

INTRAMITOCHONDRIAL GLYCOGEN GRANULES IN DIGESTIVE CELLS OF *HYDRA*

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Digestive cells of hydra contain accumulations of irregular particles, 200 to 300 Å in diameter and of low density, that stain with lead hydroxide (11). These particles were observed in clear areas of hyaloplasm and were often partially or completely enclosed by mitochondria. Similar dense particles in the hyaloplasm of digestive cells have been identified as glycogen (7, 16), although no association with mitochondria was noted. However, some other electron microscopic studies have identified glycogen within the mitochondria of the silkworm prothoracic gland (2), of sperm (1), of retinal receptor cells (9), and associated with the surface of mitochondria of muscle (17). The present paper reports further on the relationship of particles to mitochondria and presents evidence that this material is particulate glycogen.

MATERIALS AND METHODS

Hydra littoralis, cultured according to the methods of Loomis and Lenhoff (12), were used in these investigations. Intact hydra were fixed for 45 min in cold buffered 4% glutaraldehyde (15) followed by a second fixation in cold buffered 1% osmium tetroxide containing 0.4 M sucrose. Hydra were fixed after a 3-wk starvation period and 1, 2, 6, 12, and 24 hr following a feeding of the living nauplii of *Artemia salina*, the common brine shrimp. Some hydra were subjected to digestion with saliva or α -amylase (0.5% in 0.1 M phosphate buffer, pH 7, and 0.02 M NaCl) for 1 hr following glutaraldehyde fixation, but prior to osmium tetroxide fixation. The fixed hydra were dehydrated in graded concentrations of ethanol and embedded in Maraglas (6). Thin sections were cut on a Porter-Blum microtome, and some were stained lightly with lead hydroxide (5) prior to examination with an RCA EMU 3F microscope. All

observations were performed on the digestive cells of the stomach region.

RESULTS

In well fed hydra, some mitochondria at the apex of the gastrodermal cells showed a relationship to presumed glycogen particles. In these cells, a portion of the mitochondrion that abutted on the hyaloplasmic area containing the presumed glycogen particles was irregular, indented, or deficient of outer membranes (Fig. 1). This created an image which suggested that the hyaloplasm containing the particles was continuous with the interior of the mitochondrion. Other mitochondria in these cells, particularly those at the base, showed no unusual fine structural features. In addition, discrete areas of hyaloplasm contained presumed glycogen particles, but no mitochondria or other organelles were seen, in the plane of the section, adjacent to these areas (Fig. 1). Presumed glycogen particles were also scattered throughout the apical cytoplasm and not confined to glycogenic areas.

Similar mitochondria were not found in digestive cells of hydra that had been fasted for 3 wk. Instead, mitochondria in the fasted animals were intact with the usual arrangement of outer membranes and cristae (Fig. 2). None of the presumed glycogen particles were found within the mitochondria or in the hyaloplasm. Treatment of starved hydra with amylase did not remove any of the particles or granules in the cytoplasm, suggesting that the remaining particulate forms were ribosomes.

When starved hydra were fed *Artemia*, several changes were noted. Presumed glycogen particles

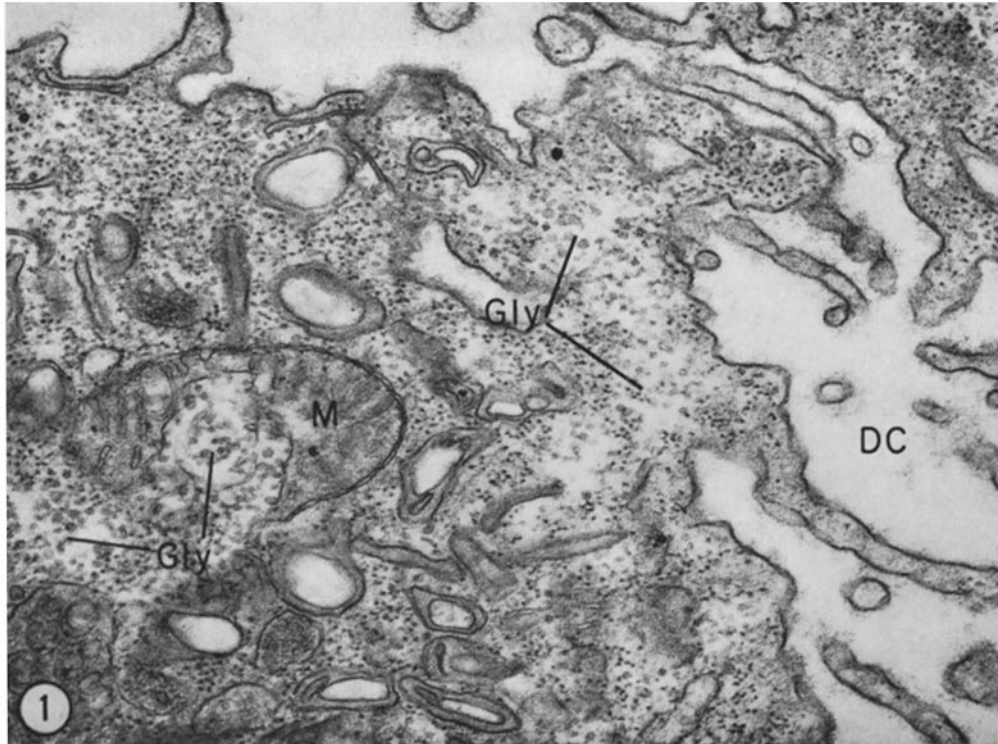


FIGURE 1 Apical surface of a digestive cell of a regularly fed hydra (*Hydra littoralis*). Fixed in glutaraldehyde and osmium tetroxide and lightly stained with lead hydroxide. Glycogen granules (*Gly*) occur in areas of the cytoplasm devoid of other organelles or are scattered about singly. One of the glycogenic areas is partially enclosed by an indented mitochondrion (*M*). Microvilli project into the digestive cavity (*DC*). $\times 46,000$.

reappeared scattered throughout the apical cytoplasm of gastrodermal cells within 1 to 2 hr after feeding. These particles were not confined to areas of the cytoplasm devoid of organelles previously referred to as glycogenic areas. Between 12 and 24 hr after feeding, changes occurred in the apical mitochondria. A clear area bounded by a single membrane appeared in the center of the mitochondrial matrix (Fig. 3). This area contained irregular granules, 200 to 300 Å in diameter, identical with the cytoplasmic glycogen particles. Although these clear centers could be interpreted as invaginations of the hyaloplasm into the mitochondria, this is probably not the case since only one membrane limited the space. In addition, in fortuitous sections the clear central space was continuous with the space between the inner and outer mitochondrial membranes and the intracristal space (Fig. 4). Thus, the membrane lining the space was continuous with the internal mito-

chondrial membrane, so that the glycogen was located in an expanded intracristal space (Fig. 4). The time or rate of occurrence of these changes was not the same in all apical mitochondria, so that different stages were present in the same section. Furthermore, at any time, in some cells almost all of the mitochondria contained granules, while in other digestive cells relatively few mitochondria showed these changes. However, on the basis of the most frequent images observed at each stage, the following general sequence of changes seemed to be the rule. Twelve hr following feeding, a small clear space appeared within the mitochondrion, causing little distortion of the organelle, and the number of contained granules was small (Fig. 3). The number of granules then increased markedly so that the space they occupied distorted the internal arrangement of the mitochondrion and at a later stage (24 hours after feeding and regularly fed hydra) sometimes caused the external

membrane to bulge at one side (Fig. 5). Following this stage, and most commonly in regularly fed hydra, the mitochondrion ruptured and the granule-laden space became continuous with the hyaloplasm, with some egress of granules from their intramitochondrial location (Figs. 5, 6). In some cases, the glycogen granules in the hyaloplasm were partially surrounded by a semicircle of mitochondrial framework, the proximal surface of which was virtually disrupted and the distal surface of which was intact (Fig. 6). In other instances, the granules appeared to be discharged from the mitochondria but were still enclosed in a membranous envelope derived from either the

inner or outer mitochondrial membrane or both (Fig. 5). After the discharge of granules, a number of the mitochondria appeared to fragment.

The particles, whether hyaloplasmic, intramitochondrial, or partially surrounded by a disrupted mitochondrion, were considered to be glycogen because they were 200 to 300 Å in size, irregular in shape, showed less native density than ribosomes, stained with lead hydroxide, and, most significantly, were entirely absent after treatment with either saliva or amylase (Fig. 7). Digestion with saliva also appeared to remove ribosomes (Fig. 7), although this was not the case with amylase.

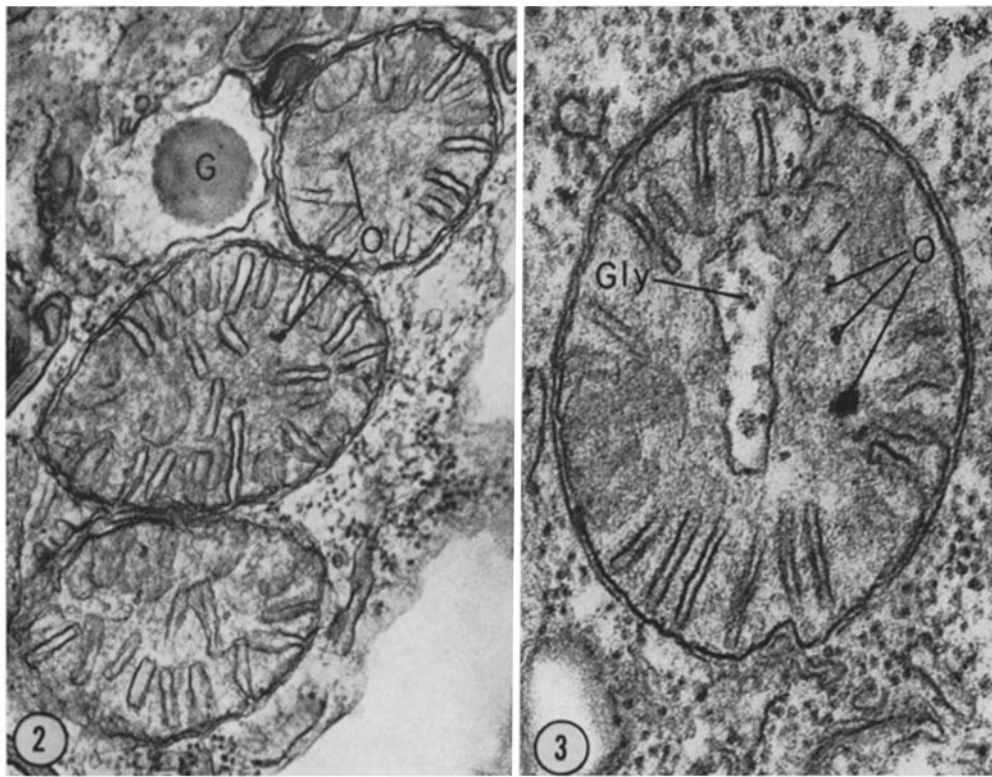


FIGURE 2 Mitochondria in a digestive cell of a hydra that had been starved for 3 wk. Note that these mitochondria show no unusual morphological features. The limiting membranes are intact and there are no indentations or central membrane-limited spaces containing granules. Opaque granules (*O*) are present in the mitochondrial matrix. The hyaloplasm contains free ribosomes, but 300Å glycogen granules are absent. A large membrane-bounded granule (*G*) of unknown nature is present in the cytoplasm. $\times 60,000$.

FIGURE 3 Mitochondrion in a digestive cell of a hydra that had been fed 24 hr previously. A central, electron-lucent space bounded by a single membrane contains irregular, moderately dense particles (*Gly*). Similar granules are also present in the surrounding cytoplasm. Opaque granules (*O*) are present in the mitochondrial matrix. $\times 103,000$.

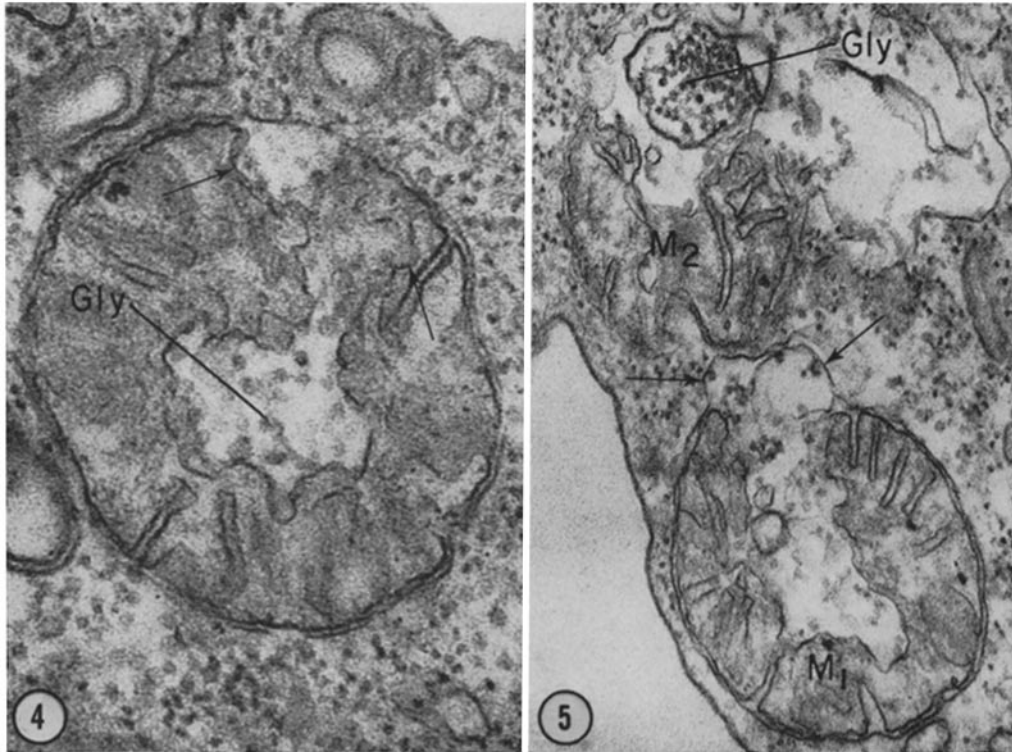


FIGURE 4 Mitochondrion in a digestive cell of a hydra that had been fed 24 hr previously. Presumed glycogen granules (*Gly*) occupy a central clear space. The membrane enclosing the central space is continuous with the inner mitochondrial membrane (arrows), so that the glycogen resides in a dilated intracristal space. Similar granules are present in the surrounding hyaloplasm. $\times 94,000$.

FIGURE 5 Mitochondria of a digestive cell of a regularly fed hydra (a daily feeding of *Artemia* for at least 1 wk). Note that the accumulation of granules within one mitochondrion (M_1) produces a bulging of the outer mitochondrial membrane (arrows). In the other mitochondrion (M_2), the granules (*Gly*) have been released into the cytoplasm but are still enclosed by a membrane while the remainder of the mitochondrion is disrupted or fragmented. $\times 57,000$.

DISCUSSION

Masses of irregular dense granules were observed adjacent to and within the apical mitochondria and in the hyaloplasm of digestive cells of fed hydra. Most of the available evidence suggests these granules represent glycogen. First, the abundance of granules in the apical border of digestive cells corresponds to the region of hydra that contains the heaviest concentration of periodic acid-Schiff-positive material that is diastase sensitive (3, 7). Glycogen has been shown to become depleted after about 2 wk of fasting (7), and in the present experiments no granules were found in animals starved for 3 wk. The granules are similar to those observed in glycogenic areas

of vertebrate tissues, including cardiac muscle, striated muscle, liver, and brown adipose tissue (4, 14). Lead hydroxide, which has an affinity for glycogen, increased the density of granules in hydra, although apparently not to the degree observed in mammalian tissues. Both mitochondrial and cytoplasmic granules were removed by treatment with saliva or amylase. Thus, on these bases, it is suggested that the 300 A mitochondrial granules represent glycogen particles. It is unlikely that the occurrence of granules within mitochondria represents an artifact of fixation, because they were not observed in mitochondria of other cell types or in the digestive cells of starved animals which were prepared in an identi-

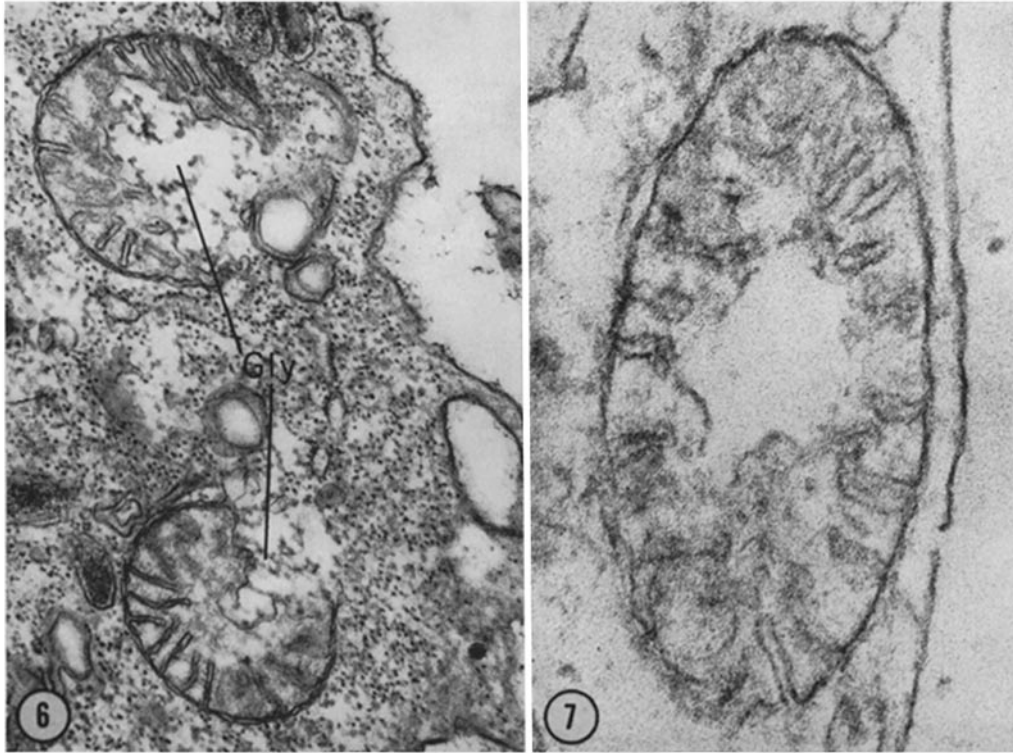


FIGURE 6 Mitochondria of regularly fed hydra. The mitochondrial membranes are deficient or interrupted at one point, so that the intramitochondrial regions occupied by the glycogen granules (*Gly*) are continuous with the hyaloplasm. The opposite sides of the mitochondria are intact, so that these organelles partially enclose the glycogenic areas. $\times 44,000$.

FIGURE 7 Mitochondrion of a regularly fed hydra which had been subjected to digestion with saliva for 1 hr following glutaraldehyde fixation, but prior to osmium tetroxide fixation. Note the absence of granules from both the central mitochondrial space and the surrounding hyaloplasm. $\times 94,000$.

cal manner. Similarly, while it is possible that the images in which a portion of the mitochondrion was disrupted with egress of glycogen into the cytoplasm were produced by glutaraldehyde fixation, it should be noted that the mitochondria of other cell types in the same section were intact.

The mitochondrial granules were identical with the cytoplasmic granules and appeared to be liberated into the cytoplasm by rupture of the mitochondria. Furthermore, the appearance of granules within mitochondria, following feeding, and subsequent release of them indicates that the mitochondria were synthesizing these granules. On the other hand, the enzymes associated with synthesis of glycogen, including α -glucan phosphorylase (8, 10), phosphoglucomutase (8), uridine diphosphate-glucose pyrophosphorylase

(13), and uridine diphosphate-glucose α -glucan glucosyltransferase (10), are located in the supernatant fraction of liver tissue. Furthermore, the overwhelming majority of electron microscopic observations have localized glycogen particles unrelated to cytoplasmic organelles. Both lines of evidence suggest that glycogen synthesis occurs in the hyaloplasm. The present observations are presented as a special case and, thereby, join several other studies (1, 2, 9) in which intramitochondrial glycogen has been noted.

In the present experiments, it becomes critical as to whether or not the clear space within mitochondria in which the glycogen granules were deposited was indented hyaloplasm. Because the membrane lining the space was continuous with the internal mitochondrial membrane, it appeared

that the granules were truly intramitochondrial. It should be noted that the mitochondria of hydra cells were not the only source of presumed glycogen-containing granules, because, following feeding, some granules appeared in the cytoplasm without corresponding mitochondrial granules. This observation implies that the hydra has other mechanisms of synthesizing glycogen and that they are probably located in the hyaloplasm. Mitochondrial synthesis of glycogen may represent a special situation occurring under optimal

nutritional circumstances or under certain functional demands. Whether these mitochondria differ in enzymatic content from other mitochondria of the digestive cells (e.g., those at the base) or from mitochondria of other cell types of the hydra cannot be stated at the present time.

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