containing 6*M* urea, 0.14*M* 2-mercaptoethanol, and 0.1 percent sodium dodecyl sulfate (SDS) and subjected to electrophoresis at *p*H 7.2, the marker histones separated into six major radioactive peaks; four of these marker peaks coincide with the ^{3}H-labeled peaks from the proteins synthesized in vitro (Fig. 2). Whereas the separation in the electrophoretic system at *p*H 4.3 is based mainly on charge, the separation at *p*H 7.2 is primarily based on molecular size (*9*).

To further establish the identity of the histones synthesized in vitro in the microsomal system with the marker histones isolated from nuclei labeled in vivo, the histone bands II and III from the *p*H 4.3 gels were isolated and again subjected to electrophoresis at *p*H 4.3 and 7.2. For this purpose the bands were first localized by rapid staining with tetrabromophenolphthalein ethyl ester (*2*), and the protein in the bands was extracted with 0.025*N* HCl. The ^{3}H-labeled proteins and the ^{14}C-labeled histone bands II and III appeared as single peaks in exactly the same position upon electrophoresis again at *p*H 4.3. At *p*H 7.2 band II as well as band III split into two stainable subbands. However, in each case the ^{3}H- and the ^{14}C-labeled proteins coincide again after reelectrophoresis under these conditions.

Our earlier studies (*2*), confirmed by Robbins and Borun (*3*), showed the close temporal coupling in the accumulative synthesis of DNA and histones. With hydroxyurea to block DNA synthesis (*10*), it was demonstrated that this relationship holds as well for the synthesis of histones in vitro. For this purpose synchronized HeLa cells were allowed to synthesize DNA for 90 minutes; DNA synthesis was then blocked in representative cultures for a further period of 90 minutes by the addition of $2 \times 10^{-3}M$ hydroxyurea. The microsomal system isolated from cells in which DNA synthesis had been blocked for 90 minutes exhibited only a marginal labeling of the histone, compared to the microsomes isolated from cells which were actively synthesizing DNA in vivo for 90 or 180 minutes (Fig. 3). The capacity for histone synthesis was not altered when the microsomes from cells in DNA synthesis were incubated in the presence of soluble supernatant fraction from hydroxyurea-blocked cells and vice versa. This indicates that the microsomal, rather than the supernatant fraction was modified by the blockade of DNA synthesis with hydroxyurea in the living cell.

Thus HeLa cell microsomes isolated from the cytoplasm of cells which are in process of DNA synthesis can synthesize histone proteins in vitro. These proteins are electrophoretically identical with marker histones isolated from nuclei labeled in vivo. The dependence of this cytoplasmic system on the synthesis in vivo of DNA also provides a means for exploring the nature of the coupling between DNA replication and histone synthesis.

DIETER GALLWITZ
GERALD C. MUELLER
McArdle Laboratory, University of Wisconsin, Madison 53706

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Microbody-Like Organelles in Leaf Cells

Abstract. *An organelle approximately 0.5 to 1.5 microns in diameter, limited by a single membrane, occurs abundantly in the chlorophyllous cells of leaves of several dicotyledonous and monocotyledonous plants. Its finely granular matrix frequently contains crystalline, fibrous, or amorphous inclusions. It is frequently appressed to a chloroplast or squeezed between chloroplasts so that its limiting membrane is in extensive contact with the outer membranes of the chloroplast envelopes. The organelle is probably identical with recently isolated leaf particles that contain enzymes involved in the metabolism of glycolate, a chloroplast product; it is interpreted as a form of plant microbody.*

We have observed that an organelle resembling a plant microbody (*1*) occurs abundantly in green leaf tissues. The organelle is a common component of the mesophyll cells of several species examined (Figs. 1 and 2) and frequently occurs appressed to chloroplasts.

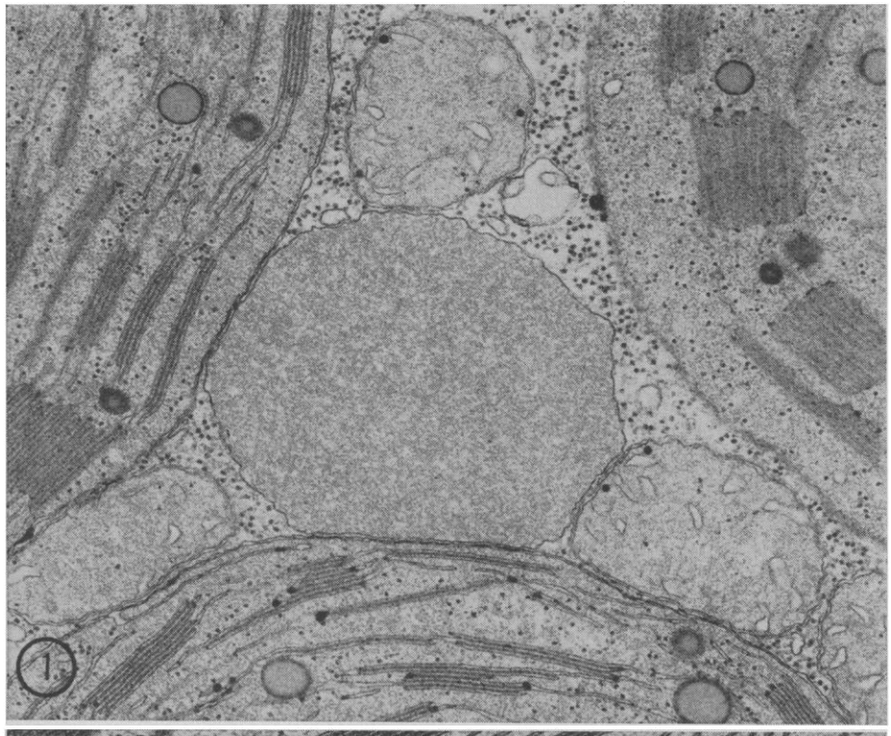
We observed the organelle, temporarily termed a "cytosome," in material fixed in glutaraldehyde and osmium tetroxide and processed for electron microscopy by standard procedures (*1*). Sections were stained with uranyl acetate and lead citrate. Although the cytosome varies in morphology somewhat in different species, it characteristically has a diameter between 0.5 and 1.5 μ, a rounded to irregular shape, and a matrix of moderate electron opacity enclosed by a single membrane. The finely granular, homogeneous matrix frequently contains dense crystalline, fibrous, or amorphous inclusions.

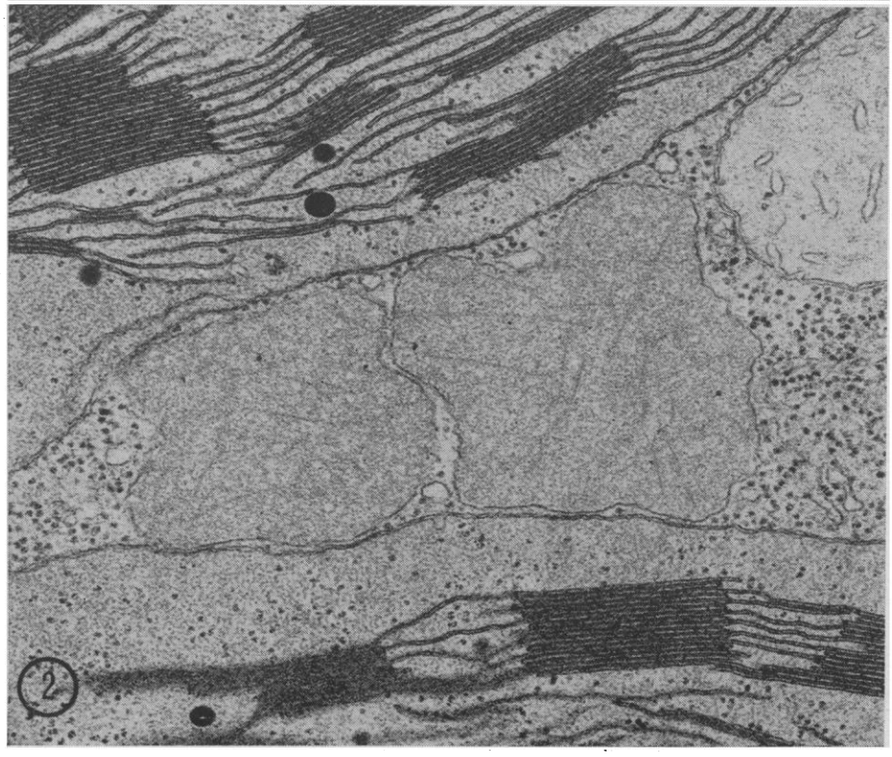
Leaf tissues of several dicotyledonous and monocotyledonous plants were examined, including bean (*Phaseolus vulgaris*), *Petunia* sp., *Torenia fournieri*, snapdragon (*Antirrhinum majus*), wheat (*Triticum vulgare*), and barley (*Hordeum vulgare*). Special emphasis was placed on mature leaves of tobacco (*Nicotiana tabacum*, var. "Wisconsin 38") and on both young and mature leaves of timothy (*Phleum pratense*). In all these species, cytosomes similar to those of tobacco and timothy were observed in many cells of both nonvascular and vascular tissues. They were particularly large and numerous in cells containing chloroplasts. In seedlings of a few other species (for example, corn), cytosomes were present but were smaller and relatively scarce. We have not determined the nature of cytosomes in mature leaves of these species.

Mature mesophyll cells have a relatively simple structure in which a layer of cytoplasm rich in free ribosomes surrounds a large central vacuole. Cisternae of the endoplasmic reticulum (ER) are present in moderate amount, but dictyosomes are relatively scarce. Chloroplasts are commonly aligned in rows in a single layer near the walls. Mitochondria are abundant around the chloroplasts. The cytosomes are similarly distributed but show a greater tendency to lie wedged tightly between adjacent chloroplasts. The relative number of cytosomes varies with species

of plant; in some species their frequency of occurrence may be as great as one third that of mitochondria. No concentration of cytosomes or other organelles around the nucleus has been noted.

The cytosomes are frequently appressed to the surfaces of chloroplasts (Figs. 1, 3, and 4), or are tightly squeezed by chloroplasts so that much of their bounding membrane is in contact with the outer membranes of the chloroplast envelopes (Fig. 2). In these instances their shapes may be highly irregular and conform to the chloroplast outlines. The association with chloroplasts apparently does not arise solely from confinement owing to limited space for often cytosomes are appressed to chloroplasts along one side when bounded by cytoplasm free of organelles on the other (Fig. 3). Contact of mitochondria with cytosomes and chloroplasts is also extensive, but is not as marked as that between cytosomes and chloroplasts. The physical associations between these three organelles are not shared by the ER or dictyosomes, and are not observed between plastids, microbodies, and mitochondria in other cells of the plant body.





The cytosomes of different species vary in content. Crystalline inclusions in cytosomes are common in leaves of several species examined, and are striking in tobacco (see cover photo), where they are localized in the spongy parenchyma cells. In cells of the palisade layer, the cytosomes of tobacco usually either lack specialized regions (Figs. 1 and 3) or possess dense, rounded amorphous or faintly crystalline nucleoids (Fig. 4). The nucleoid usually occurs adjacent to the limiting membrane. Since it is small relative to the organelle and is encountered frequently, it may occur in a high proportion or in all of the cytosomes.

The cytosomes in leaves of timothy and other plants have smaller crystalline inclusions with a lattice structure different from that of tobacco. The inclusions appear to become less frequent as the cells mature. For example, crystalline deposits are common in the leaf cytosomes of 5-day-old timothy seedlings, but are scarce in 11-day-old seedlings. Another form of inclusion, fibers distributed randomly in the matrix (Fig. 2), has been seen only in monocots, including wheat, timothy, and barley. The fibers, which are various lengths and about 50 Å thick, show evidence of a regular substructure.

Morphological variations in cytosomes such as those mentioned above are recognizable within a particular

Fig. 1. Portion of mature parenchyma cell of tobacco leaf, showing in the center a rounded cytosome considered to be a form of plant microbody. It is surrounded by mitochondria and chloroplasts. Note the finely granular matrix and single bounding membrane of the microbody. The membrane is in contact with the surface of two of the chloroplasts (× 40,000). Fig. 2. Two microbodies (center) wedged between chloroplasts in a mature leaf of timothy. Fine fibers, typical of the leaf microbodies of several monocots examined, occur at random in the matrix. Part of a mitochondrion is visible at upper right (× 55,000).

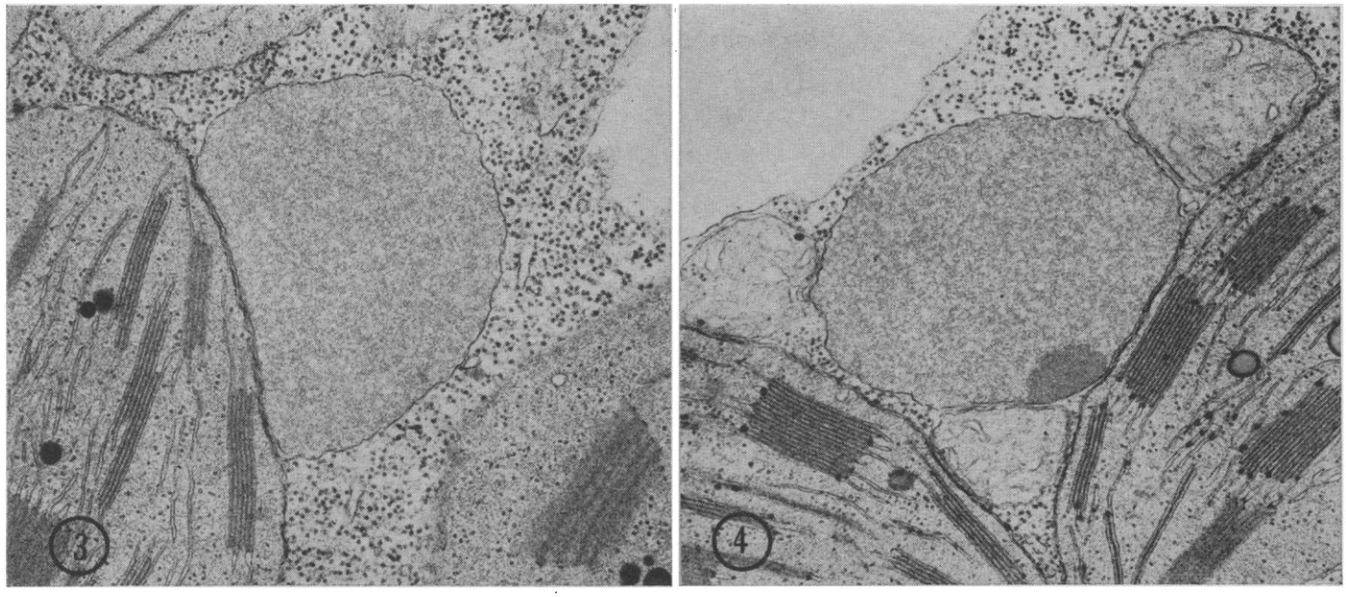


Fig. 3. Microbody appressed to a chloroplast in a parenchyma cell of a mature tobacco leaf. The close association between the organelle and the chloroplast does not appear to result from space limitations. Darkly stained regions occur where the membranes of the two organelles are in contact (× 38,000). Fig. 4. Tobacco leaf microbody (center) with a dense, amorphous inclusion next to the limiting membrane at lower right. The microbody is adjacent to three mitochondria and two chloroplasts (× 37,000).

species and can be correlated to some extent with cell type or stage of development. Another variation, observed in both timothy and tobacco as well as in other species, and potentially of considerable functional significance, is a marked increase in cytosome size as the cells mature.

Tolbert *et al.* (*2–4*) have obtained particles rich in catalase and in enzymes concerned with metabolism of glycolate and related compounds from leaf tissues of several species. The particles, termed "peroxisomes," were isolated from broken chloroplast fractions of leaf homogenates. Although their appearance *in situ* was not described, electron-microscopic examination of the pellet fraction showed that the organelles are bounded by a single membrane (*3*). We believe they may be identical with the bodies we have observed, particularly since our observations of the fine structure of tobacco (a species from which Tolbert *et al.* isolated peroxisomes) clearly demonstrate that microbody-like cytosomes are the only unfamiliar organelles present. Furthermore, their observed association with chloroplasts suggests interaction between the two organelles, a proposal consistent with a possible role of the cytosomes in glycolate metabolism.

Appreciable peroxisome activity has not been found in the leaves of all species examined but only in those which possess photorespiration (*4*). If the leaf cytosomes are identical to peroxisomes, it would not be surprising if they were smaller or lacking in species which do not photorespire. We have extensively studied only photorespiring plants; however, preliminary observations indicate that seedling leaves of corn, a plant which lacks photorespiration (*5*), have small and relatively few cytosomes, and that these are not closely associated with the chloroplasts.

Recently we have characterized a class of organelles bounded by a single membrane and closely associated with one or more cisternae of rough ER, at least in early stages of their development (*1*). They are morphologically similar to animal microbodies (*6*), and appear to be identical with the organelles termed "plant microbodies" (*7*). Cytosomes described above resemble these plant microbodies but are considerably larger and are ordinarily not associated with the ER.

Cytosomes may develop from typical microbodies present in the very young leaf cells. In young leaf cells of timothy the cytosomes are similar in size to the microbodies observed in other parts of the plant and are sometimes associated with ER cisternae. Furthermore, cytosomes with crystalline inclusions, for example, those in tobacco leaves, obviously correspond to the "crystal-containing bodies" (*8*), which are modified microbodies (*1*).

Thus, we suggest that the leaf cytosomes be considered microbody variants and that microbody terminology be extended to include them. This term seems preferable, as it follows historical precedent in naming organelles on a morphological basis. It is not implied, however, that the leaf microbodies must be functionally equivalent to their structural and ontogenetic counterparts in other plant organs. Plant microbodies may vary in enzyme complement according to cell type and state of differentiation (*1*). In green leaf tissues, it appears likely that they enlarge and differentiate so as to function in the metabolism of glycolate and related compounds.

SUE ELLEN FREDERICK
ELDON H. NEWCOMB
Department of Botany, University of Wisconsin, Madison 53706

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