CYTOCHEMICAL LOCALIZATION OF CATALASE IN LEAF MICROBODIES (PEROXISOMES)

SUE ELLEN FREDERICK and ELDON H. NEWCOMB

From the Department of Botany and Institute of Plant Development, The University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT

Segments of mature tobacco leaves were fixed in glutaraldehyde, incubated in medium containing 3,3′-diaminobenzidine (DAB) and hydrogen peroxide, and postfixed in osmium tetroxide. Electron microscopic observation of treated tissues revealed pronounced deposition of a highly electron-opaque material in microbodies but not in other organelles. The coarsely granular reaction product is presumably osmium black formed by reaction of oxidized DAB with osmium tetroxide. Reaction of the microbodies with DAB was completely inhibited by 0.02 M 3-amino-1,2,4-triazole and was considerably reduced by 0.01 M potassium cyanide. These results, when considered in light of recent biochemical studies, strongly suggest that catalase is responsible for the reaction. Sharp localization of this enzyme in microbodies establishes that they are identical to the catalase-rich "peroxisomes" recently isolated from leaf cell homogenates. A browning reaction that occurred in leaves during the incubation step was inhibited by cyanide but not by aminotriazole and therefore could not have been caused by the same enzyme. This reaction and a slight deposition of dense material within primary and secondary walls are ascribed to oxidation of DAB by soluble and wall-localized peroxidases.

INTRODUCTION

It is now well established that liver and kidney cells of vertebrates contain a distinctive organelle, the "microbody", which is bounded by a single membrane and is closely associated with the endoplasmic reticulum. As reviewed by de Duve and Baudhuin (9), it has been shown in biochemical studies that certain microbodies contain catalase and several hydrogen peroxide-producing oxidases. Organelles with this enzyme complement have been termed "peroxisomes" (8), a name emphasizing their role in hydrogen peroxide metabolism. The peroxisomes, found also in the protozoan *Tetrahymena* (cf. 28), are quite distinct from the lysosomes, another class of single membrane-bounded particles in animal cells that contain acid hydrolases.

There is now considerable biochemical and cytochemical literature (reviewed by Frederick et al. [13]) indicating that in plant cells also, various oxidases and hydrolases are segregated within particulate components. Unfortunately much of this literature, especially as it relates to the localization of hydrolytic enzymes within particles, is confused by lack of fine structural characterization of the presumptive particles and by application to the particles of numerous terms that for plant tissues have not been adequately defined on the fine structural level.

However, recent studies have demonstrated convincingly that there is a class of plant organelles related both fine structurally and biochemically o animal microbodies. The widespread occurrencet of plant organelles ultrastructurally similar to animal microbodies was first pointed out by Mollenhauer et al. (27). The fine structural characteristics of plant microbodies were described in some detail in this laboratory (13).

On the biochemical level, it has been demonstrated with two quite different plant organs that single membrane-bounded particles rich in two of the enzymes found in animal peroxisomes can be obtained from cell homogenates. Breidenbach and Beevers (4) and Breidenbach et al. (5) have isolated "glyoxysomes" from fatty seedlings, while Tolbert et al. (32, 33) have isolated peroxisomes from green leaf tissues. Glyoxysomes contain enzymes involved in the net conversion of acetyl CoA to succinate via the glyoxylate pathway, while the leaf peroxisomes possess enzymes concerned in the metabolism of glycolate, a photosynthetic product. Both particles contain an α-hydroxy acid oxidase and catalase, enzymes characteristic of animal peroxisomes.

Recently, it has been shown that enlarged forms of microbodies are common in the leaf mesophyll cells of tobacco and several other photorespiring plants, where they are frequently appressed to the chloroplasts (12). Since these microbodies are the only relatively unfamiliar organelles observed in the leaf cells, it has been suggested that they must be identical to the peroxisomes isolated from leaf homogenates. In order to establish this unequivocally, however, it is necessary to show cytochemically that one or more of the enzymes localized in the isolated peroxisomes is also localized in the microbodies *in situ*.

The equivalence between leaf peroxisomes and leaf microbodies has been shown in the present study by employing the electron cytochemical reagent diaminobenzidine (DAB). This compound, used recently to demonstrate peroxidatic activity of animal microbodies containing catalase (2, 10, 11, 29), also reveals that pronounced activity is associated with leaf microbodies. Inhibitor studies strongly indicate that this activity is due to catalase, a characteristic peroxisome enzyme. Thus, biochemical studies of these organelles can now be complemented by ultrastructural cytochemical investigations in order to clarify certain aspects of their occurrence and role in green plant tissues.

MATERIALS AND METHODS

Preparation and Fixation of Tissues

Mature green leaves were obtained from nonflowering plants of *Nicotiana tabacum* (cv. Wisconsin 38) grown in the greenhouse. The lower epidermis was stripped from regions between major veins of the leaves to allow better penetration of fixative and incubation medium, after which the stripped pieces of leaf tissue were segmented in the fixing solution into squares approximately 1mm on a side. The fixing solution consisted of 3% glutaraldehyde in 0.05 M potassium phosphate buffer at pH 6.8. Fixation was at room temperature for 1.5–2.0 hr. At the end of this time, leaf segments were rinsed for 15–20 min in at least three changes of phosphate buffer of the same composition as that of the fixative.

Incubation

After glutaraldehyde fixation and rinsing, the leaf segments were incubated with DAB (3,3′-diaminobenzidine, Sigma Chemical Co., St. Louis, Mo.). Routine incubations were carried out in small, covered, glass Petri dishes or in corked vials for 50–60 min at 37°C. The containers were agitated periodically to insure that the leaf segments remained immersed.

Variations in the procedure included incubations for 30 min only, or at room temperature. Incubations were also carried out in a nitrogen atmosphere.

The standard incubation medium, that of Novikoff and Goldfischer (29), as described by Beard and Novikoff (2), was always prepared immediately before use. It contained 10 mg DAB, 5 ml of 0.05 M propanediol buffer (2-amino-2-methyl-1,3-propanediol, Sigma Chemical Co.) at pH 10.0, and 0.1 ml of 3% H_2O_2. The pH was adjusted to 9.0 prior to addition of the tissue segments.

The following procedures served as controls to determine whether any observed activity could be ascribed reasonably to catalase: (*a*) incubation in the standard DAB medium minus H_2O_2; (*b*) preincubation for 20–30 min with propanediol buffer containing 0.02 M aminotriazole (3-amino-1,2,4-triazole, Aldrich Chemical Co., Inc., Milwaukee, Wis.), followed by incubation in standard DAB medium also containing 0.02 M aminotriazole; (*c*) preincubation for 20 min in propanediol buffer containing 0.001 or 0.01 M KCN, followed by incubation in standard DAB incubation mixture containing 0.001 or 0.01 M KCN; (*d*) incubation under anaerobic conditions in standard DAB medium or standard DAB medium minus H_2O_2. In all experiments, a check on the DAB reactivity was run by incubating the tissue in the usual way in complete medium without poison.

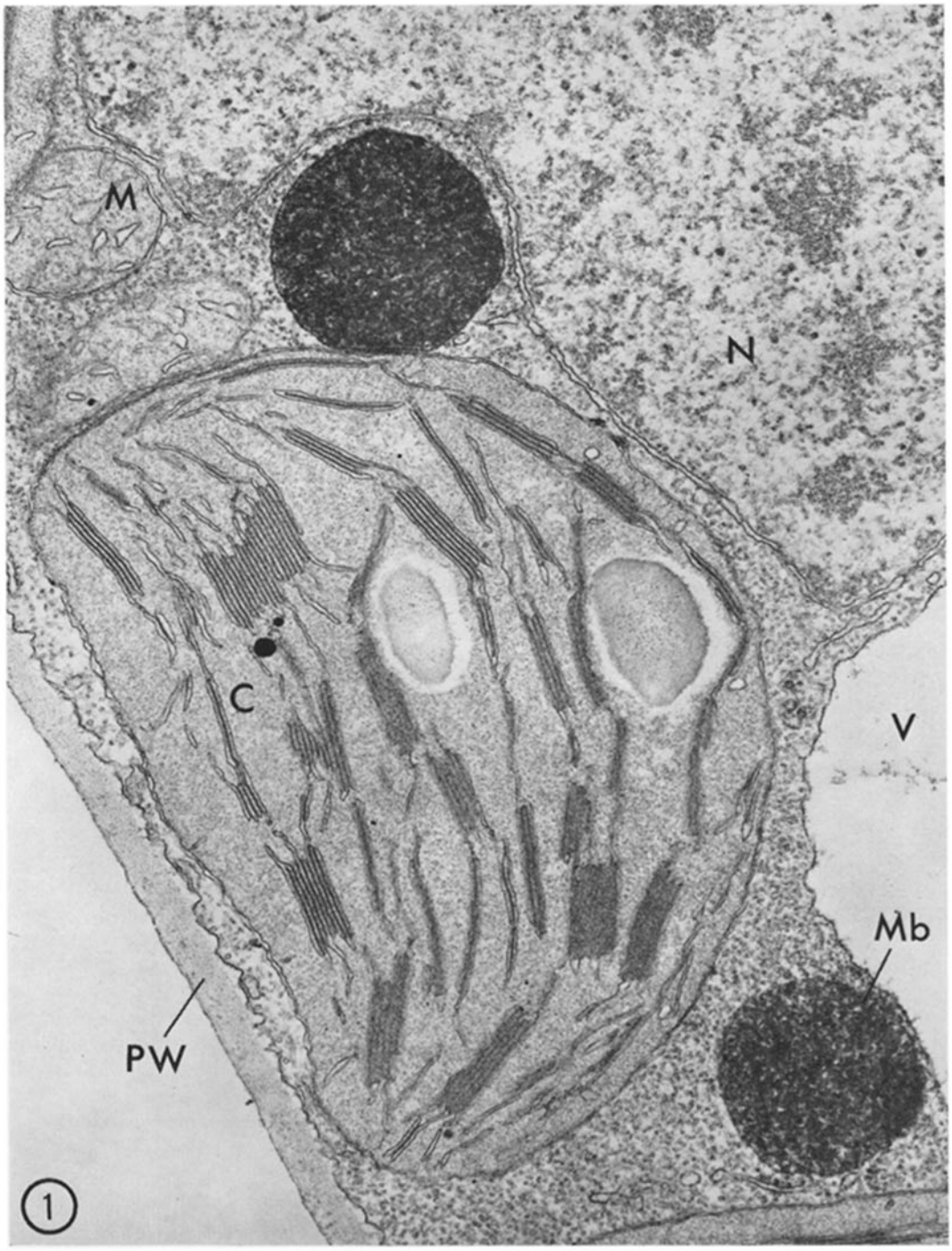


FIGURE 1 Portion of a tobacco leaf mesophyll cell that has been incubated in medium containing DAB. Dense reaction product, attributable to activity of catalase, has completely permeated the two microbodies (*Mb*). This highly electron-opaque product (osmium black) is sharply confined to the microbodies and is not observed in the nucleus (*N*), mitochondrion (*M*), chloroplast (*C*), primary cell wall (*PW*), or vacuole (*V*). × 37,000.

For the anaerobic control, leaf segments were placed in a small amount of buffer in the side arm of a Warburg flask containing 5 ml of standard DAB medium in the central compartment. The flask was then attached to a manometer arm and gassed with nitrogen for 20 min, after which the manometer stopcock was closed and the flask was removed from the manometer. The tissue was then mixed with the medium by tilting, and was incubated for 60 min.

Following the incubations, tissues were rinsed for 15–20 min with several changes of 0.05 M phosphate buffer. After the anaerobic incubation, the flask was flushed with nitrogen gas while the tissue was rinsed by pipette with nitrogenated buffer. This was immediately followed by postfixation in OsO_4.

Postfixation and Preparation of Tissues for Viewing

Tissues were postfixed in 2% OsO_4 in 0.05 M phosphate buffer at pH 6.8 for 2 hr. They were then dehydrated in an acetone series followed by propylene oxide, and were embedded in Araldite-Epon. Silver-gray sections were cut on a Sorvall MT-2 ultramicrotome (Ivan Sorvall Inc., Norwalk Conn.), mounted on copper grids, and were left unstained or were stained in 2% aqueous uranyl acetate for 10 min followed by lead citrate for 5 min. Sections were viewed in a Hitachi HU 11-A electron microscope at an accelerating voltage of 75 kv with a 30 μ objective aperture.

RESULTS

Incubation of glutaraldehyde-fixed tobacco leaf segments in media containing DAB and hydrogen peroxide resulted in pronounced deposition of electron-opaque material within structures clearly recognizable as microbodies. In Fig. 1, two microbodies are shown as they typically appear in tissues incubated in DAB. As discussed later, their enhanced electron opacity resulting from deposition of the reaction product is assumed to indicate the presence of catalase. The difference in opacity is readily apparent when these microbodies are compared with those in tissue fixed by routine procedures and not exposed to this reagent (Fig. 2). The microbodies not treated with DAB appear as discrete rounded to oval bodies with a matrix of moderate electron opacity. The matrix frequently contains a crystalline inclusion.

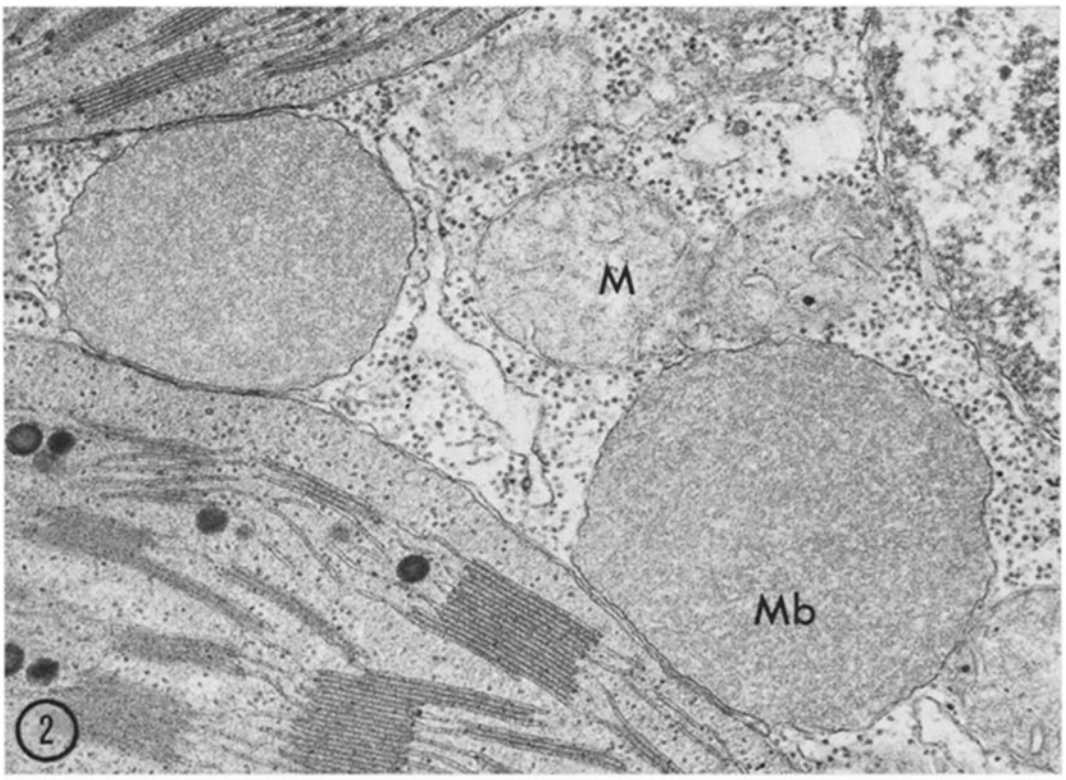
The matrix of microbodies in tissues incubated in DAB is completely permeated by the reaction product, which normally occurs as a highly electron-opaque, coarsely granular deposit (Fig 1). Frequently the membrane also becomes thicker and more opaque in appearance as a result of the incubation, perhaps due to accumulation of product along its surface. In addition, electron-opaque material often accumulates exterior to the membrane, particularly at places where a microbody lies close to or against a chloroplast, resulting in dense granulation in the space between the organelles or an opaque layer at the interface established by their contact.

The reaction product is always sharply localized in the microbodies or in the microbodies and the immediately adjacent cytoplasm. Fig. 1, which is representative of the many sections examined for DAB reactivity, illustrates this point clearly. In this section there is no evidence for staining either of the general cytoplasmic matrix or of other organelles, including the mitochondria and chloroplasts. In some sections a slight deposition of dense material is observed within the secondary walls of xylem, and to a lesser extent within primary walls and intercellular spaces.

The sharp restriction of DAB staining to microbodies is further illustrated by sections that have not been contrasted with lead citrate and uranyl acetate (Fig. 3). Omission of poststaining emphasizes differences in electron opacity between structures with and without accumulated reaction product, since the density of the oxidized DAB is caused mostly by reaction with osmium tetroxide (31) and is not greatly enhanced by the additional staining step. In Fig. 3, only the microbodies and the osmiophilic globules of the chloroplasts are of substantial electron opacity. The cytoplasmic matrix, the chloroplast lamellar system and stroma, and the mitochondrial matrix and cristae are considerably more transparent.

The intensity of the catalase reaction varies when certain modifications are made in the routine experimental procedure. The differences in accumulation of reaction product can be correlated with the duration of the incubation and the temperature at which it is carried out. Although changes in these and other factors were not studied extensively, certain generalizations can be made. Incubation at 37°C for 50–60 min with the DAB mixture described gives optimal staining of microbodies, while incubation for only 30 min results in a lesser amount of reaction product. Little or no dense material can be observed in the microbodies after incubations at room temperature.

Among the control procedures designed to eliminate staining of the microbodies with DAB, only addition of aminotriazole to the incubation medium is completely effective. This compound,



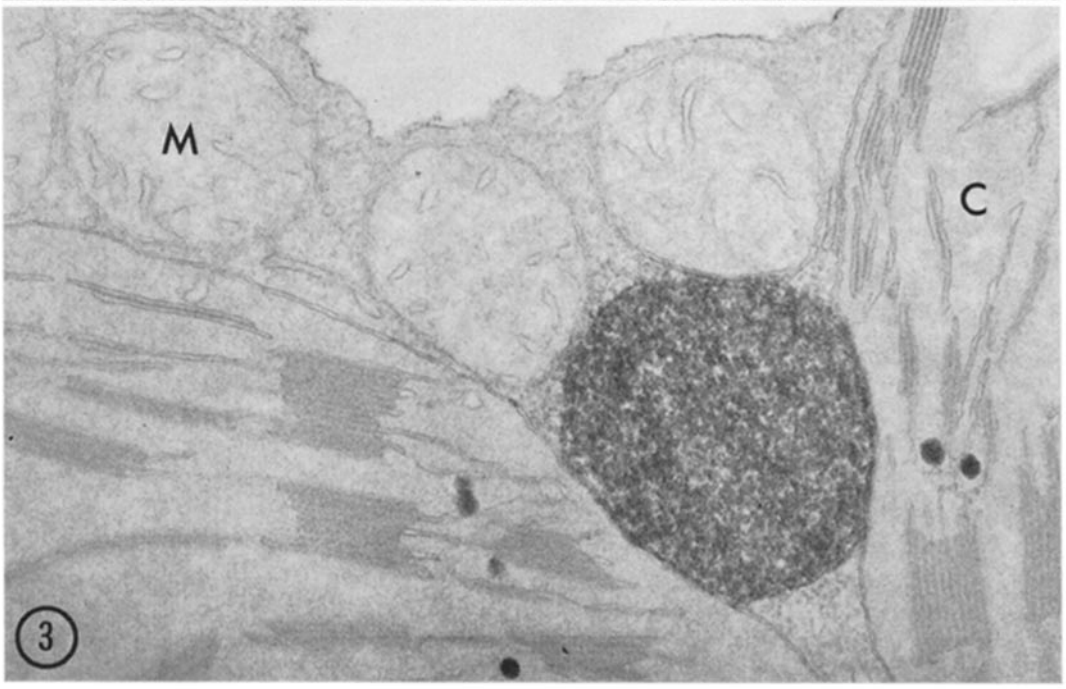


FIGURE 2 Portion of a young tobacco leaf cell showing the appearance of microbodies (*Mb*) when tissue is fixed and poststained in the usual manner without incubation in the DAB medium. Compare their finely granular matrix of moderate density with that of the stained microbodies in Fig. 1. The microbodies are appressed to chloroplast envelopes. Several mitochondria (*M*) lie nearby. × 46,000.

FIGURE 3 Portion of a tobacco leaf cell incubated with DAB as in Fig. 1, but section is not poststained in uranyl acetate and lead citrate. Note that all cytoplasmic structures except the microbody are quite electron transparent. This provides further evidence that catalase is localized in the microbodies. *C*,

an inhibitor of catalase activity (see Discussion), completely eliminates microbody staining at a final concentration of 0.02 M, while still allowing slight accumulation of dense product in cell wall structures. A microbody and other organelles in tissue incubated in the presence of this inhibitor are shown in Fig. 4. Comparison with Figs. 1 and 2 demonstrates that there has been no detectable staining of the microbody by the DAB reaction. It should also be noted that the ribosomes in Fig. 4 are relatively indistinct compared to those of tissue fixed by normal procedures and not exposed to DAB (Fig. 2). This appearance is characteristic of all ribosomes in material that has been incubated in the DAB medium (Figs. 1, 4, 5, and 7).

Omission of hydrogen peroxide from the incubation medium reduces the staining of microbodies very little when the reaction is carried out aerobically. Fig. 5 shows a microbody as it appears typically after aerobic incubation in the standard DAB medium minus hydrogen peroxide. The intensity of staining is comparable to that of microbodies from tissues incubated in medium that contains hydrogen peroxide (compare Figs. 1 and 5).

When the reaction is carried out anaerobically, omission of the hydrogen peroxide noticeably lessens the degree of staining. However, because the staining intensity is reduced, variations in darkening dependent on distance of the microbodies from the tissue surface become more evident. For this reason, results cannot be illustrated with a single representative micrograph.

Addition of 0.001 M potassium cyanide to the incubation medium has no apparent effect on the staining of microbodies, but addition of 0.01 M cyanide causes partial inhibition. Again the degree of staining is more variable than it is for the complete medium or the medium with aminotriazole, making it impossible to choose a representative micrograph.

Microbodies containing crystalline inclusions are highly reactive toward DAB, and much of the product is localized within the crystals (compare Figs. 6 and 7). It is clear that the reaction must take place throughout the crystal bodies during incubation, since sections of them always show a uniformly heavy distribution of dense material. The crystalline lattice evident in the unstained microbody (Fig. 6) is usually obscured by the abundance of reaction product (Fig. 7). Generally, the larger the crystal, the less reactive the granular or amorphous matrix.

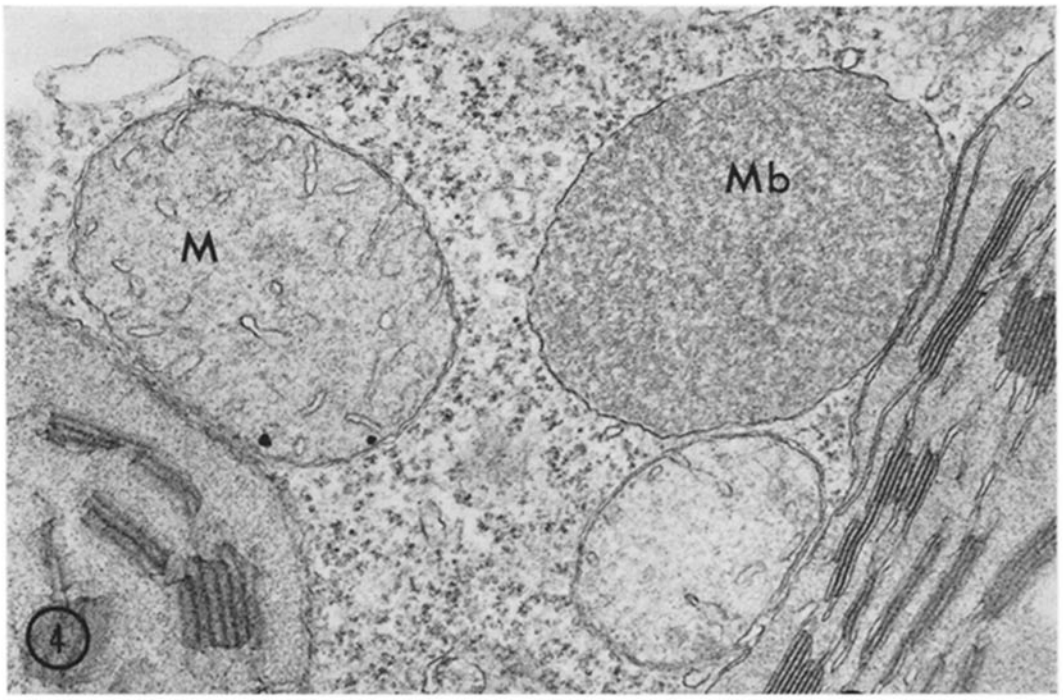
Observations of changes in the appearance of the leaf tissues during incubation with DAB are also of interest. It is observed that leaf segments rapidly turn brown to black during their exposure to the standard DAB mixtures. The browning reaction is reduced or prevented, however, by some of the variations in procedure mentioned above. Omission of hydrogen peroxide eliminates tissue darkening under both aerobic and anaerobic conditions, yet allows staining of the microbodies. Similarly, both 0.01 and 0.001 M potassium cyanide eliminate the darkening of the leaf fragments, which normally occurs during incubation. On the other hand, 0.02 M aminotriazole does not noticeably reduce the tissue darkening, although it prevents the staining of microbodies.

It is clear from these results that the reaction which causes staining of the microbodies during incubation in DAB media is not the same as that responsible for the browning of segments of leaf tissue. An interpretation of the nature of the processes is given in the Discussion.

DISCUSSION

An organelle identified as a plant microbody was described recently in green leaf cells of several plant species, where it occurs in abundance and is often appressed to chloroplasts (12). The electron microscopic cytochemical studies reported herein have localized catalase within this organelle. Demonstration of the occurrence of this enzyme in the leaf microbodies supports the suggestion made in the earlier paper that they are identical to peroxisomes, the particles recently isolated from leaf homogenates by Tolbert et al. (32, 33). It has been shown that peroxisomes contain catalase and several other enzymes, most of which are involved in some aspect of the metabolism of glycolate, a product of photosynthetic CO_2 fixation.

The electron microscopic cytochemical method of localizing catalase shows clearly that only the microbodies of the leaf cells contain appreciable activity that can be attributed to this enzyme. The other cellular organelles are devoid of dense reaction product after incubation in DAB under conditions that favor pronounced microbody staining. These observations plus the biochemical findings of Tolbert and associates that significant proportions of the total leaf catalase are contained within the peroxisomes (32, 33) go far toward dis-



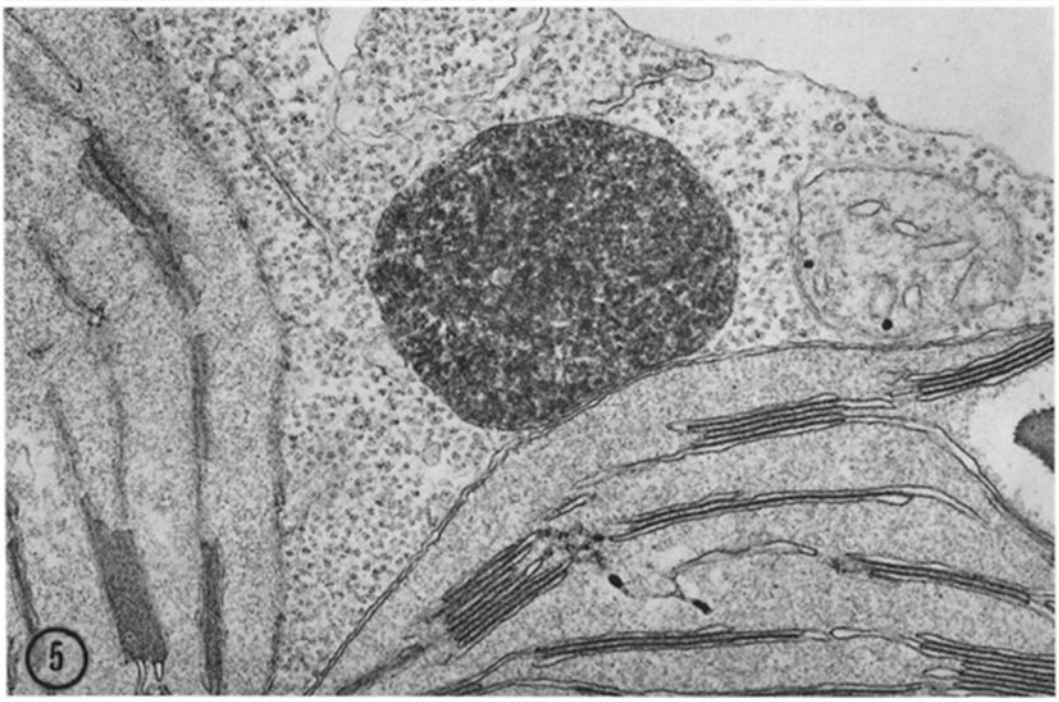


FIGURE 4 Appearance of a microbody (*Mb*) and other organelles of a tobacco leaf cell when incubation in the DAB medium is carried out in the presence of 0.02 M aminotriazole. The general appearance of the microbody matrix and its density relative to that of the nearby chloroplasts and mitochondria (*M*) closely resemble those of cells not exposed to DAB (Fig. 2). Aminotriazole has inhibited any observable activity of catalase. × 38,000.

FIGURE 5 Section through cell of tobacco leaf tissue incubated in DAB medium from which hydrogen peroxide has been omitted. The staining intensity of the microbody is not noticeably less than that of microbodies incubated in the presence of hydrogen peroxide, as in Fig. 1. × 45,000.

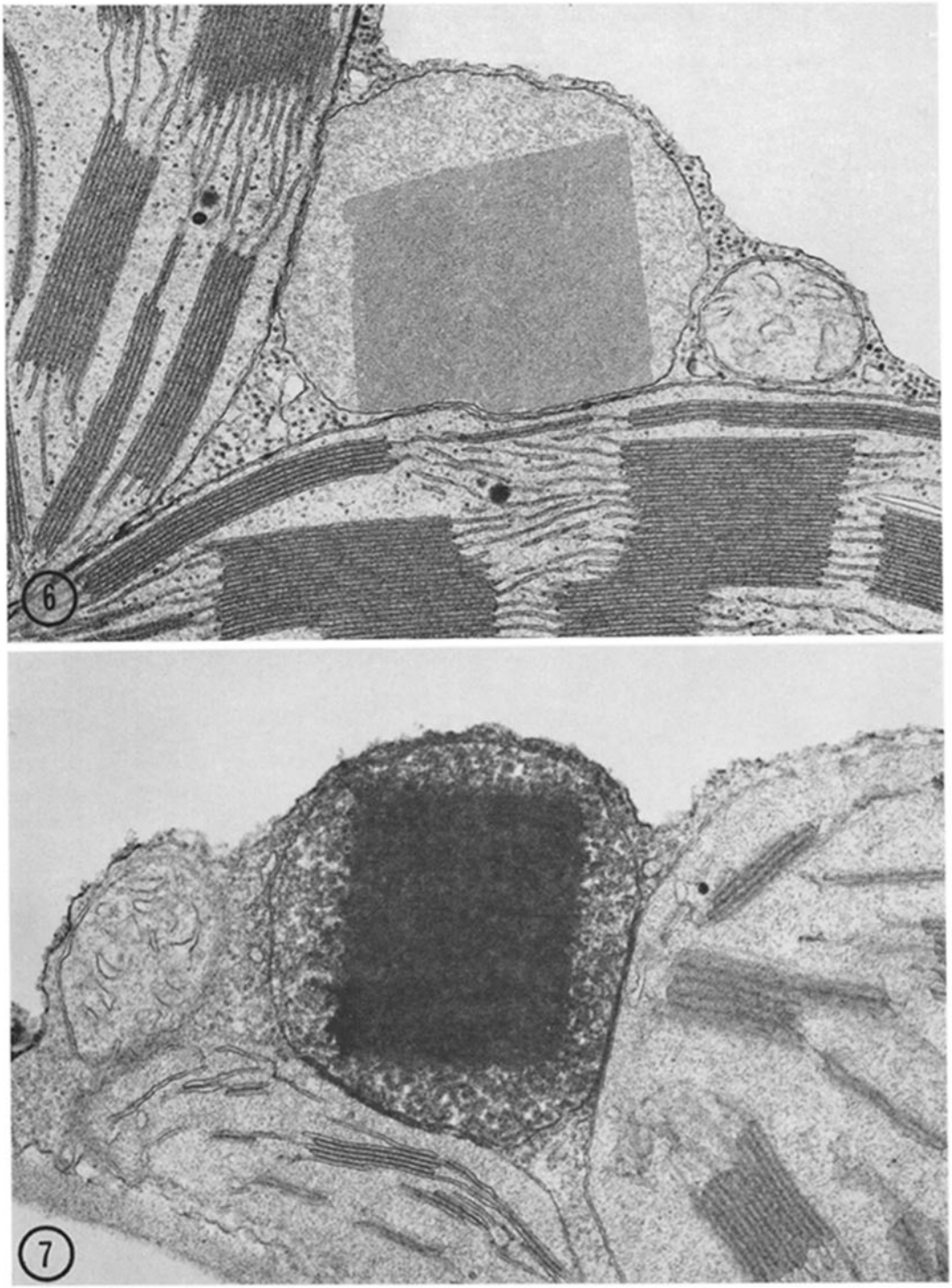


FIGURE 6 Portion of a tobacco leaf cell fixed and poststained in the usual manner (i.e., without incubation in DAB medium). A microbody with a large crystalline inclusion is appressed to two chloroplasts. A small mitochondrion lies to the right of the microbody. × 51,000.

FIGURE 7 Portion of a tobacco leaf cell incubated in the DAB medium. The microbody contains a large crystalline inclusion which shows a heavy deposition of osmium black throughout. Some reaction product is visible in the microbody matrix around the crystal, in the cytoplasm immediately adjacent to the microbody, and at the interfaces between microbody and chloroplasts. There is no product in the chloroplasts themselves or in the mitochondrion. × 44.000.

proving the claims of earlier years that catalase is localized mainly in chloroplasts or in the soluble cytoplasm. Although the biochemical studies still showed some catalase in nonparticulate fractions, there is a strong possibility that this enzyme was liberated from peroxisomes broken during homogenization. In disclosing the sharp localization of catalase activity, the methodology of ultrastructural cytochemistry is able to contribute importantly to this question.

The use of diaminobenzidine to localize various hemoproteins with the electron microscope depends upon its oxidation to a polymeric form that can interact with osmium tetroxide to yield electron-opaque osmium black (31). The oxidation of DAB, often peroxidatic in nature, can be catalyzed by several hemoprotein compounds, including peroxidase (14, 17, 21), cytochrome oxidase (31), and myoglobin (16). The reagent has thus been useful in localizing peroxidatic activity in a number of cytoplasmic structures and organelles. These include eosinophil leucocyte granules that contain peroxidase (1, 3) and animal microbodies that contain catalase (2, 10, 11, 29). The list can now be extended to include leaf microbodies (present study), and the microbodies of castor bean endosperm and the oat coleoptile (34).

In view of the nonspecific nature of the DAB reaction, it may be questioned whether the accumulation of electron-opaque product in the leaf microbodies might not be due to the activity of a hemoprotein other than catalase. The absence of reaction product from the mitochondrial cristae largely eliminates the already unlikely possibility that cytochrome oxidase is responsible for any part of the staining observed in microbodies. Moreover, detection of this enzyme, and of peroxidase as well, has usually been accomplished at a pH lower than that used in the present study (17, 31). As there have been no reports of heme compounds resembling myoglobin in leaf tissue, it can be assumed that the DAB reaction of the microbodies is due either to catalase, which is known to be capable of peroxidatic reactions with a variety of substrates (7, 22), or to peroxidase itself. These two enzymes, often grouped together in the general category of hydroperoxidases, are so similar in their peroxidatic activity that distinction between them by cytochemical means is quite difficult.

Evidence that accumulation of reaction product in leaf microbodies is mediated primarily by catalase rather than peroxidase is largely indirect and has been provided in part by experiments employing aminotriazole. This compound, a potent inhibitor of catalase in several plant and animal tissues (18, 25, 26, 30), completely prevented detectable microbody staining at a concentration of 0.02 M. The same concentration of this reagent has been found to inhibit reaction of rat liver microbodies with DAB (10, 11). The ability of aminotriazole to inhibit catalase without also inhibiting peroxidase in plants is not well documented, but this sort of specificity has been shown in animals, where leucocyte catalase can be inhibited 96% by a concentration of aminotriazole that does not affect peroxidase (30). It must be recognized, however, that aminotriazole may be less specific in other animal tissues, such as those of thyroid, where it may directly or indirectly reduce peroxidase activity by as much as 50% (19).

Further indirect evidence that the observed reactivity of leaf microbodies with DAB is due to catalase rather than peroxidase is provided by observations of Tolbert et al. (32) on the distribution of the two enzymes when leaf cell homogenates are fractionated. While a large part of the catalase activity was found in a particulate fraction containing a single membrane-bounded organelle (the peroxisome), peroxidase activity was found mainly in the soluble fraction, with a small proportion in the chloroplast and mitochondrial fractions. These results are consistent with the interpretation of the cytochemical work reported herein, in which the strong reaction of the microbodies is attributed to catalase.

In view of the large body of evidence that several plant parts contain both soluble and wall-localized peroxidases (15, 20, 24, 32), it seems plausible that the browning of leaf segments during the incubation and the slight deposition of reaction product within walls are due to this enzyme. The browning reaction could be accounted for by oxidation of DAB by these peroxidases to a dark product, which might accumulate generally throughout the cytoplasm. This suggestion is supported by studies using potassium cyanide, which is an inhibitor of both catalase and peroxidase (6), and aminotriazole, which is more effective against catalase. The latter compound did not reduce the brown color, while potassium cyanide eliminated almost all of it. Although no electron-opaque product corresponding to soluble peroxidase activity could be observed in the cytoplasm, such product might have been distributed too diffusely to be detectable in thin sections.

In leaf peroxisomes catalase appears to function

principally in the catalatic destruction of hydrogen peroxide (23), whereas in animal peroxisomes peroxidatic consumption of hydrogen peroxide with concomitant oxidation of other substances is believed to be more important (9). In both cases, however, the enzyme is responsible for the elimination of hydrogen peroxide produced by oxidases localized within the organelles. The hydrogen peroxide in the leaf peroxisomes results from the oxidation of glycolate, a photosynthetic product excreted from the chloroplasts (23, 32). The oxidation of glycolate by glycolate oxidase produces glyoxylate, which may then be converted to glycine. The latter compound subsequently may be released from the peroxisomes and converted to carbon dioxide elsewhere in the cell (23). This metabolism of glycolate produced in photosynthesis constitutes a respiratory pathway quite distinct from that of mitochondria, and presumably accounts for the enhanced oxygen uptake in light (photorespiration), which is characteristic of many plant groups.

Since the mechanism of DAB oxidation is assumed to be peroxidatic and therefore dependent on the presence of hydrogen peroxide, it might be expected that omission of the latter from the incubation medium would eliminate staining of microbodies. No such effect is observed, however, presumably because endogenous production of hydrogen peroxide occurs (information on the staining of animal microbodies by DAB in the absence of hydrogen peroxide is given in refs. 10, 11, and 29). If glycolate oxidase remains active after glutaraldehyde fixation, then the oxidation of stored glycolate will provide a plentiful supply of hydrogen peroxide to serve as a substrate in the peroxidatic oxidation of DAB. Attempts to eliminate the production of endogenous hydrogen peroxide by incubating the tissue under anaerobic conditions were partially successful, as they brought about a considerable reduction in microbody staining.

It is noteworthy that the crystals of microbodies stain intensely throughout as a result of the DAB incubation. Of further interest is the observation that the larger the crystal, the less intensely the amorphous matrix in the remainder of the microbody is stained. These results suggest that the inclusions represent the progressive crystallization of catalase from the matrix. The size of the crystals indicates that in some of the microbodies, at least, catalase must constitute a considerable proportion of the total microbody protein, an observation consistent with the biochemical results on the catalase content of leaf peroxisomes (32, 33).

Since it has now been shown that the leaf microbodies observed *in situ* possess catalase and correspond beyond reasonable doubt to the isolated particles characterized as peroxisomes, it seems justifiable to consider them to be peroxisomes also. However, since they also belong among the organelles defined as microbodies on morphological grounds, it is appropriate to designate them as such in fine structural studies.

This study was supported by NSF Grant GB-6161 awarded to Dr. E. H. Newcomb and a NASA predoctoral traineeship awarded to S. E. Frederick.

Received for publication 9 May 1969, and in revised form 16 June 1969.

REFERENCES

1. BAINTON, D. F., and M. G. FARQUHAR. 1967. Segregation and packaging of granule enzymes in eosinophils. *J. Cell Biol.* **35**:6A.
2. BEARD, M. E., and A. B. NOVIKOFF. 1969. Distribution of microbodies in the nephron of the rat: A cytochemical study. *J. Cell Biol.* **42**:501.
3. BEHNKE, O. 1969. Demonstration of endogenous peroxidase activity in the electron microscope. *J. Histochem. Cytochem.* **17**:62.
4. BREIDENBACH, R. W., and H. BEEVERS. 1967. Association of the glyoxylate cycle enzymes in a novel subcellular particle from castor bean endosperm. *Biochem. Biophys. Res. Commun.* **27**:462.
5. BREIDENBACH, R. W., A. KAHN, and H. BEEVERS. 1968. Characterization of glyoxysomes from castor bean endosperm. *Plant Physiol.* **43**:705.
6. BURRIS, R. H., and H. N. LITTLE. 1949. Oxidases, peroxidases, and catalase. *In* Respiratory Enzymes. H. Lardy, editor. Burgess Publishing Company, Minneapolis. 170, 173.
7. CHANCE, B. 1951. The iron-containing enzymes. *In* The Enzymes. J. B. Sumner and K. Myrbäck, editors. Academic Press Inc., New York. **2**:428.
8. DE DUVE, CH. 1965. Functions of microbodies (peroxisomes). *J. Cell Biol.* **27**:25A.
9. DE DUVE, CH., and P. BAUDHUIN. 1966. Peroxisomes (microbodies and related particles). *Physiol. Rev.* **46**:323.
10. FAHIMI, H. D. 1968. Cytochemical localization

of peroxidase activity in rat hepatic microbodies (peroxisomes). *J. Histochem. Cytochem.* **16**:547.

11. FAHIMI, H. D. 1968. Peroxidase activity of microbody catalase. *J. Cell Biol.* **39**:42A.
12. FREDERICK, S. E., and E. H. NEWCOMB. 1969. Microbody-like organelles in leaf cells. *Science (Washington).* **163**:1353.
13. FREDERICK, S. E., E. H. NEWCOMB, E. L. VIGIL, and W. P. WERGIN. 1968. Fine-structural characterization of plant microbodies. *Planta.* **81**:229.
14. FRIEND, D. S., and M. G. FARQUHAR. 1967. Functions of coated vesicles during protein absorption in the rat vas deferens. *J. Cell Biol.* **35**:357.
15. GAGNON, C. 1968. Peroxidase in healthy and diseased elm trees investigated by the benzidine histochemical technique. *Can. J. Bot.* **46**:1491.
16. GOLDFISCHER, S. 1967. The cytochemical localization of myoglobin in striated muscle of man and walrus. *J. Cell Biol.* **34**:398.
17. GRAHAM, R. C., JR., and M. J. KARNOVSKY. 1966. The early stages of injected horseradish peroxidase in the proximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* **14**:291.
18. HEIM, W. G., D. APPLEMAN, and H. T. PYFROM. 1956. Effects of 3-amino-1,2,4-triazole (AT) on catalase and other compounds. *Amer. J. Physiol.* **186**:19.
19. HOSOYA, T. 1963. Effects of various reagents including antithyroid compounds upon the activity of peroxidase. *J. Biochem. (Tokyo).* **53**:381.
20. JANSEN, E. F., R. JANG, and J. BONNER. 1960. Binding of enzymes to Avena coleoptile cell walls. *Plant Physiol.* **35**:567.
21. KARNOVSKY, M. J. 1965. Vesicular transport of exogenous peroxidase across capillary endothelium into the T system of muscle. *J. Cell Biol.* **27**:49A.
22. KEILIN, D., and E. F. HARTREE. 1936. Coupled oxidations of alcohol. *Proc. Roy. Soc. (London) Ser B.* **119**:141.
23. KISAKI, T., and N. E. TOLBERT. 1969. Glycolate and glyoxylate metabolism by isolated peroxisomes or chloroplasts. *Plant Physiol.* **44**:242.
24. LIPETZ, J., and A. J. GARRO. 1965. Ionic effects on lignification and peroxidase in tissue cultures. *J. Cell Biol.* **25**:109.
25. MARGOLIASH, E., and A. NOVOGRODSKY. 1958. A study of the inhibition of catalase by 3-amino-1:2:4-triazole. *Biochem. J.* **68**:468.
26. MARGOLIASH, E., and A. NOVOGRODSKY. 1960. Irreversible reaction of 3-amino-1:2:4-triazole and related inhibitors with the protein of catalase. *Biochem. J.* **74**:339.
27. MOLLENHAUER, H. H., D. J. MORRÉ, and A. G. KELLEY. 1966. The widespread occurrence of plant cytosomes resembling animal microbodies. *Protoplasma.* **62**:44.
28. MÜLLER, M., J. F. HOGG, and CH. DE DUVE. 1968. Distribution of tricarboxylic acid cycle enzymes and glyoxylate cycle enzymes between mitochondria and peroxisomes in *Tetrahymena pyriformis. J. Biol. Chem.* **243**:5385.
29. NOVIKOFF, A. B., and S. GOLDFISCHER. 1968. Visualization of microbodies for light and electron microscopy. *J. Histochem. Cytochem.* **16**:507.
30. RECHCIGL, M., and W. H. EVANS. 1963. Role of catalase and peroxidase in metabolism of leucocytes. *Nature (London).* **199**:1001.
31. SELIGMAN, A. M., M. J. KARNOVSKY, H. L. WASSERKRUG, and J. S. HANKER. 1968. Nondroplet ultrastructural demonstration of cytochrome oxidase activity with a polymerizing osmiophilic reagent, diaminobenzidine (DAB). *J. Cell Biol.* **38**:1.
32. TOLBERT, N. E., A. OESER, T. KISAKI, R. H. HAGEMAN, and R. K. YAMAZAKI. 1968. Peroxisomes from spinach leaves containing enzymes related to glycolate metabolism. *J. Biol. Chem.* **243**:5179.
33. TOLBERT, N. E., A. OESER, R. K. YAMAZAKI, R. H. HAGEMAN, and T. KISAKI. 1969. A survey of plants for leaf peroxisomes. *Plant Physiol.* **44**:135.
34. VIGIL, E. L. 1969. Intracellular localization of catalase (peroxidatic) activity in plant microbodies. *J. Histochem. Cytochem.* **17**:425.