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AN ELECTRON MICROSCOPIC STUDY OF DYE-SENSITIZED, LIGHTED TETRAHYMENA PYRIFORMIS LG

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Some micro-organisms containing pigments or dyestuffs of particular structure react to light more intensively than usual: their behaviour as well as their motility change suffer damage and generally perish. This phenomenon was observed comparatively early (Raab, 1900; Straub, 1904; Tappeiner and Jodlbauer, 1904; Metzner, 1921); its cause, however, could be explained reasonably only somewhat later (Blum, 1941). It was ascertained that some strongly light-absorbent pigments, or dyestuffs, the so-called photosensitizers exert a photodynamic effect in the presence of O_2 and in interaction with various macromolecules (Eidus and Kondakowa, 1959; Livingston, 1967; Biczók, 1969a). Experience shows that in the complex formation of substrate and colouring matter the proteins as well photo-oxidizable materials play an important role (Smetana, 1938; Freeman and Giese, 1952; Haurowitz; 1963; McLaren and Shugar, 1964). The localization of the process is in the matrix of cells, or in the single organels, thus in the mitochondria and lysosomes (Cooperstein et al., 1960; Allison et al., 1966; Morgan et al., 1966; Slater and Riley, 1966). The photodynamic effect is followed by photodamage, consequently, it was expected that some alterations would occur in our experimental animals, in the specimens of Tetrahymena pyriformis LG sensitized by rose bengale, eosin, methylene blue, in the sites of localization. This expectation was justifed by simultaneous indications of simple light microscopic examinations concerning *Uroleptus* (Calkins, 1929) and *Paramecium* (Soong et al., 1937), as well as by electron microscope researches (Wise, 1965). The light effect itself followed by a temporary increase in velocity (Saier and Giese, 1966; Biczók, 1969a), the alterations expected in the sensitized cells were considered as indications of changes in movement.

Materials and Methods

The sterile culture bred in darkness, inoculated in every three weeks and centrifuged from 4—6 days old culture was transferred into an ion milieu defined and buffered with phosphate to pH 6,9 (Biczók, 1961), set with methylene blue (Màrck; colour index 52,015), eosin (Fluka; colour index 45,400), and rose bengale, respectively (Fluka; 45,435) in a dilution of 1 : 50 000. The time interval of staining with methylene blue was 30 minutes, with rose bengale and eosin 60 minutes. The samples of *Tetrahymenae pyriformes* were irradiated with a Tungsram bulb of 25 000 lux until the accelerated motion declined

conspicuously. (In unstained substance, no conspicuous decrease was observed even after 40—45 minutes).

After being fixed in 1 per cent OsO_4 for one hour, the lighted and centrifuged animals were dehydrated in the usual alcohol series and, contrasted for 20 minutes, with 3—4 per cent uranylacetate dissolved in 70 per cent alcohol. The animals were embedded in araldite (DURKUPAN; FLUKA), polymerized in a thermostat of 37 °C temperature for one hour and then at 56 °C for two days. Ultra-thin sections were made by PORTER—BLUM ultra-microtome. After staining with Reynold's lead-citrate the sections were examined under Tesla BS 242 D type electron microscope.

Results

The electron microscopic structure of *Tetrahymena pyriformis* is well known (METZ and WESTFALL, 1954; PITELKA, 1963; TOKUYASU and SCHERBAUM, 1965; ALLEN, 1967; LEVY and ELLIOTT, 1968). By this fact, the appreciation of the change in the electron microscopic structure of the unlighted dyesensitized *Tetrahymenae* studied by us, and their comparison with the motion velocity curves obtained by shooting films (BICZÓK et al., 1968) as well as by direct measurements, have considerably been facilitated. This curve, expressing a light-activated oriented photophobatic motion cannot be identified with the motion curve of the native *Paramecium* treated with UV (SAIER and GIESE, 1966), the former one having explicitely two maxima while the curve of *Paramecium* has only one apex. The size of photodynamic effect is expressed by the somewhat steep descending branch of the curve. It is the steepest after being sensitized by rose bengale, then follows the curve of animals stained with eosin, and methylene blue, respectively. Accordingly, the most expressive photodamage could be observed in animals sensitized with rose bengale.

In the electron-optical photographs of the unstained *Tetrahymena* specimens, lighted for 60 minutes, essentially the structural conditions of the non-lighted ones can be observed, only the mitochondria are a little more swollen (T. III, Fig. 11). Basically similar conditions prevail in the sensitized, non-lighted specimens, too, the presence of dyestuff causes no particular change in the intracellular structures (T. I, Figs. 1, 2; T. II, Fig. 9); while in the lighted ones definite changes can be observed.

1. Cortical membrane structures: The cytoplasm of *Tetrahymena pyriformis* is covered by a pellicle consisting of three unit-membrane-

Table I

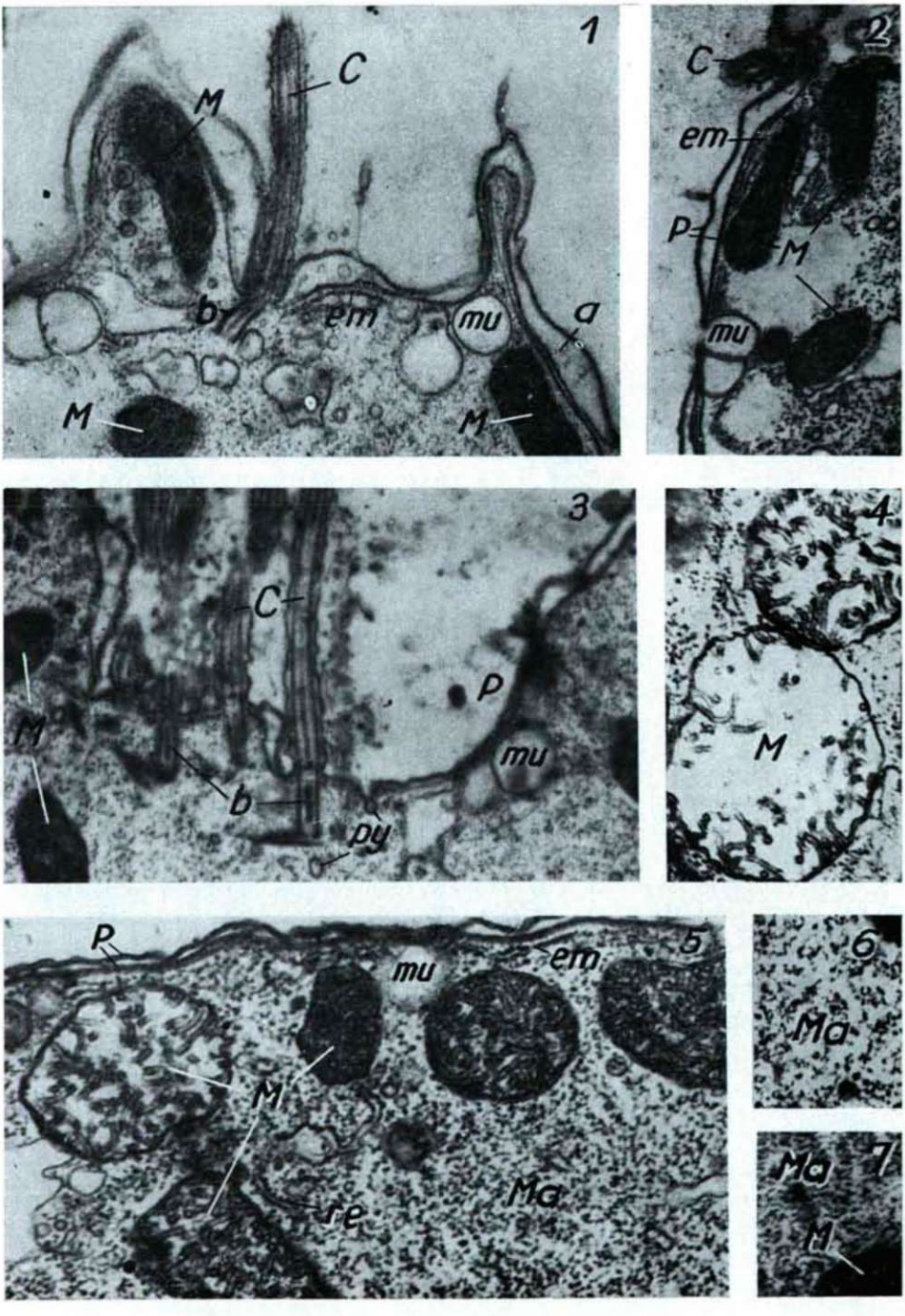
Figs. 1—3. Triple membrane of the pellicle (P) and cilium (C) with basal body (b) of non-lighted *Tetrahymena pyriformis* sensitized with rose bengale (Rb). The mitochondria (M) are intact, the mucinogenic bodies (mu) generally empty. In the cavity of the oral region (Fig. 1) pinocytotic vesicles (py) can be observed. Fig. 1: 25,000 x; Figs. 2 and 3: 18,000 x.

Fig. 4. Photodynamic damage in the tubules of mitochondria (M), in the last phase of the decreasing speed of motion. 28,000 x.

Fig. 5. Intact and partly damaged mitochondria (M), after the development of the light-activated maximum motion speed. The pellicle (P) membranes, the amorphous ectoplasmatic (em) and cytoplasmatic (Ma) substance, as well as the rough endoplasmatic reticulum (re) are essentially unchanged. 18,000 x.

Figs. 6 and 7. The change of the cytoplasmatic granules ribosomes of the sensitized, non-lighted animals (Fig. 7) in aggregation and size, on the effect of light (Fig. 6) 28,000 x.

TABLE I



like layers (ALLEN, 1967). By osmium fixation under the 90—100 Å thick membrane, covering the cilia too, a 75—85 Å thick middle, resp. inner membrane can be seen, separated from each other with a well-descernible lacuna (T. I, Figs. 1, 5; T. II, Fig. 8). On the side of the latter facing the cytoplasm, an electron dense substance is found of about half unit-membrane in thickness, which may double at place whose function is yet unkown (*Fibrogranular* layer at *Paramecium aurelia:* JURAND and SELMAN, 1968) T. I, Figs. 1, 2, 5; T. II, Fig. 9; T. III, Fig. 15). These structures are sensitizers; therefore, they do not show, even in the presence of rose bengale, any photoinduced destructions mentioned by some researchers (BLUM, 1941; GIESE, 1967), a phenomenon preparing the permeation of xanthens. In the arranged cortical membrane structures a desorganization occurs only during the swelling of the body after the light-activated motion having ceased (T. III, Fig. 15).

2. Nuclear membrane: On the cross-section of macronucleus, the double membrane separating cytoplasm and nucleoplasm and between them the less dense (PITELKA, 1963), so called perinuclear space can be observed distinctly. The continuous membrane is interrupted by pores. These structures in the sensitized *Tetrahymenae* seems to be strongly lightresistant at the applied degree of illumination. They have not shown any photodynamic damage even in cases where in other structures the sings of desorganization could be well observed (T. III, Figs. 12, 13).

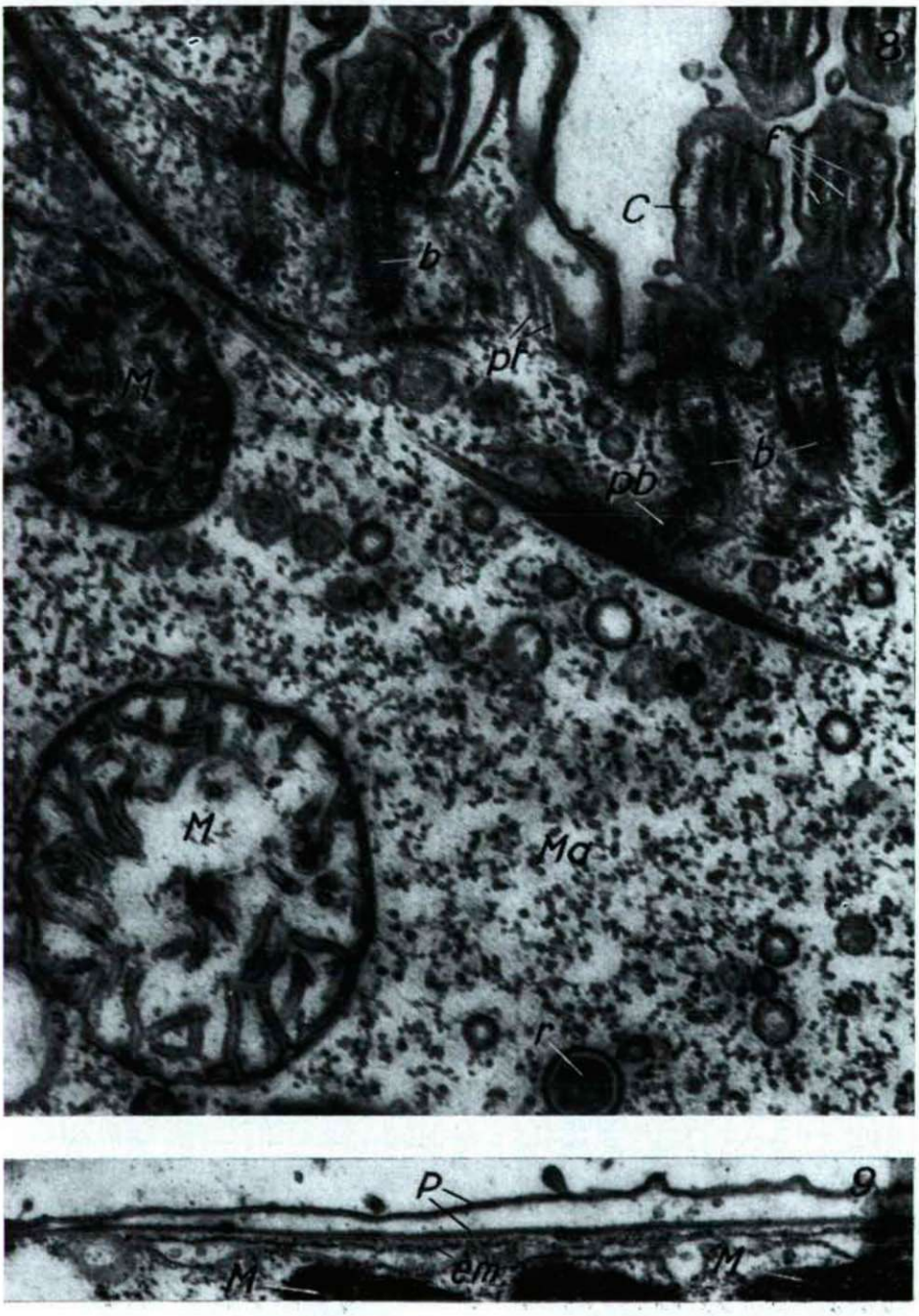
3. Mitochondria: From the double unit membranes of the mostly ovoid or elongated sponge-finger-vake-like organel (T. I, Figs. 1—2; T. III, Fig. 11) separated by a fissure of low electron density, mainly the outer one shows a quality of light resistance. It is conspicuous and characteristic that a considerable part of these organels is oriented mostly into the cavities of the 17 ribs of ectoplasm, in the vicinity of cilia (ALLEN, 1967, p. 554, T. I, Fig. 1). The single sensitizers may supposedly work their way among the macromolecules, and possibly into them, establishing contact with the enzymes of the respiratory chain (CHALAZONIZIS, 1964). It was therefore not surprizing that the most striking photodamage could be observed on the inner membrane in the mitochondria, mainly in their tubules (T. I, Figs. 4, 5; T. II, Fig. 8; T. III, Figs. 14, 15). Tie tubules have sometimes desintegraed almost entirely, the electron dense substance was strongly reduced. The phenomenon is similar to the laser- induced mitochondrium damage in some protozoa, after some vital dyestuffs, primarily by the permeation of JANUS GREEN B known as a particular mitochondrium dyestuff (STORB, AMY, and WERTZ, 1966). It is conspicuous that staining with rose bengale a photodestruction in the organel takes place immediately after illumination following the appearance of the

Table II

Fig. 8. Damage of the mitochondria of sensitized, lighted specimens in the phase of decreasing speed of motion. The central and peripheral fibrils (f) of cilia (C), the basal body (b) the peribasal bodies in its vicinity (pb) with the tubular and amorphous components, and the postciliary microtubules (pt) suffered no photodamage. The granules of cytoplasm (Ma) are aggregated: 56,000 x.

Fig. 9. and T. III, Fig. 11. In the unstained lighted animals the pellicle (P), the amorphous ectoplasmatic substance (em), the mitochondrial (M) membranes, the lipid-like bodies (L), are intact. Fig. 2: 18,000 x; T. III, Fig. 2: 36,000 x.

TABLE II



first velocity maximum of motion (T. I, Fig. 5); in others, however, it occurs only after motionless period, when the animal is incapable of any reactivation even if the light-effect had ceased. This phenomenon may obviously be attributed to the differences between the functional states of the single mitochondria.

4. Lipid and other bodies: In almost every part of cytoplasm we can sometimes find a great number of lipid-like, round or oval dense bodies of about the size of mitochondria (T. III, Figs. 10, 11, 12) the genesis and function of which could only be partly explained (ALLEN, 1967: TOKUYASU and SCHERBAUM, 1965; LEVY and ELLIOTT, 1968). They are Oil Red O-positive, their majority is homogeneous sometimes with the faint signs of an organization. They are supposed to be formed within the cisterns of the endoplasmatic reticulum (LEVY and ELLIOTT, 1968). This supposition may arouse reflection when we consider that the cytoplasm is rich in lipid bodies, formed rapidly and at the some time poor in endoplasmatic reticulum (particularly of the smooth surfaced type). The riper form of body is covered with a simple membrane. It is remarkable how resistant the lipid bodies and their membranes are to photodamage in case of animals lighted after sensitization, too. The situation is similar to that of the socalled residual bodies and mucoid bodies (mucoid cysts), often containing concentrical myelinlike membranes. The former ones occur sporadically in the intermitochondrial spaces (T. II, Fig. 8; T. III, Figs. 11, 15) as degenerative products (SWIFT and HRUBAN, 1964). The mucoid or mucinogenic bodies, mucocystic vacuoles surrounded by a limiting membrane (TOKUYASU and SCHERBAUM, 1965) are nearly entirely missing from the matrix but they are found in a considerable number under the pellicle (T. I, Figs. 1, 2, 3, 5; T. III, Fig. 15). The wall of these vacuoles remained all the while intact, their amorphous dense substance seems, however, to be fully evacuated as a result of light effects.

5. Cilia: Their attractive structure, function has lately got more and more into the focus of interest (PITELKA, 1963; ROTH, 1958, 1964; ARGENTSINGER, 1965; ALLEN, 1967). The electron optical photographs of their longitudinal section are not showing any essential difference in cases of animals unstained and lighted after being dye-sensitized (T. I, Figs. 1, 3; T. II, Fig. 8; T. III, Fig. 11). Nor are the outer membrane surrounding them, the central and periphal fibrils and the basal body incorporated in the sytoplasm, the probasal body and postciliary microtubules which consist of tpubules and amorphous components (T. II, Fig. 8) at the double membrane approximating the distal part of basal body and at its proximal part, too.

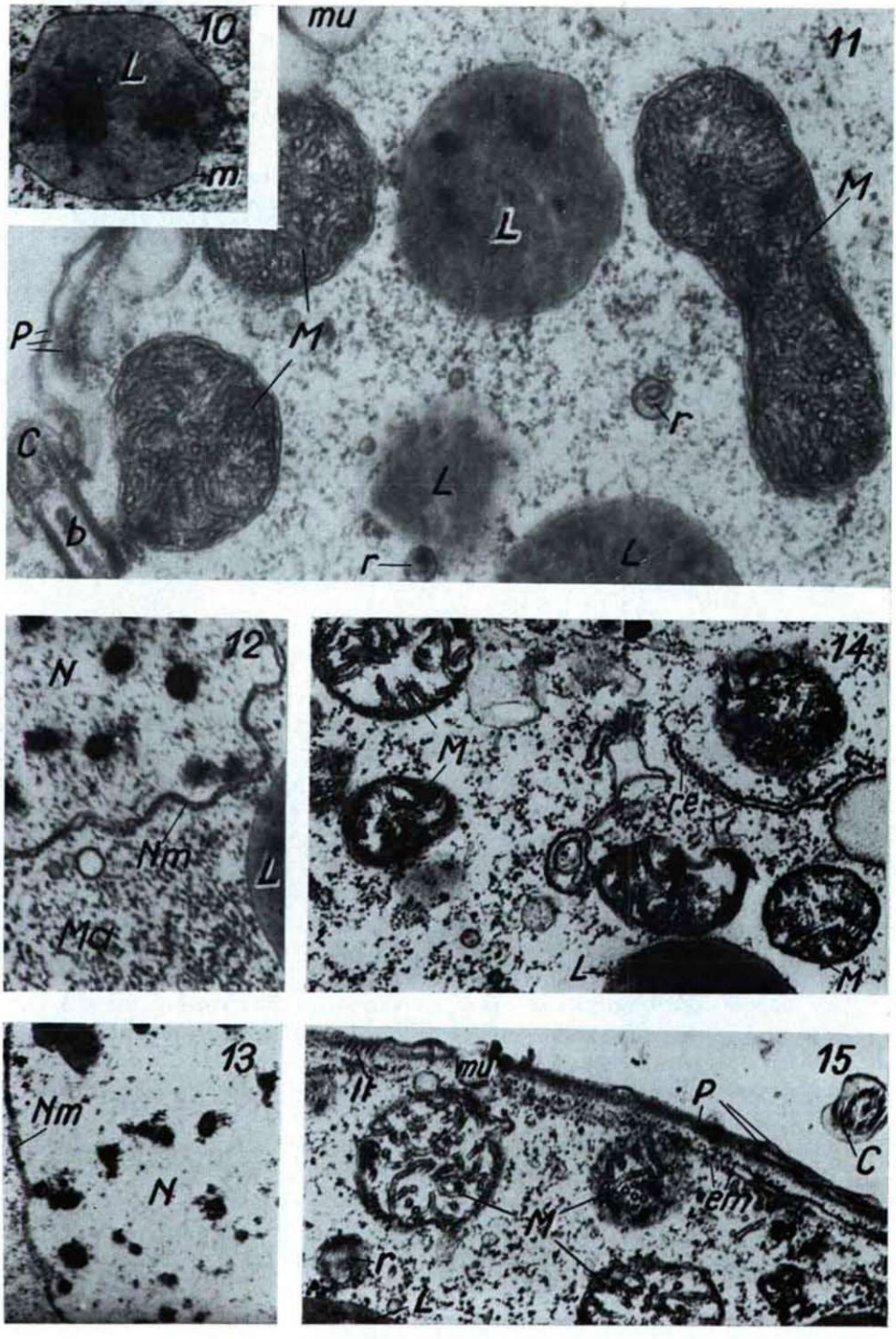
Table III

Fig. 10. The lipid-like body (L) showing some traces of organization, with its simple membrane (m), has been resistant to photodynamic damage: 28,000 x.

Figs. 12. and 13. The intact nuclear membrane of the sensitized lighted animals (Nm). Fig. 12: 28,000 x; Fig. 13: 18,000 x.

Figs. 14. and 15. Photodestruction of electron microscopic structures at sensitized *Tetrahymena pyriformis*, immediately before cessation of photoactivated motion. Apart from mitochondria (M), also the cytoplasmatic granules, pellicular membranes (P) and even the rough endoplasmatic reticulum (re) are damaged: 18,000 x. Sensitized with rose bengale.

TABLE III



6. Cytoplasmatic granula and other components: The cytoplasm of *Tetrahymena piriformis* is granulated with an even density. Concerning their size, the majority of these granules is ribosoma (T. I, Figs. 2, 5, 7; T. III, Figs. 12, 14). Their minority are components of the rough endoplasmatic reticulum (T. I, Fig. 5; T. III, Fig. 5). This reticulum is an efficient means in protein synthesis. It occurs in a conspicuously small amount, in the same way as the smooth surfaced endoplasmatic reticulum. Both forms are strongly resistant to light, even in sensitized specimens. On the other hand, the free ribosomes and other granules differring from them in size, show a considerable variation, forming smaller or bigger nodules, aggregations, and their distribution is uneven (T. I, Fig. 6; T. III, Fig. 14, 15). These observations lead not only to the conclusion that the granules and ribosomes both suffer damage but also to that, that the polypeptides forming the basic structure of cytoplasm become denaturated, in structure whose changes have been emphasized by several authors (MAST, 1932; ERDMANN, 1955; HYMAN and HOWLAND, 1940; DATTA, 1960; MONTGOMERY et al. 1961).

Discussion

A characteristic symptom of the photodynamic effects of various rays is the damage and often destruction of some cells, erythrocytes (BLUM, 1951), spermatozoa, phagocytes, and inside them of lysosomes (ALLISON, A. C. et al., 1966), as well as that of mitochondria (FREDERIC, 1958, etc.). The effect owing to the membrane destruction following the photo-oxidation, releases destruction-inducing enzymes. For an intracellular effect like that, it is thougth to be necessary that the desorganised membrane should transmit the sensitizers, flourescent xanthen-dyes against which it had earlier formed a barrier (BLUM, 1941; ALLISON et al., 1966). The situation is, in fact, that the triple membrane of pellicle, and especially its outer part reaching the cilia, remains intact even in the phase of decreasing speed of motility, when a considerable photodynamic effect in the mitochondria can be observed. Consequently, it is not a precondition of the cells being penetrated by xanthen sensitizers — at least by rose bengale and eosin — that the permeation should have been prepared by the photodamage elicited by these dyestuffs adhering to the membrane. Anyway, this is not the only possibility for an uptake of dyestuffs. We have to take into consideration also the peculiar activity of the intact membrane, the pinocytosis, that according to the light and electron microscopic investigations plays an important role in the intercellular dyestuff enrichment (SCHMIDT, 1962), which is strongly activated in case of *Amoebae* by light (CHAPMAN and ANDERSEN, 1962). The formation of pinocytotic vesicle found in the oral region and its presence in the ectoplasm lead us to beleive that we have to reckon with this form of dyestuff uptake through the pellicle composed by the three membranes (T. I, Fig. 3.).

We have mentioned as a photoactivated phenomenon the provisional increase of the speed of motion as a result of light, followed by a decrease in the speed of motion. We related this phenomenon to the photodestruction

of *Tetrahymena*, resp. of its electron microscopic structure. Excellent investigations have been carried out in connection with the motion of such a character in case of *Paramecium* (SAIER and GIESE, 1966), where the decrease in the speed of motion and in the lashes of cilia was explained by the damage of cilia. The photodynamic damage is analogous in many respect to the destructions caused by various rays. Nevertheless, in case of the sensitized phobophototaxic motion decrease we have looked in vain for the sings of desorganization in cilia. The mitochondria, however, were damaged. It has been obvious, so far, that there may be some connection between the function of cilia and mitochondria (OLLSON, 1962) as these organels form longitudinal series under the pellicle along the kinetosomes, and are in contact with the inner, peribasal layer of the pellicle (CHEISSIN and MOSEVICH, 1962); which may be well observed in our electron optical photographs, too. The ciliary motion is an energy-consuming process (KAMIYA, 1959), the damage of mitochondrium responsible for the oxidative phosphorilation is not indifferent (BEYER, 1960), disturbing the ATP pruduction indispensable for the ciliary function and, in this way, excluding the possiblity of any ciliary activity. This is supported by the O_2 consumption following the light-activated sensitized motion reactions that may be parallelled somewhat with the damage of mitochondria, and with the change in the speed of motion; in the beginning it is conspicuously increasing but later on remarkably decreasing (BICZÓK, 1969a, b).

Summary

The electron microscopic structures have been damaged by light, in the sequence of Rb > E > Mb, in *Tetrahymena pyriformis* LG sensitized by rose bengale (Rb), eosin (E), and methylene blue (Mb). The composed membranes (pellicles) and from them mainly the outer one; the cilia, their fibrillary components, the microtubules, the smooth and rough endoplasmatic reticulum, as well as the lipid and residual bodies often limited by simple membrane were not desorganized perceptively by the photodynamic effect. However, the tubular crystae of mitochondria, the cytoplasmatic basic structures, granula and ribosomes were severely damaged.

The photodamage was manifested more strongly in the phase of the decrease in motion following the increase of light-activated motion (after the second maximum of the motion curve). It is, therefore, obvious that:

The xanthen sensitizers (Rb, E) enter the cytoplasm without damaging the membrane structure. One of the ways of dyestuff uptake may be the pinocytosis that as a dye- and light- activated phenomenon can be manifested through the pellicle, too.

The phase of decreasing speed of the light-activated motion cannot be a result of the decrease of lashing caused by the damage of cilia but much more that of the disturbance and absence of the oxidative phosphorylation as a consequence of the damage of mitochondria in the vicinity of cilia.

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