

GLYCOGEN-MEMBRANE COMPLEXES WITHIN MOUSE STRIATED MUSCLE CELLS

PHILIAS R. GARANT. From the Harvard School of Dental Medicine, Boston, Massachusetts 02115

INTRODUCTION

During the course of an investigation of the fine structure of mouse tongue striated muscle, an unusual glycogen-membrane complex was frequently observed within the peripheral sarcoplasm. Since the role of smooth membranes in glycogen metabolism is the subject of considerable disagreement, it was felt that a report of these glycogen-membrane complexes would be of interest. Glycogen-membrane arrays resembling the complexes described herein, were reported within liver cells of ethionine-intoxicated rats by Steiner et al. (18). Similar structures have been observed in human hepatocytes of patients with alcoholic hepatitis (2,

14), cirrhosis (2), and obstruction of the bile duct (2). It appears that glycogen-membrane complexes have not been previously reported within striated muscle cells.

MATERIAL AND METHODS

Specimens obtained from the anterior two-thirds of the tongue of twelve 10-day-old Swiss albino mice were fixed for 2 hr at 4°C in a 4% glutaraldehyde solution buffered to a final pH of 7.4 with Millonig's phosphate buffer. The tissue was then rinsed in buffer and subsequently postfixated at 4°C in 1% osmium tetroxide pH 7.4 (Millonig's phosphate buffer). Dehydration was rapidly carried out in a graded series of ethanol at 4°C followed by embedding in Epon. Ultrathin sections were cut on a

Porter-Blum microtome equipped with a diamond knife, stained with both uranyl acetate and lead citrate at room temperature, and examined in an RCA-EMU-3G electron microscope at 50 kv.

RESULTS

The peripheral sarcoplasm of the striated muscle cells contained several nuclei, large numbers of mitochondria, moderate glycogen deposits, isolated profiles of rough endoplasmic reticulum, and an occasional Golgi region usually located in a juxtannuclear position. Glycogen-membrane complexes were frequently observed in the peripheral sarcoplasm closely associated with mitochondria and glycogen stores (Figs. 1, 3). Although a complex was sometimes located adjacent to the Golgi apparatus (Fig. 1), a consistent relation with any specific cytoplasmic organelle, other than mitochondria, was not observed. The glycogen-membrane complexes were composed of a semicircular (Fig. 1), circular (Fig. 2), or flat (Fig. 5) arrangement of alternating smooth-membraned cisternae and narrow cytoplasmic bands. Each cisterna con-

sisted of a pair of parallel membranes demarcating an inner cavity approximately 300–450 Å wide containing a fine granular material of moderate electron opacity (Fig. 2). On occasion, this granular material seemed to be concentrated into a membrane-like structure in the center of the cavity, producing cisternal segments with an apparent quintuple-layering of three membranes and two intermembranous spaces (Fig. 2). Vesicular enlargements occurred at irregular intervals along each cisterna, and towards the outer zones of the complex they appeared as larger saccular dilations containing delicate fibrillar material (Figs. 2, 4, and 5).

Glycogen particles, 250–350 Å in diameter, were always disposed in a single file within the narrow cytoplasmic regions located between adjacent cisternae (Figs. 3–5). The glycogen within the complex was always of the mono-particulate variety whereas larger poly-particulate rosettes of glycogen were often seen near the periphery of the complex (Figs. 1 and 4).

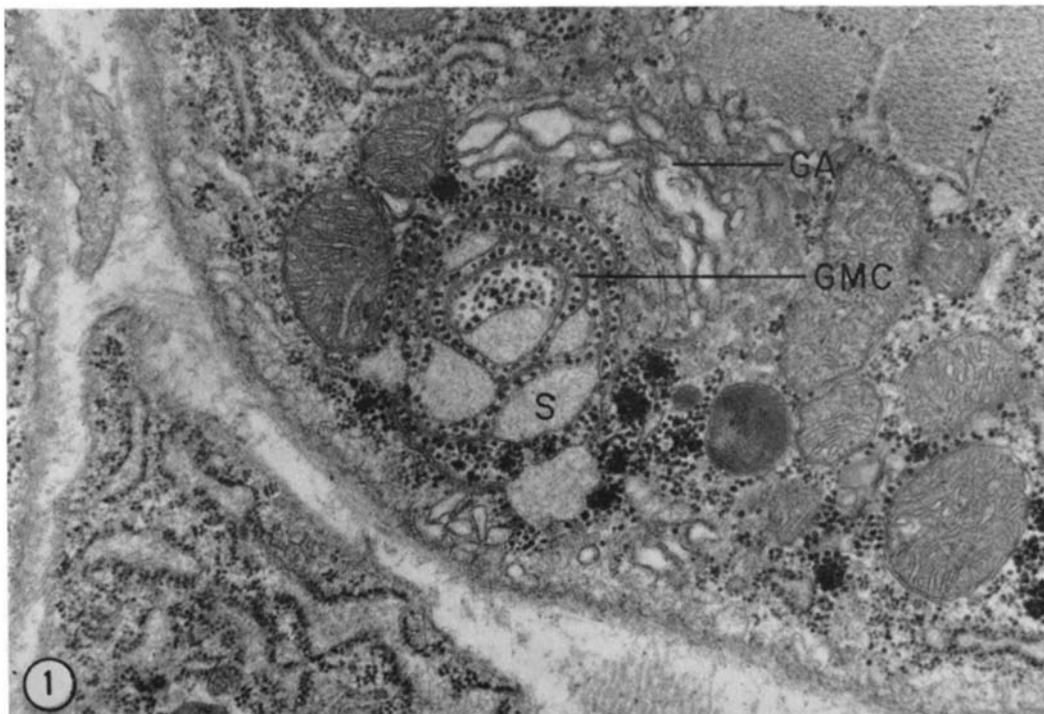


FIGURE 1 Glycogen-membrane complex (*GMC*) situated near some vesicular elements of the Golgi apparatus (*GA*). Note the saccular dilations (*S*) containing fibrillar material. $\times 29,000$.

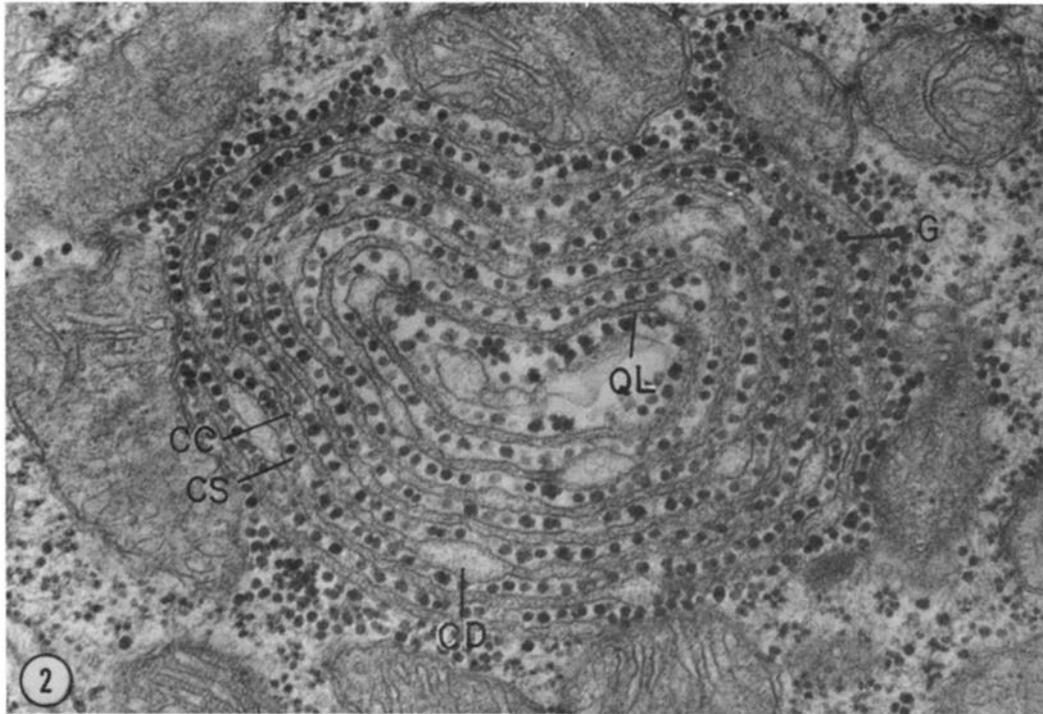


FIGURE 2 Glycogen-membrane complex composed of a circular arrangement of alternating smooth-membraned cisternae (CC) and narrow bands of cytoplasm (CS) containing glycogen granules (G). Cisternal dilations (CD) and quintuple-layered regions (QL) occur at irregular intervals along the length of each cisterna. Note the close apposition of several mitochondria to the outermost cisternae. $\times 55,000$.

Although mitochondria were often closely opposed to the outermost cisternae (Figs. 1-4), no continuities between the two were ever noted, nor were any observations made which might suggest possible continuity between the complexes and the membranes of the sarcotubular system or of the Golgi apparatus.

DISCUSSION

Because of the close morphological relationship between glycogen particles and smooth membranes, it is often tempting to conclude that the membranes play a significant role in glycogen metabolism. Electron microscopic studies of hepatocytes from rats which have been exposed to fasting (4, 11, 12, 15) refeeding (4, 11), and carcinogens (15), have led to the conclusion that the smooth membranes of the agranular endoplasmic reticulum play an immediate role in glycogen metabolism. Recently Coimbra and Leblond (3), using electron microscopic radioautography to

trace the uptake of glucose- ^3H by liver cells, have suggested that the smooth endoplasmic reticulum may be involved in the early stages of glycogen granule formation.

Several electron microscopic studies (6, 7, 13, 17), however, have shown that the storage and breakdown of glycogen in the liver occur without any apparently related changes in the smooth endoplasmic reticulum. In addition, it has been shown that brown adipose tissue and avian glycogen bodies are capable of storing large amounts of glycogen although they do not contain a system of smooth membranes (16).

Glycogen synthetase and ADPG pyrophosphorylase, two enzymes in the pathway from glucose-1-phosphate to glycogen, have been located in the supernatant fraction of liver cells (8, 10, 19). Since glycogen synthetase was not bound to microsomal membranes, Luck (10) concluded that the smooth endoplasmic reticulum of liver cells had no direct enzymatic role in glycogenesis. It has been

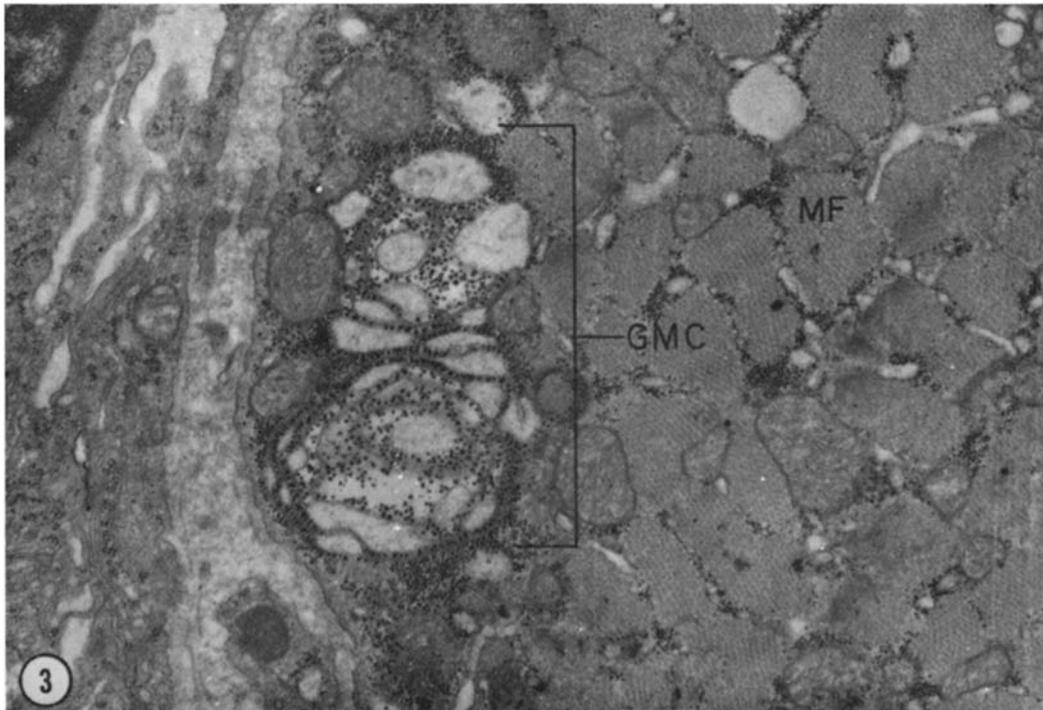


FIGURE 3 Portion of striated muscle cell showing myofibrils (*MF*) cut at an oblique angle and a large glycogen-membrane complex (*GMC*) located within the peripheral sarcoplasm. $\times 17,000$.

demonstrated, however, that a smooth membrane-rich fraction of liver cells contains a factor capable of activating glycogen synthetase (5). Thus, it would seem that although the principal enzymes required for glycogen synthesis are not attached to the agranular endoplasmic reticulum of liver cells, the smooth membranes might function indirectly in glycogenesis by controlling the activity of glycogen synthetase. In fact, London (9), in discussing the results of an investigation of a mathematical model of hepatic glycogen metabolism, has suggested that the assumption that the reactions occur in a homogeneous phase without compartments is not a good one.

It is evident from the above discussion that, despite numerous advances in our knowledge, the role of membranes in hepatic glycogen metabolism is still far from being completely resolved. In addition, the possibility that the results obtained from liver cells can be applied to other cell types, and in particular to muscle cells, has yet to be worked out. For example, Andersson-Cedergren and Muscatello (1), contrary to what one would expect from the data obtained from liver cells,

have reported that glycogen synthetase is present in the sarcotubular fraction and not in the supernatant fraction of striated muscle cells. They concluded that membranes of the sarcotubular system play a direct role in glycogenesis.

Whether the glycogen-membrane complexes described in this paper represent a purely fortuitous arrangement of glycogen granules and smooth membranes or a functional unit cannot be answered at present. Work has been undertaken to determine whether or not the complexes are present in muscle cells before birth and what changes, if any, might occur during the first few days postnatal. The possibility of inducing changes in the complexes by means of hormones is also under consideration.

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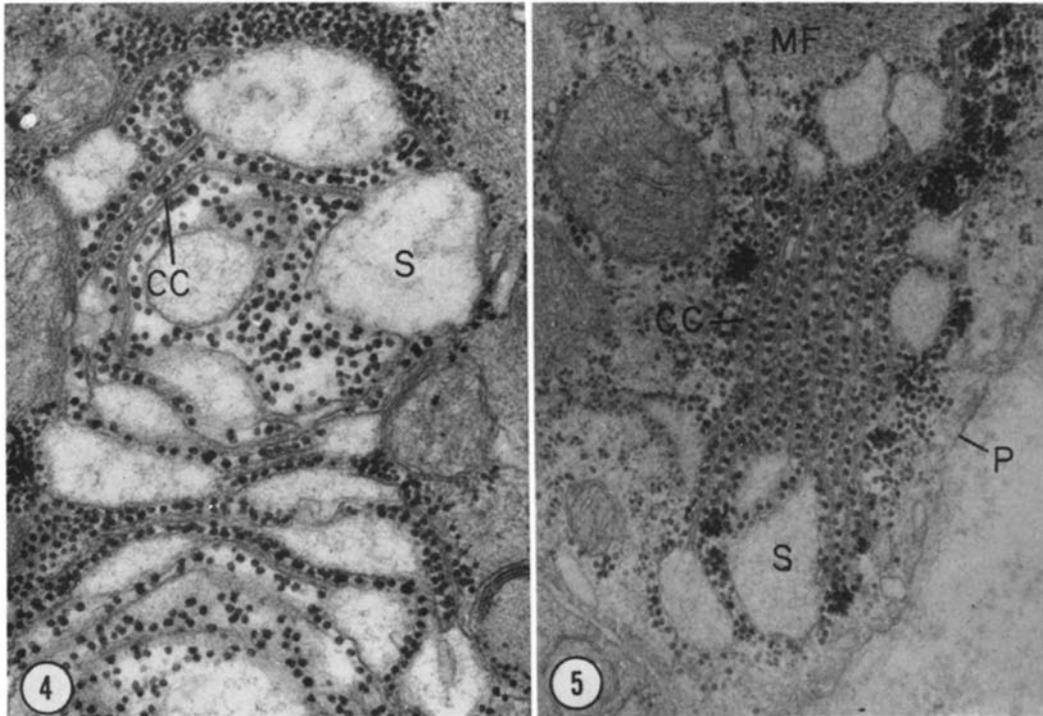


FIGURE 4 Enlarged view of part of the glycogen-membrane complex depicted in Fig. 3. Continuities between cisternal cavities (CC) and the saccular dilations (S) are clearly visible. $\times 45,500$.

FIGURE 5 Glycogen-membrane complex of the flat type located just within the plasmalemma (P). Saccular dilations (S) arise from the cisternae at both ends of the complex. MF, myofibrils. $\times 35,000$.

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