CYTOCHEMICAL LOCALIZATION OF ACID PHOSPHATASES IN *EUGLENA GRACILIS*

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ABSTRACT

The localization of induced and constitutive acid phosphatase activity in *Euglena* was studied by light and electron microscopy, using two different cytochemical methods. Cells grown in high phosphate medium have constitutive acid phosphatase activity in three regions: in the Golgi complex, around the paramylum bodies, and in the peri-reservoir vesicles. Cells that have formed an induced acid phosphatase by exposure to a phosphate-deficient medium have, in addition to the constitutive activity localized exactly as in the uninduced cell, a strong activity in the pellicle. The induced activity is not uniformly distributed over the pellicle, but is localized at the notch of each pellicle complex, near a group of about four fibrils and near a characteristic vesicle of the endoplasmic reticulum. In the cytostome, where fission begins during division, there is an alternation of large and small pellicle complexes, both of which have induced phosphatase activity. A similar alternation is seen over the entire pellicle of dividing cells.

INTRODUCTION

In recent years, several studies have been made on the localization of phosphatase activity in a variety of cell types. Malamy and Horecker, for example, (1) found that lysis of the cell wall of *E. coli* released the induced alkaline phosphatase into the medium and concluded that this enzyme was located outside the cell membrane. In yeast, it has been shown that growth in low phosphate medium causes an increase in both acid phosphatase and alkaline phosphatase activity (2). Evidence from a variety of cell fractionation techniques suggests that the alkaline phosphatase activity is intracellular, while the acid phosphatase activity is external to the cell membrane (3–5). In some mammalian cell systems (6), and in the protozoan *Tetrahymena pyriformis* (7), it is known that there are several phosphatases present, but there is little evidence concerning their location within the cell. In an accompanying paper (8), evidence was presented that *Euglena* has a constitutive acid phosphatase whose detailed biochemical properties differ from those of another acid phosphatase which can be induced by growing the cells in a low phosphate medium. Two observations suggested that these enzymes might differ not only in their biochemical properties, but also in their location in the cell. First, it was observed that treatment of induced cells with toluene increased the apparent phosphatase activity. Second, even after extensive disruption of induced cells by ultrasound, and removal of paramylum granules and mitochondria by centrifugation, much of the activity could be recovered in particulate form by higher speed centrifugation. It seemed possible, therefore, that the induced activity might be located in the pellicle, and a detailed cytochemical study was undertaken in an effort to localize the induced and constitutive enzymes to particular regions of the cell.

MATERIAL AND METHODS

GENERAL REMARKS: The cells used in this study

were a streptomycin-bleached strain of *Euglena gracilis*, var. *bacillaris*, strain SML-1. Induced and uninduced cells were grown as described in detail in an accompanying paper (8). Some experiments were also done on *Astasia longa* (Jahn strain), and these will be described in detail when necessary.

Fresh samples were first examined with the phase microscope (Leitz, Heine condenser) and photographed. Subsequently, frozen-dried and air-dried smears were made, with or without glutaraldehyde fixation. The freeze-dry method was chosen for the purpose of direct correlation of the *intra vitam* phase image of intracellular organelles with their cytochemical reactions (Figs. 1 and 2). Unfortunately, whenever paramylum bodies were abundant, such structural correlations were thwarted. Since pilot studies suggested that the induced enzyme was localized at the cell surface, or pellicle, unspecific surface contamination of the single organisms by precipitates in the Gomori incubation medium (see below) had to be eliminated. This was achieved by embedding the cells in agar prior to incubation in the Gomori medium (9). To avoid errors that might arise from differential handling, both induced and uninduced cells were sampled simultaneously for the cytochemical studies.

Two methods, the Gomori (10) and the Burstone (11), were used to identify the sites of acid phosphatase activity. In the Burstone method, a red azo dye, and not phosphate is the observed reaction product; therefore, the Burstone method served as an independent control for the lead phosphate method of Gomori. Special attention was paid to washing the cells after fixation, to remove free phosphate which, especially in the uninduced cells, could lead to dense lead phosphate precipitates even in the absence of added substrate.

Preparation of Cells for Electron Microscopy

For the electron microscopic demonstration of acid phosphatase activity, the technique of Holt and Hicks (12) was adapted to the particular needs of the experiment.

Cells were concentrated by centrifugation at approximately 2,000 RPM for 3 minutes in a clinical centrifuge, and the supernatant discarded. The cells

FIGURE 1 Uninduced living cell, positive phase contrast. *NU*, nucleus; *P*, paramylum; *R*, reservoir; *PRV*, peri-reservoir vacuoles; *PF*, paraflagellar body; *GT*, gullet; *F*, flagellum; *AX*, axonemes; *D*, diverticulum. × 1069.

FIGURE 2 Same cell as in Fig. 1, after freeze-drying without fixation, and incubation in the Gomori medium without substrate for 90 minutes. *R*, reservoir; *NU*, nucleus; *LS*, lead sulfide. × 1183.

FIGURE 3 Induced cell after freeze-drying, 2 hours fixation in glutaraldehyde, and incubation in the Burstone medium for 1 hour. *NU*, nucleus; *RP*, reaction product (red precipitate, appears black in photomicrograph). Note faint striation of reaction product at *ST*. × 1163.

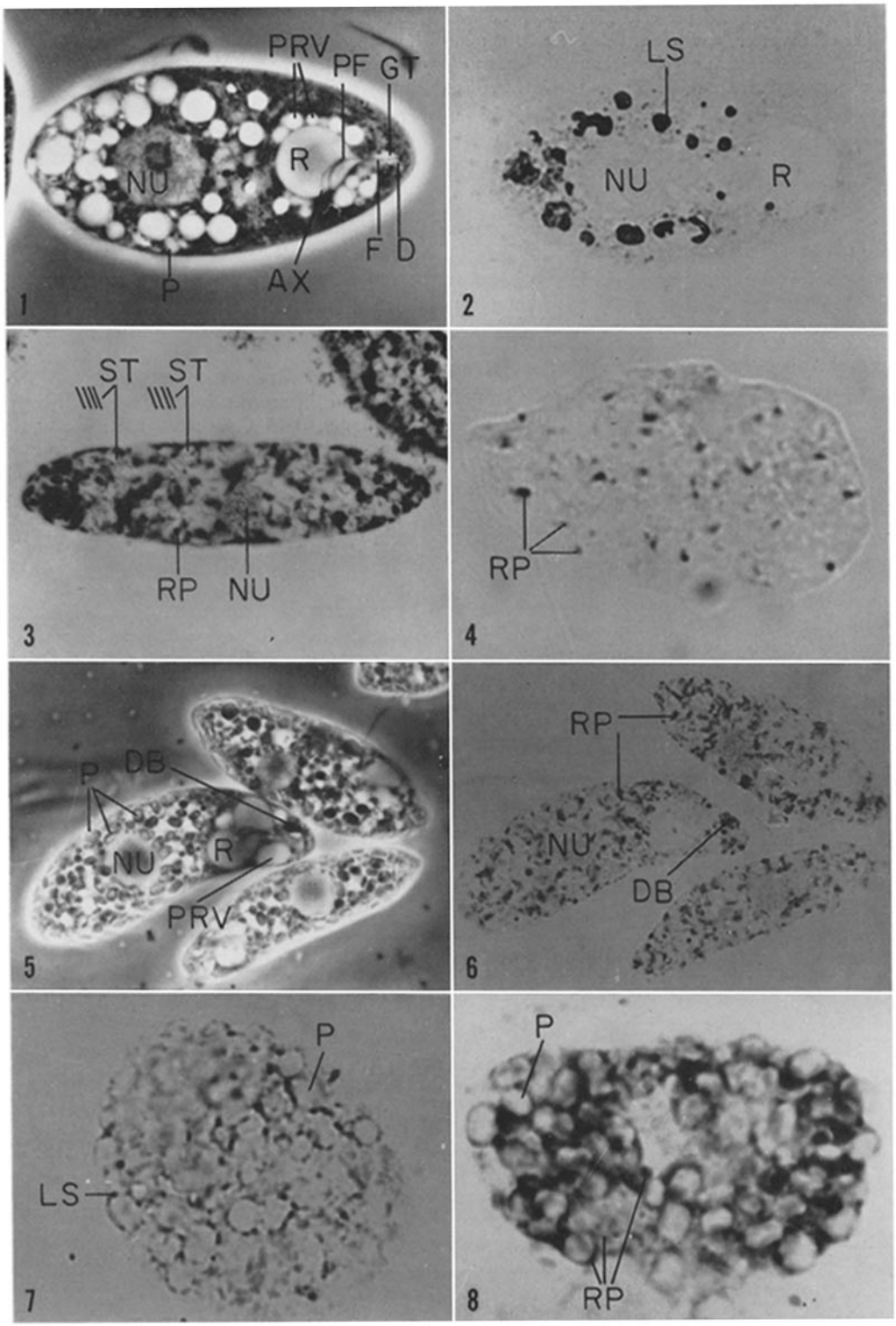
FIGURE 4 Uninduced cell, prepared as in Fig. 3, but incubated in the Burstone medium for 3.5 hours. *RP*, reaction product. × 2200.

FIGURE 5 Uninduced living cell, positive phase contrast. *NU*, nucleus; *DB*, dark body; *PRV*, peri-reservoir vacuole; *R*, reservoir; *P*, paramylum. Most intracellular organelles are obscured by the paramylum bodies. × 813.

FIGURE 6 Same cell as in Fig. 5 after freeze-drying, 30 minutes glutaraldehyde fixation, and incubation in the Burstone medium for 30 minutes. The dark body of Fig. 5 correlates with an accumulation of reaction product in the area indicated *DB*. *RP*, reaction product. × 890.

FIGURE 7 Induced cell, 2 hours fixation in glutaraldehyde, and incubation in the Gomori medium for 30 minutes without substrate. Note granular lead sulfide (*LS*) around the paramylum bodies (*P*). × 1405.

FIGURE 8 Induced cell, neither fixed nor washed, incubated in the Burstone medium for 20 minutes. Note the reaction product (*RP*) surrounding the paramylum bodies (*P*). × 1490.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were resuspended in a cacodylate-buffered (0.05 M; pH 7.2) solution of 4 per cent glutaraldehyde with 5 per cent sucrose, and left at 0–2°C for 30 minutes with occasional stirring. After 1 hour, the cells were washed two times in malate buffer (0.05 M, pH 5.0) containing 5 per cent sucrose, (hereinafter referred to as malate-sucrose buffer), resuspended in cold buffer, and washed twice during the next 45 minutes. After the final wash the cells were centrifuged briefly, the supernatant fluid decanted, and the cell pellet stirred with a glass rod. To this cell suspension an equal volume of a 4 per cent agar solution at 58–60°C was added and mixed by vigorous shaking. The agar mixture was centrifuged for about 2 minutes in the clinical centrifuge, thus forming a concentrated cell pellet embedded in agar. The pellet was removed from the tube with a bent glass rod and 40-micron sections were cut with a Superhistofreeze microtome. These sections were collected in cold malate-sucrose buffer, and then placed into incubation media (described below) for the demonstration of acid phosphatase activity. After incubation, the sections were briefly washed in malate-sucrose buffer, fixed in 1 per cent osmium tetroxide containing 5 per cent sucrose (13), and embedded in Epon (14) or Maraglas (15). After cutting with glass knives on the standard Porter-Blum ultramicrotome, the thin sections were put on collodion- and carbon-coated copper grids (200 mesh). The thin sections on the grids were then stained by Karnovsky's first lead hydroxide method (16) for 5 minutes in a nitrogen atmosphere. This procedure did not interfere with the localization of lead phosphate. Some samples of both induced and uninduced *Euglena* were fixed in glutaraldehyde for 6 hours, but otherwise processed as described above. The longer glutaraldehyde fixation resulted in better tissue preservation, but had no appreciable effect on the localization of the reaction product. It was established that fixation of cells in glutaraldehyde did not greatly inhibit the acid phosphatase activity.

From each culture of cells that was used for cytochemical studies, some cells were immediately fixed in 1 per cent osmium tetroxide containing 5 per cent sucrose. These cells were used for studies on the ultrastructure of *Euglena*.

The sections were viewed with either a Hitachi HS-7 or a RCA EMU-3G electron microscope.

Preparation of Samples for Light Microscopic Cytochemistry

Cells were concentrated by use of a clinical centrifuge. Frozen-dried preparations were made as previously described (17). Smears on acid-cleaned and thoroughly rinsed glass slides were made by streaking out a drop of cell suspension with the edge of a coverglass. The preparations were then air dried. Since the organisms did not adhere well to the glass slides, some care had to be taken during the changing of fluids in subsequent steps. The slides were fixed in glutaraldehyde, washed in malate buffer, and incubated in the Gomori medium exactly as described for electron microscopy. In addition, alternate slides were incubated at room temperature in the Burstone medium for various times, ranging from 30 seconds to several hours. From the agar blocks which were prepared for thin sectioning and electron microscopic study, frozen sections were also cut for light microscopic observation after incubation in the Burstone or Gomori media.

Incubation Media for Acid Phosphatase Localization

The lead nitrate method of Gomori (10) was employed. Cells were incubated for times ranging up to 30 minutes, at 37°C, in solution containing 10 mM β-glycerophosphate, 4 mM lead nitrate, and 50 mM sodium acetate, (pH 5.0). An acetic acid rinse was omitted. For light microscopy, the lead phosphate reaction product was transformed to lead sulfide by a short rinse in dilute ammonium sulfide. The incubation medium was always prepared within a half hour of its use, but was not preheated to 37°C prior to the start of tissue incubation. In some experiments, specified below, 10 mM *p*-nitrophenylphosphate was substituted for β-glycerophosphate in the Gomori medium. In other experiments, also specified below, 10 mM thiamine pyrophosphate was used as the substrate at pH 7.2 in the Wachstein-Meisel medium (18). Control experiments were run by omitting substrate from the media.

The diazo coupling method of Burstone (11) was used without modification, employing AS-MX naphthol phosphate and fast red LB salt. The Burstone medium was always used within 1 hour of its preparation. Early experiments showed that no red color ever developed if the substrate was left out, and such controls were not run in subsequent experiments.

RESULTS

Light Microscopic Findings

Burstone's Diazo Coupling Method

The reaction product (Figs. 3, 4, 6) generally appeared as straight or semilunar shaped rodlets, as stellate or ring-like structures, or, occasionally, as small round granules. In uninduced cells, a few red foci (*i.e.*, reaction product) were found randomly distributed throughout the cells (Fig. 4). These reaction centers were usually visible only after a few hours of incubation. With increasing time of incubation there was a slight increase in the

size and number of these foci. In any given sample of uninduced cells there was an occasional cell which showed all the features of an induced cell, as described below. Heating the slides to 100°C for 10 seconds prior to incubation abolished the acid phosphate reaction, while heating at 60°C for 20 minutes produced only negligible inhibition, in accordance with the expected thermal denaturation characteristics of the constitutive acid phosphatase (8).

In induced cells, a strikingly different behavior was apparent (Fig. 3). Instead of requiring an hour or more before reaction product could be demonstrated, as was found for uninduced cells, the reaction product in induced cells was frequently noticeable after 30 seconds incubation. After a few minutes incubation, the distribution of reaction product resembled that seen in uninduced cells after several hours incubation. With increasing time of incubation, numerous, faint, minute granules appeared. These were especially well discernible over the optically empty reservoir. When viewed under such favorable conditions (*cf.* Fig. 11), the arrangement of these minute red granules was very regular, the granules being roughly equidistant from one another in two planes. With further incubation, the granules appeared as continuous parallel streaks. Elsewhere throughout the cell, individual foci increased in size, especially around the reservoir. Wherever cells had been disrupted, debris consisting of small rods and spicules always showed the reaction product.

In living cells observed with positive phase optics, dark bodies were observed adjacent to the reservoir and elsewhere (Figs. 5, 6). These dark bodies showed marked acid phosphatase activity in both induced and uninduced cells. In uninduced cells, such bodies were small and infrequently seen. In induced cells, these bodies were larger and more numerous.

Gomori Lead Sulfide Method

Results obtained with the Gomori method differed only slightly from the results obtained with the Burstone method. Even after heating at 100° for 10 seconds, certain large polymorphous bodies, which remained unstained in the Burstone method, were blackened by lead sulfide (Figs. 2 and 14), even in the absence of added substrate. It is possible that these foci are related to the metaphosphate-containing granules analyzed by Sager (19). In accordance with this suggestion is the observation that a similar localization of lead sulfide was observed in cells incubated in Gomori medium without substrate (Fig. 7). Their polymorphous appearance was probably due to the confluence of one or more vacuoles in which the material was contained (*cf.* Figs. 1, 2).

In both induced and uninduced cells the edges of the paramylum bodies were often surrounded by the reaction product of both the Burstone and the Gomori methods (Figs. 7 and 8). In the case of the Gomori method, a lead sulfide precipitate was also observed in the absence of added substrate (Fig. 7). When paramylum bodies were seen outside ruptured cells after incubation in Gomori medium without substrate, they also had lead sulfide precipitates around them. Since reaction product was seen with the Burstone method as well as with the Gomori method, it seems probable that some constitutive acid phosphatase activity is associated with the paramylum bodies. The appearance of a lead sulfide precipitate in the absence of added substrate, probably indicates the existence of an endogenous source of phosphate in the paramylum bodies.

In induced cell preparations treated by the Gomori method, the cells, in addition to the areas described for the uninduced cells, showed a remarkable striation of the lead sulfide deposits, corresponding to the ridges of the pellicle (Figs. 9 to 11). The striations converged to a vortex at both the anterior and posterior end of the cells, and were also visible in induced cells incubated in the Burstone medium (Figs. 3, 12). Pressure on the coverglass led to the disruption of some cells and the consequent spillage of their intracellular contents, leaving only the pellicle. Examples of such cells are shown in Figs. 9 and 10. The helical pattern of these striations, already visible in phase contrast, is considerably accentuated in the induced cell by the deposition of reaction product.

While in uninduced cells there was no tendency for the lead sulfide to be localized around the reservoir, such a localization was an early, consistent, and prominent feature in the induced cells. The flagella of induced cells were frequently seen as brownish-black filaments.

The distribution of lead sulfide was the same whether thiamine pyrophosphate at pH 7.2 or *p*-nitrophenylphosphate at pH 5.0 was used instead of β-glycerophosphate.

Cytochemical Changes During the Course of Induction and Reversion of Acid Phosphatase Activity

Although most of the experiments were performed on uninduced cells (*i.e.*, cells growing exponentially in a high phosphate medium) or on fully induced cells (*i.e.*, cells which had stopped dividing because no phosphate remained in their environment), it was considered that a time study of the cytochemical changes in a single culture would permit closer correlation of the cytochemical findings with the biochemical ones. For this purpose, 10 ml from a dense but exponentially growing culture (650,000 cells/ml) were added to 1 liter of culture medium containing 5 μM phosphate. The change in cell number and phosphatase activity (assayed at pH 5, *p*-nitrophenylphosphate substrate) was followed for the next 9 days, by which time the cell number had stopped increasing and the culture was partially induced. On the 9th day, the cells were transferred to fresh medium containing high phosphate and the time course of phosphatase reversion was followed by assaying samples of these cells at approximately 8-hour intervals. Cells were taken for cytochemical study at various times throughout the course of induction and reversion. Two days after the beginning of the induction process, the phosphatase activity was still 4.8 mμ moles/min/10^6 cells, the level characteristic of uninduced cells (8). At this time, although no reaction product was visible along the pellicular ridges, small granular reaction product was prominent around the reservoir (Fig. 13). On the 7th, and especially on the 9th day, when the phosphatase activity had risen to 13.5 mμ moles/min/10^6 cells, reaction product was clearly visible in the pellicle of most of the cells (Figs. 11, 12).

Eight hours after the readdition of phosphate to this partially induced culture, the activity had fallen to 7.1 mμ moles/min/10^6 cells; and now the reaction product of the Gomori method was no longer visible in the pellicular ridges (Fig. 14), although the Burstone reaction product was still fairly prominent. Twenty-four hours after phosphate repletion, the activity had fallen to 3.6 mμ moles/min/10^6 cells and the reaction product of the Burstone method was distributed as in uninduced cultures.

Electron Microscopic Findings

In uninduced cells, the reaction product (lead phosphate) was found in the Golgi lamellae and

FIGURE 9 Induced fresh cell, positive phase contrast. The cellular contents have been spilled, leaving an essentially empty pellicle, the striation of which is visible. *PE*, pellicle; *P*, paramylum; *PR*, pellicular ridges and grooves. × 1123.

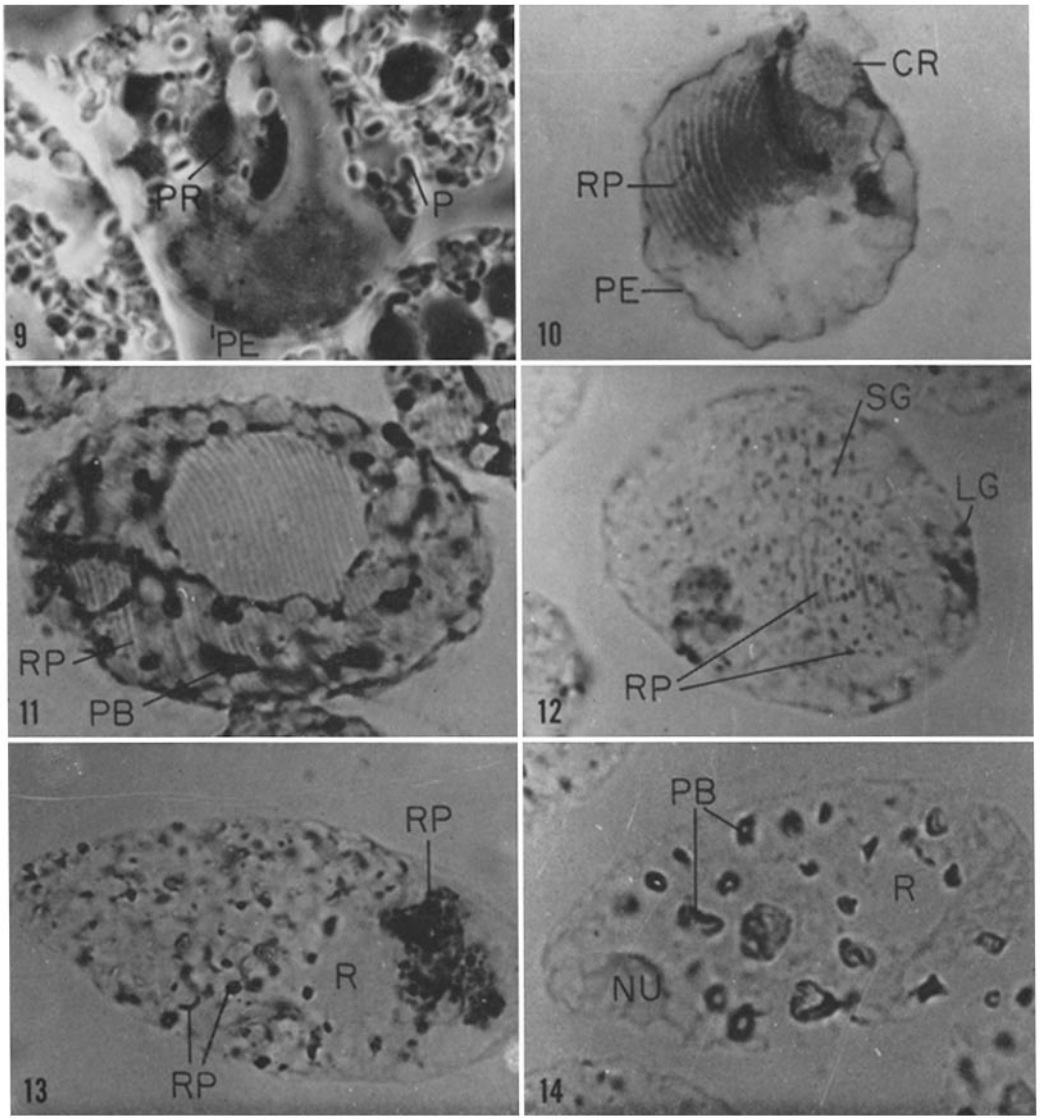
FIGURE 10 Induced cell after freeze-drying, 2 hours glutaraldehyde fixation, and incubation in the Gomori medium with β-glycerophosphate as substrate. *PE*, pellicle; *RP*, reaction product on pellicle; *CR*, area in which pellicle on each side of the cell is optically superimposed. × 1205.

FIGURE 11 Induced cell, incubated (after freeze-drying) in the Gomori medium for 1 hour with *p*-nitrophenylphosphate as substrate. Reaction product along the grooves of the pellicle is demonstrated particularly well over the reservoir. Lead sulfide is also seen in polymorphous bodies, (*PB*). × 1345.

FIGURE 12 Induced cell after freeze-drying without fixation, and incubation in the Burstone medium for 1 hour. The reaction product (*RP*) is deposited in small (*SG*) and large (*LG*) granules, as well as in streaks. × 1405.

FIGURE 13 Second day after withdrawal of phosphate. After freeze-drying, the cells were fixed for 30 minutes in glutaraldehyde, washed, and incubated for 10 minutes in the Gomori medium with β-glycerophosphate as substrate. Note the prominent accumulation of reaction product (*RP*) around the reservoir (*R*). × 1165.

FIGURE 14 Eight hours after the readdition of phosphate to induced cells. The cells were prepared as described in Fig. 13. *NU*, nucleus; *R*, reservoir; *PB*, polymorphous body. Pellicular reaction product is absent. × 1405.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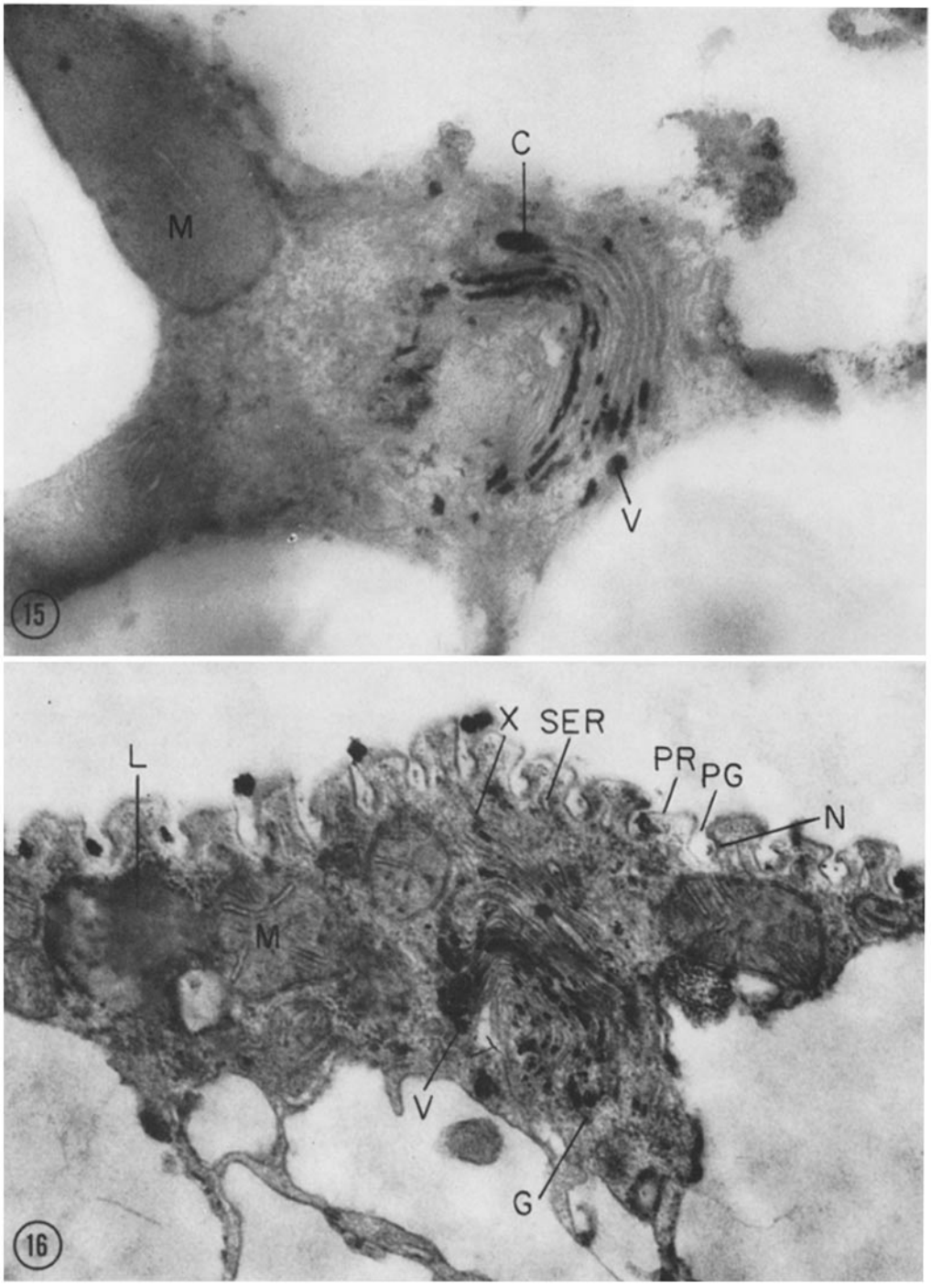


FIGURE 15 Golgi complex of uninduced cell, incubated in the Gomori medium for 30 minutes with β-glycerophosphate as substrate. Epon. *C*, cisterna; *V*, vesicle; *M*, mitochondrion. Original, × 13,600; final, × 54,400.

FIGURE 16 Induced cell, prepared as in Fig. 15, but incubated 10 minutes. Maraglas. *G*, subpellicular Golgi complex; *L*, lysosome-like dense body; *SER*, subpellicular ER; *PR*, pellicular ridge; *PG*, pellicular groove; *N*, notch; *V*, Golgi vesicle; *M*, mitochondrion; *X*, point at which the Golgi lamellae seem to be continuous with the subpellicular ER. Original, × 11,000; final, × 40,700.

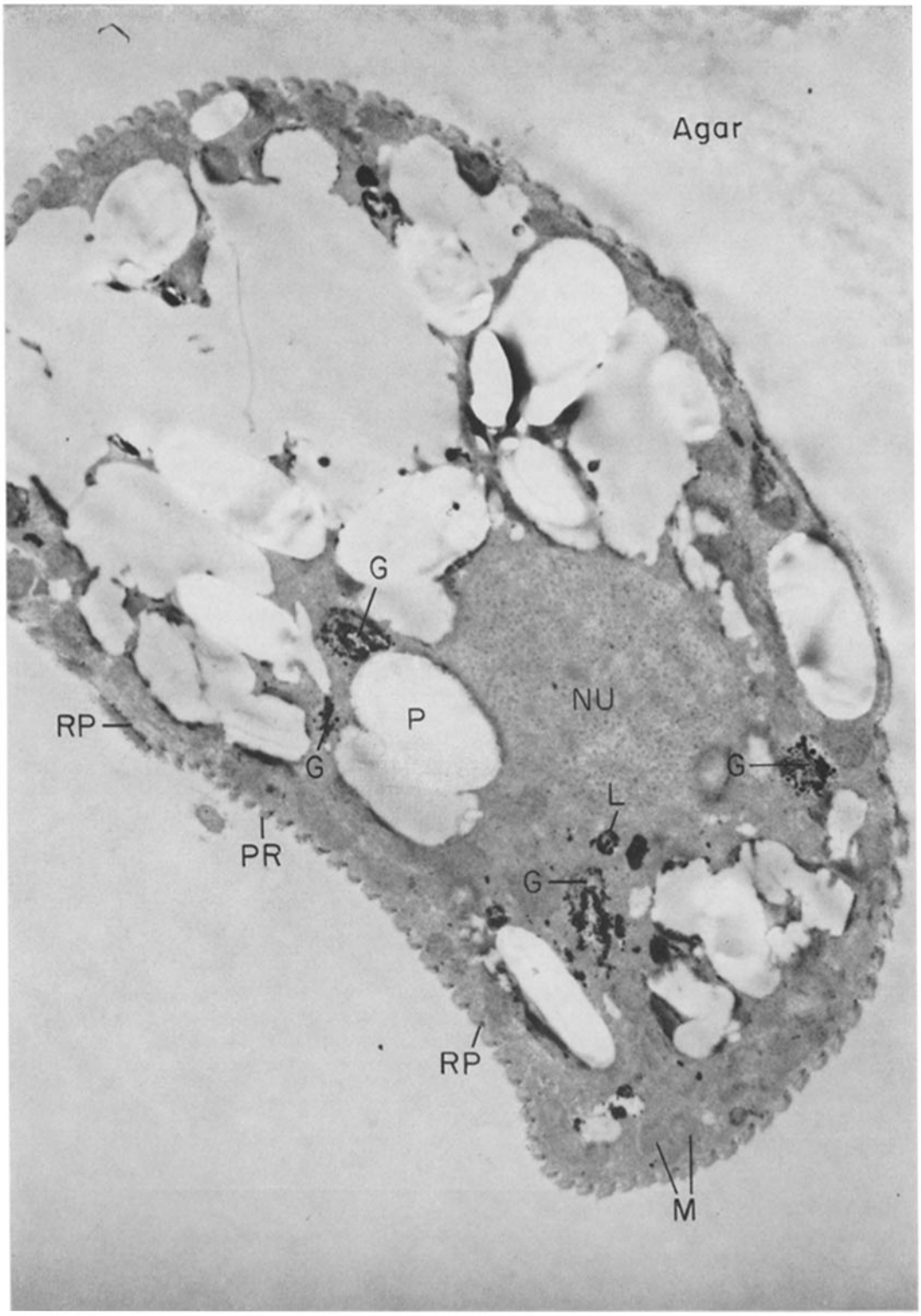


FIGURE 17 Induced cell, prepared as in Fig. 16. Epon. *NU*, nucleus; *P*, paramylum; *G*, Golgi complex; *L*, lysosome-like body: *PR*, pellicular ridge; *RP*, reaction product; *M*, mitochondria. Note reaction product in the notches of the pellicle. No reaction product can be seen in the agar. Original, × 2,800; final, × 24,360.

the Golgi vesicles, regardless of the location of the Golgi zone with respect to the nucleus, the pellicle, or the reservoir (Fig. 15). The Golgi complex of induced cells (Fig. 16) was similar to that of uninduced cells. In many instances, the first few Golgi lamellae closest to the convexity of the always semilunar-shaped Golgi complex were devoid of reaction product (Fig. 15). Occasionally, the entire Golgi complex carried the reaction product (Fig. 17). Reaction product was also found in vesicles and vacuoles around the reservoir, in the region in which "contractile vacuoles" periodically develop (Fig. 18). In some instances, continuity of the Golgi cisternae with these early components of the "contractile vacuole" was evident. Reaction product was sometimes demonstrable inside the reservoir (Fig. 18), as well as around the flagellum, but no reaction product was noticed on the cell membrane lining the reservoir. No reaction product was found in the pellicle of uninduced cells (Fig. 18), except in a rare cell which presumably corresponded to the ocasional "induced" cells seen in uninduced cultures with the Burstone and Gomori techniques in light microscopy. Identical results were obtained with *p*-nitrophenylphosphate or thiamine pyrophosphate as substrates. No reaction product was found in the absence of substrate, but black precipitates, presumably lead, were seen at the edges of a large number of dense bodies, usually occurring near or in paramylum bodies (Fig. 19). These bodies, found in both induced and uninduced cells, were of variable shape, sometimes round, triangular, or rectangular (*cf.* Fig. 18).

As expected from the light microscopic observations already presented, the major difference between induced and uninduced cells resided in the pellicle. In all electron microscopic studies utilizing agar, the agar closely surrounded the cells, except in areas in which it had been slightly separated from the cells (Fig. 20). The greatest concentration of reaction product was immediately adjacent to the pellicle and decreased with increasing distance from the pellicle. With short incubation time, or in the earliest stages of induction, reaction product was seen only in the pellicle, and not in the adjacent agar (Fig. 17).

The pellicle of *Euglena* is a complex structure (20). In cross-section, the pellicle appears as a repeating set of ridges and grooves. When induced cells were incubated for a long time (*e.g.* 60 minutes) in Gomori medium, the grooves of the pellicle were usually filled with reaction product. Some reaction product also continued over the ridges between the grooves. With shorter incubation times, however, the reaction product was localized to only one side of the grooves (Figs. 16, 17, 20 to 24). It was also observed that the first reaction product (not noticeable by light microscopy) to appear, 2 days after the beginning of induction (Fig. 17), was seen on only one side of the grooves, more specifically, in one corner of the grooves (Figs. 17, 23, 24). From within the cell, this corner was always near a tubule of the endoplasmic reticulum (ER), (Fig. 23 and reference 20). Adjacent to this tubule, up to four fibrils were seen in cross- or longitudinal section (Fig. 23). In some instances, Golgi complexes were found close to the pellicle, and there was an occasional observation that reaction product was present in the characteristic subpellicular tubules of the ER (Figs. 16 and 22). It is thus conceivable that there is a continuity between the subpellicular ER and the Golgi complex. A more detailed analysis of the morphology of the tubular system peculiar to the region of the pellicle, in which the induced acid phosphatase occurs, is presented in an accompanying paper (20).

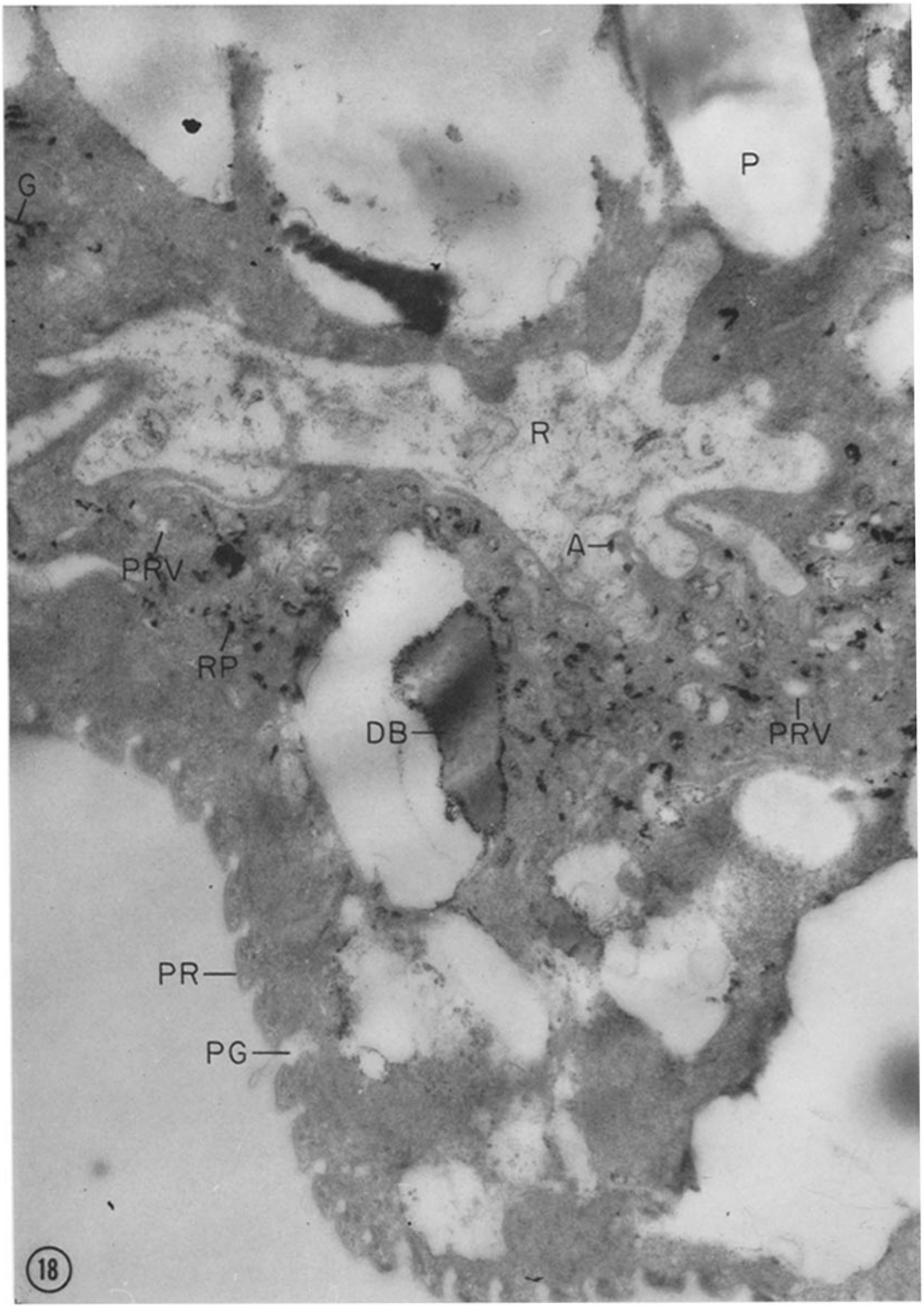
Induced Activity in Presumptive Ridges

Fig. 24 shows a section through the cytostome of an induced cell. It can be seen that there is an alternation of large and small ridges, and that reaction product is localized in the notch of each ridge. It is noteworthy that each pair of large and small ridges shares a tubule of the subpellicular ER.

Observations with Polarization Optics

During the course of these studies, it was noted

FIGURE 18 Uninduced cell, prepared as in Fig. 15, but with *p*-nitrophenylphosphate as substrate. Epon. *PR*, pellicular ridge; *PG*, pellicular groove; *G*, Golgi complex; *DB*, dense body; *R*, reservoir; *PRV*, peri-reservoir vacuoles; *RP*, reaction product in peri-reservoir vacuoles; *P*, paramylum; *A*, reaction product inside the reservoir. Note absence of reaction product in the pellicle and in the limiting membrane of the reservoir. Original × 13,600; final × 24,480.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that living unstained *Euglena* had several small bright foci when viewed in polarized light. It was then noticed that induced cells seemed to have more such foci, and a quantitative count of these foci was undertaken in both induced and uninduced cells. It was observed that uninduced cells averaged about 4.5 polarization foci per cell, with double or triple that value in induced cells. We have as yet no insight into the meaning of this observation.

Experiments with Astasia Longa

Astasia longa is a naturally bleached flagellate which is closely related to *Euglena*. In fact, Pringsheim has classified *Astasia* as a naturally bleached *Euglena* (21). It was, therefore, of interest to examine *Astasia* for the possible presence of an induced phosphatase. It was found that *Astasia* grown in high phosphate medium had an acid phosphatase activity of about 1.3 mμ moles *p*-nitrophenylphosphate hydrolyzed/min/10^6 cells at pH 5.0 and 30°C. When the *Astasia* were transferred to low phosphate medium, and followed until growth had ceased for several days, it was found that there was no change in acid phosphatase activity. Thus, *Astasia* has a lower level of constitutive acid phosphatase activity than *Euglena* and does not form induced acid phosphatase. The general distribution of lead phosphate reaction product in electron microscopy was the same as described above for uninduced *Euglena* cells, and no reaction product was ever observed in the pellicle.

DISCUSSION

Precipitation techniques for the demonstration of acid phosphatase activity on the light microscopic level, as well as in the electron microscope, have been increasingly employed in recent years (22). Acid phosphatase activity has been described in Golgi lamellae, Golgi vesicles, and in lysosomes of various cells; and interesting speculations have been put forward concerning the morphologic and functional relationships of these organelles (23).

In the present study, two types of acid phosphatase activity were localized. Constitutive acid phosphatase activity was observed in the Golgi complex, around the paramylum granules, and in components of the peri-reservoir ER. These sites react both with β-glycerophosphate and *p*-nitrophenylphosphate at pH 5 and with thiamine pyrophosphate at pH 7.2. In agreement with the biochemical deductions (8), these constitutive enzymes are relatively insensitive to thermal denaturation. In addition, it was possible to localize an induced acid phosphatase in a specific repeating region of the pellicle.

In *Euglena*, the constitutive activity observed in the Golgi complex was occasionally seen to be continuous with activity observed in both the subpellicular and peri-reservoir ER, suggesting the possibility of some functional interrelation between these structures. As already noted, the constitutive activity around the paramylum granules may be associated with an internal phosphate source within these granules. The vesicles of the peri-reservoir ER, the third location of constitutive acid phosphatase activity, resemble Golgi vacuoles much more than they resemble lysosomes. In general, however, the localization of the constitutive phosphatase activities is in agreement with other observations (23).

Studies with the light microscope demonstrated that the induced acid phosphatase was located in the pellicle. Electron microscopic observation then revealed that the induced enzyme was not located uniformly throughout the pellicle. Instead, it is located in the notch of each pellicle complex in close conjunction with a set of approximately four fibrils, and near a characteristic vesicle

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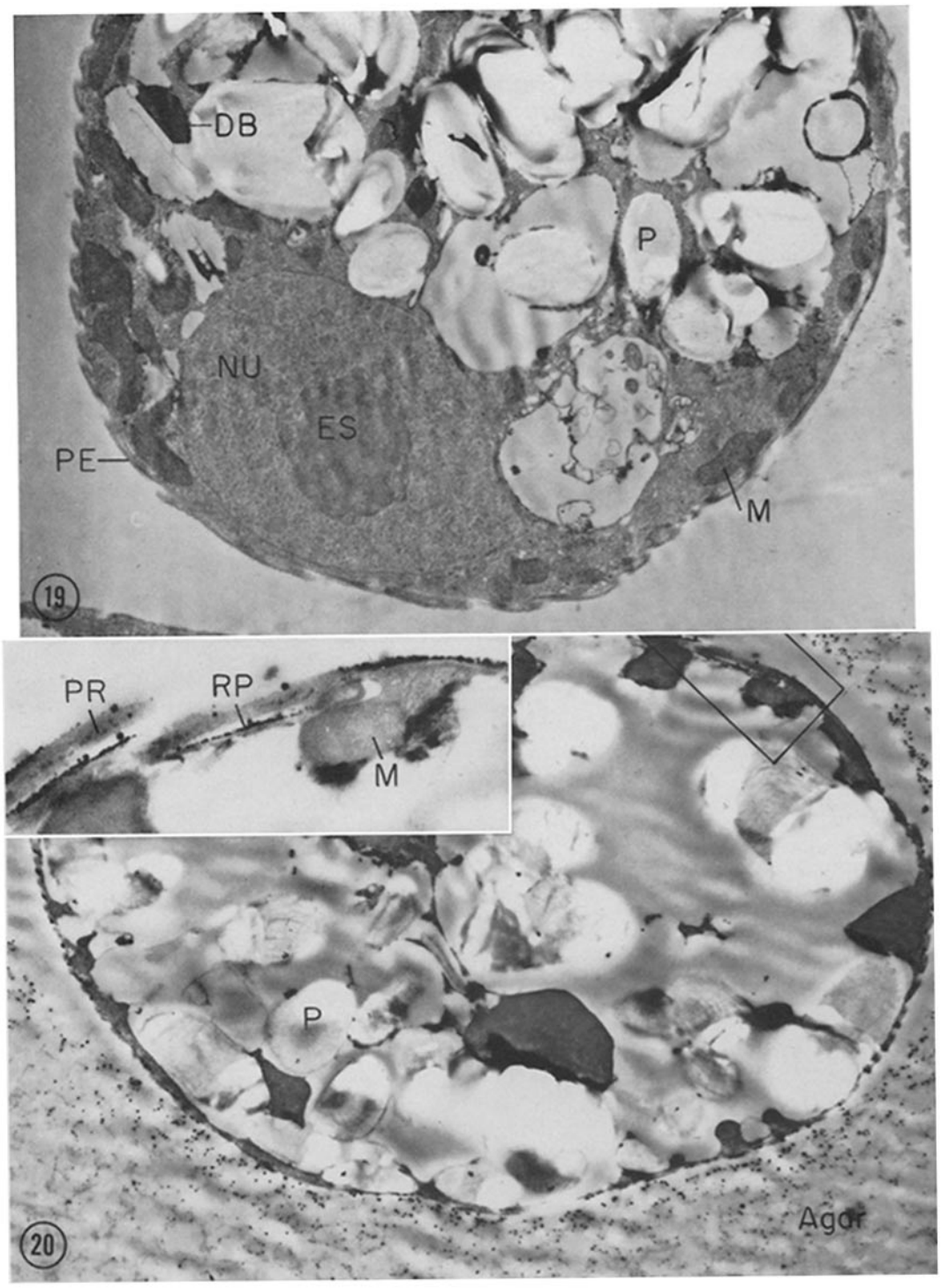
FIGURE 19 Uninduced cell, prepared as in Fig. 15, but without substrate. Epon. *ES*, nucleolus; *NU*, nucleus; *DB*, dense body; *PE*, pellicle; *M*, mitochondrion; *P*, paramylum. Lead phosphate precipitate is present around the dense body. Original, × 10,400; final, × 18,720.

FIGURE 20 Induced cell, incubated with thiamine pyrophosphate in the Wachstein-Meisel medium at pH 7.2 for 30 minutes. Epon. *P*, paramylum; *M*, mitochondrion; *RP*, reaction product; *PR*, pellicular ridge. Note that the largest concentration of reaction product is at the pellicle and diminishes in the surrounding agar with increasing distance from the pellicle. Original, × 3,200; final, × 8,840; insert, × 14,500.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of the subpellicular ER. Induced activity is not observed on the cell membrane of the gullet or reservoir, but begins at the junction of the gullet and the cytostome, where the first pellicle complexes appear. The exact significance of the conjunction of the induced activity with the subpellicular elements is not clear. It is well known that protein synthesis occurs on ribosomal complexes that are often associated with the ER, and it is possible that the tubule of the subpellicular ER, located at the notch of each pellicle complex, is the site of synthesis of the induced acid phosphatase. No function can, at present, be ascribed to the fibrils in the notch, and it could be that their close association with the site of induced phosphatase activity is fortuitous.

The localization of the induced enzyme activity near the surface of the cell is in accord with its presumed function as a non-specific hydrolase of organic phosphates in the absence of sufficient orthophosphate.

In an accompanying paper (20), an electron micrograph of a cell in division is presented. In this photograph it is seen that small ridges alternate with tall ones. Studies on synchronously dividing *Astasia longa* (to be published elsewhere) have shown that this alternation of short and tall ridges is a characteristic feature of cell division in these organisms. It is, therefore, of considerable interest that induced acid phosphatase activity is demonstrable in the notches of both short and tall ridges in the cytostome. The fine structure of pellicle complexes of *Euglena* is discussed in more detail in an accompanying paper (20).

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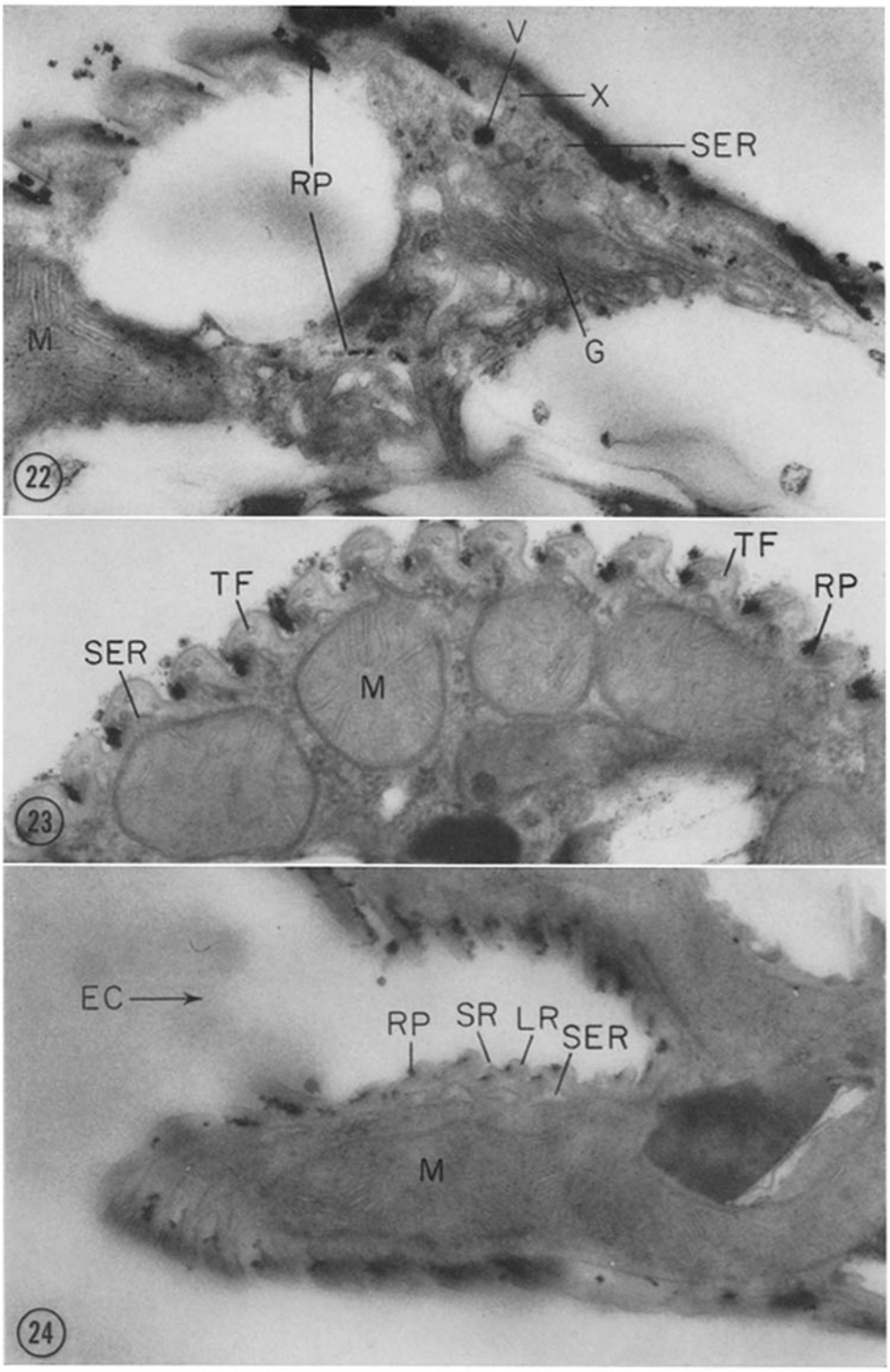
FIGURE 21 Induced cell, prepared as in Fig. 16. Epon, *RP*, reaction product; *TF*, fibril; *M*, mitochondrion. The reaction product is confined to one side of the pellicular ridges and is in close contact with one cell membrane. Original, × 11,000; final, × 45,000.


M
TF
RP
21

FIGURE 22 Induced cell, prepared as in Fig. 16. Epon, *RP*, reaction product; *G*, Golgi complex; *SER*, subpellicular ER; *X*, point at which a direct connection between the Golgi complex and the *SER* is suggested; *M*, mitochondrion; *V*, Golgi vesicle. Original, × 11,000; × 41,800.

FIGURE 23 Induced cell, prepared as in Fig. 20. *TF*, fibril; *SER*, subpellicular ER: *M*, mitochondrion. Note that reaction product (*RP*) is located at the notch of the pellicle complex. Original, × 11,000; final, × 44,000.

FIGURE 24 Induced cell, prepared as in Fig. 18. *EC*, entrance to cytostome; *RP*, reaction product; *SR*, small pellicular ridge; *LR*, large pellicular ridge; *SER*, subpellicular ER; *M*, mitochondrion. Note reaction product at the notches of both the large and small pellicular ridges. Original, × 10,200; final, × 42,840.


V
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G
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TF
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