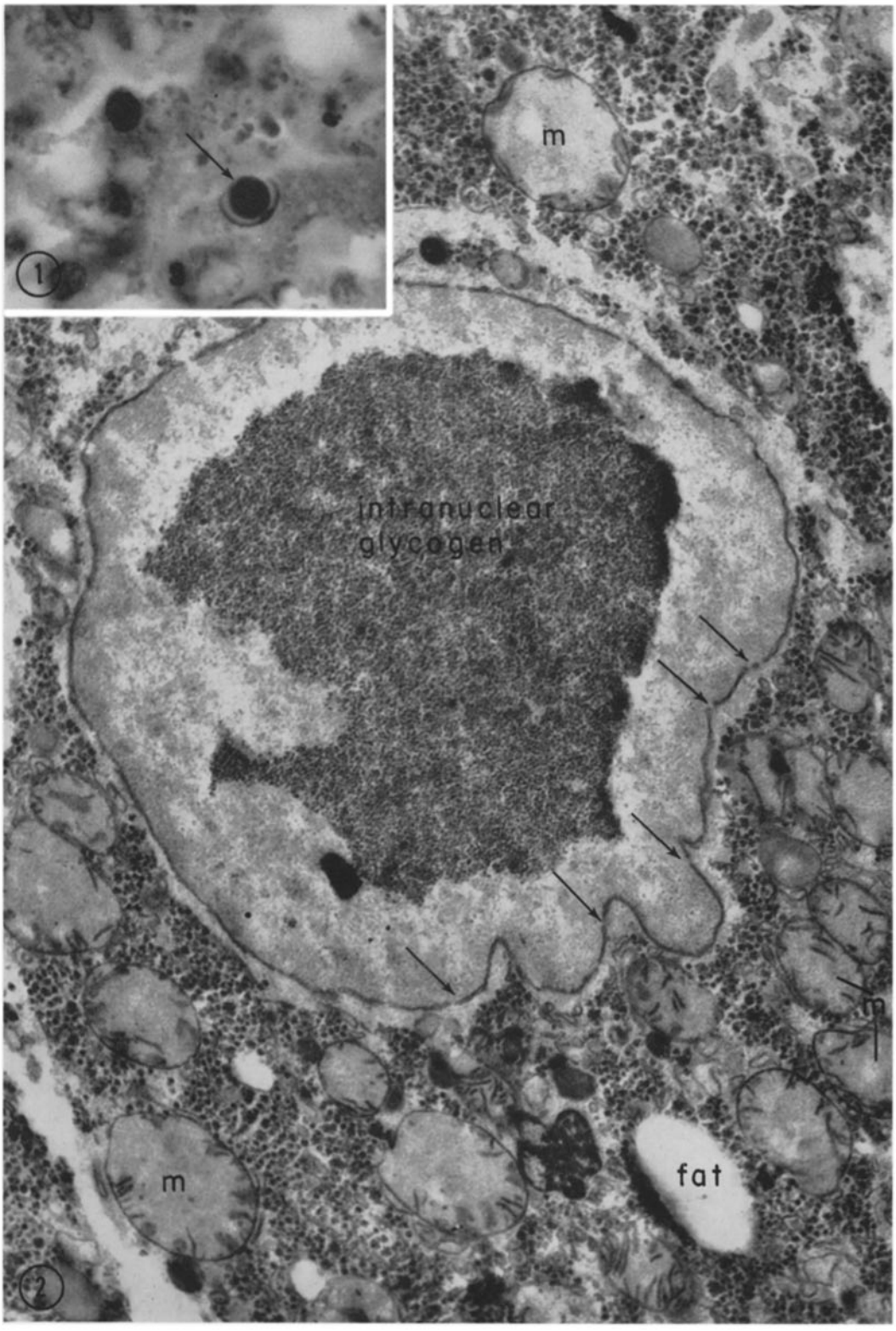
[1] Glycogen storage diseases are inborn errors of metabolism in which there is defective metabolism of glycogen due to deficiency of various enzymes associated with glycogenolysis. Several different biochemical lesions have been described since the original description of a deficiency in glucose-6-phosphatase (2, 10).

FIGURE 1

This light micrograph of a section of liver from glycogen storage disease, type I, was stained with Best's carmine stain for glycogen. It shows the presence of glycogen within the nucleus of a liver cell (at arrow). Magnification, approximately 1000.

FIGURE 2

This electron micrograph of a thin section of liver from the same patient shows the appearance of the glycogen in the cytoplasm and within the nucleus of the cell. Even at this comparatively low magnification one can see a difference in the particle sizes. The nuclear envelope shows numerous interruptions in its circumference (arrows). The mitochondria (*m*) in this cell do not differ from those which have been described in normal liver cells. This specimen was fixed in potassium permanganate. Magnification, approximately 11,000.


1
m
intranuclear
glycogen
m
m
fat
2

uniform in size; they measure about 950 A, and conform to the descriptions of cytoplasmic glycogen in thin sections as reported by Drochmans (3), Karrer (4), Millonig and Porter (7), and Revel, Napolitano, and Fawcett (9). After establishing the generally similar appearance of the glycogen particles in stained specimens which had been fixed with either osmium tetroxide or potassium permanganate, we found it convenient to study chiefly the permanganate-fixed material.

A single liver cell is shown in Fig. 2, where abundant cytoplasmic glycogen can be seen. The mitochondria are not remarkable, and the elements of the endoplasmic reticulum, both rough and smooth surfaced, are scanty in these liver cells. Most cells contain appreciably more fat than is shown here, and many cells contain dense bodies which may be lysosomes. The nucleus in the cell in Fig. 2 is bounded by a convoluted envelope in which well defined nuclear pores can be seen. The majority of the cytoplasmic glycogen particles appear larger than the stomata of the nuclear pores. In the centre of the nucleus, separated from the nuclear envelope by a clear band of nucleoplasm, there is an agglomeration of dense, granular glycogen material which appears particulate at higher magnifications (Fig. 3).

The intranuclear glycogen is different from the cytoplasmic glycogen in that the particles are demonstrably smaller than the rosettes in the cytoplasm. They are approximately 300 A in diameter. It is possible that the material in the nucleus only appears particulate, and that the discrete nature of the granules may be an illusion of the thin section. Conceivably, the particles may be attached to one another like pearls on a string. The particles of intranuclear glycogen have a smaller diameter than the openings in the nuclear envelope. There are also small (50 A) densities in both the cytoplasmic and intranuclear glycogen particles, as described previously by Revel, Napolitano, and Fawcett (9).

DISCUSSION

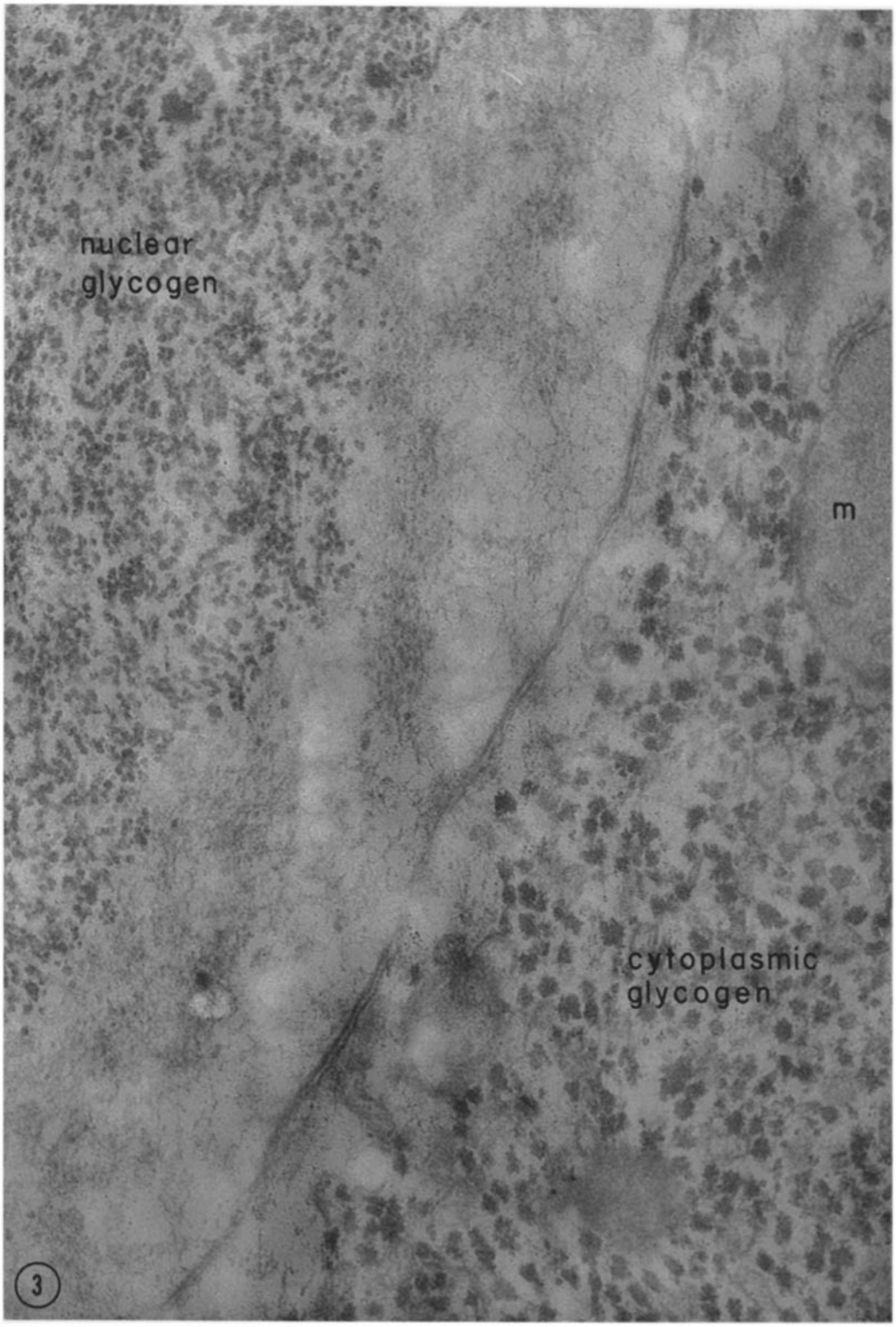
A variable appearance of glycogen in thin sections has been described by those investigators who have shown that different methods of preparation result in differences in its appearance (4, 7, 9). In the present study we need to account for different appearances in the same cell where such factors as autolysis, osmolarity, pH, temperature and nature of the fixative, and embedding material are constant. Even the fact that a large particle may only be partly included in a given section cannot explain the difference in appearance between the populations of cytoplasmic and intranuclear glycogen granules. Because glycogen is known to vary in its molecular weight, and a range of particle sizes has been visualized in other studies, the suggestion has been made that the larger particles may represent aggregates of smaller units (4). This could be true in the present instance, but it seems odd then that there are no large particles among the small ones in the nucleus.

There are reasons to expect variations in the morphology of glycogen molecules; it has been said that no two molecules are identical. In any cell it would be difficult to imagine a population of glycogen molecules in which none were being either synthesized or degraded. Stetten states that there are probably many glycogens, and glycogen might better be used as a generic rather than a specific term (11).

A situation in which one might expect to find a more homogeneous population of glycogen molecules is any one of the glycogen storage diseases. Since glycogen can be synthesized but not broken down, it accumulates within the cell. In type I glycogen storage disease, on which the present observations were made, there is a deficiency of glucose-6-phosphatase. Deficiency in this enzyme permits the synthesis of normal glycogens

FIGURE 3

This electron micrograph of a thin section of liver from glycogen storage disease, type I, shows the appearance of intranuclear and cytoplasmic glycogen at higher magnification. A mitochondrion is shown at *m*. The nuclear envelope runs from the upper right to the lower left. This specimen was fixed in osmium tetroxide. Magnification, approximately 32,000.


nuclear
glycogen
m
cytoplasmic
glycogen
3

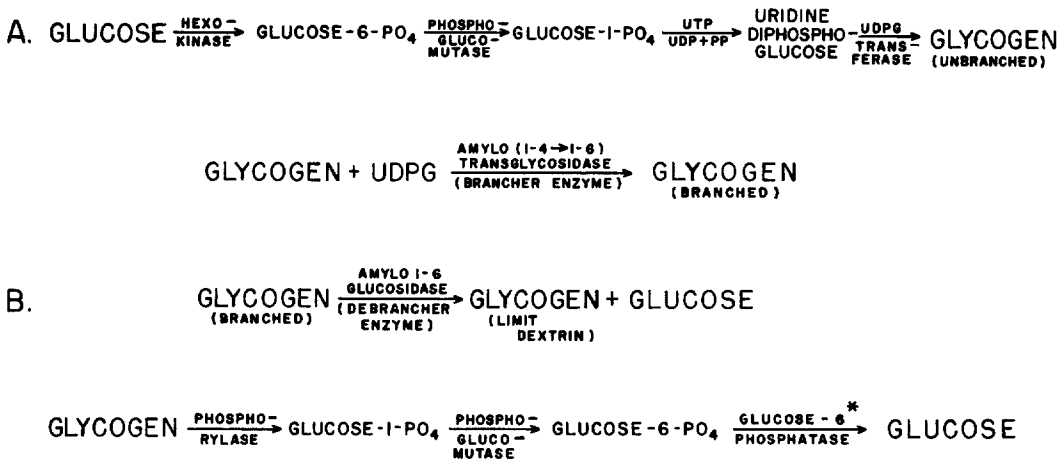


FIGURE 4

This simplified scheme for glycogenesis (*A*) and glycogenolysis (*B*) illustrates the role of some of the enzymes which have been identified in glycogen synthesis and degradation. From the diagram one can see that a branched glycogen is formed by the action of amylo (1–4 → 1–6) transglycosidase; such a branched glycogen can be debranched by amylo (1–6) glucosidase. Deficiency of either of these enzymes may result in a glycogen storage disease, as may a deficiency in phosphorylase. In the present case, glucose-6-phosphatase (*), the last step in the glycogenolytic pathway (*B*) is absent.

as shown in Fig. 4 in which a simplified scheme for the pathways of glycogenesis and glycogenolysis is outlined. Our observations indicate that the cytoplasmic glycogen particles in type I glycogen storage disease do not differ significantly from the glycogen of the normal liver. Perhaps the material in the nucleus represents an unbranched or short-chained glycogen which has been sequestered therein, since small molecules can pass through the nuclear pores while the larger, branched molecules cannot. Alternatively, perhaps the physical nature of the contents of the nucleus or the presence or absence of enzymes within the nucleoplasm may result in an intranuclear glycogen which differs in some respects from the cytoplasmic glycogen.

Further studies on these naturally occurring but rare inborn errors of metabolism may demonstrate to what extent the disposition and appearance of glycogen vary *in situ*, and may provide a means whereby differences in the appearance of cellular organelles can be correlated with the presence or absence of enzymes concerned with glycogenesis and glycogenolysis.

SUMMARY

Light and electron microscope observations on liver cells from a case of glycogen storage disease in which there is a deficiency of glucose-6-phosphatase show the presence of glycogen within the nucleus of many cells. Electron micrographs demonstrate that the particles of glycogen within the nucleus are uniformly smaller than those in the cytoplasm.

Note Added in Proof. A recent study on glycogen storage disease has come to our attention (SALAMON, J. C., HABIB, R., and BERNHARD, W., Étude au microscope électronique de la glycogénose hépatique, *Path. et Biol.*, 1961, **9**: 1251) in which intranuclear glycogen deposits are identified but no comment is made on the differing size of the populations of intranuclear and cytoplasmic particles.

The technical assistance of P. M. Spicer, A. Strömblad, and B. Jönsson is gratefully acknowledged.

This study was supported by Grant A-4422 from the United States Public Health Service, National Institute of Arthritis and Metabolic Diseases, and by Dominion Provincial Health Grant 604-13-58. Dr. Sheldon is a Markle Scholar in Medical Science.

Received for publication, January 25, 1962.

REFERENCES

1. CHIPPS, H. D., and DUFF, G. L., Glycogen infiltration of the liver cell nuclei, *Am. J. Path.*, 1942, **18**, 645.
2. CORI, G. T., Glycogen structure and enzyme deficiencies in glycogen storage disease, *Harvey Lectures*, 1953, **48**, 145.

3. DROCHMANS, P., Mise en évidence du glycogène dans la cellule hépatique par microscopie électronique, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 553.
4. KARRER, H. E., Electron microscope observations on chick embryo liver: Glycogen, bile canaliculi, inclusion bodies and haematopoiesis, *J. Ultrastruct. Research*, 1961, **5**, 116.
5. LUFT, J. H., Improvement in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
6. MILLONIG, G., A modified procedure for lead staining of thin sections, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 736.
7. MILLONIG, G., and Porter, K. R., Structural elements of rat liver cells involved in glycogen metabolism, Proceedings of European Regional Conference on Electron Microscopy, Delft, De Nederlandse vereniging voor Electronenmicroscopie, 1960, 655.
8. POLLISTER, A. W., and HIMES, M. H., A comparison of nucleolus and intranuclear glycogen body, abstract, American Society for Cell Biology, Chicago, 1961, 169.
9. REVEL, J. P., NAPOLITANO, L., and FAWCETT, D. W., Identification of glycogen in electron micrographs of thin tissue sections, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 575.
10. SIDBURY, J. B., CORNBLATH, M., FISHER, J., and HOUSE, E., Glycogen in erythrocytes of patients with glycogen storage disease, *Pediatrics*, 1961, **27**, 103.
11. STETTEN, D., JR., and STETTEN, M. R., Glycogen metabolism, *Physiol. Rev.*, 1960, **40**, 505.
12. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals. II. Application of solutions containing lead and barium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 727.
13. ZETTERQVIST, H., The Ultrastructural Organization of the Columnar Absorbing Cells of the Mouse Jejunum, Stockholm, Aktiebolaget Godril, 1956.