Cellular Organelles and Lipids

JEROME J. WOLKEN, Biophysical Research Laboratory, Carnegie Institute of Technology, Pittsburgh, Pennsylvania 15213

Abstract

These structural and biochemical studies indicate that the internal structure of the cell is a complex system of macromolecules which are organized into organelles for function. These organelles consist of complicated internal membranes, the structure of which in molecular dimensions is best represented by a double layer of lipid and protein, referred to as the unit membrane (Fig. 3b, 3d, 4d, 5d). Such a structure provides large surface areas for the orientation of pigment molecules as in the photoreceptors (Fig. 8) and sites for enzymatic reactions. Since these membranes contain high concentrations of lipids, their synthesis and molecular organization become important.

Such ordered organelle structures behave like liquid crystals for they undergo phase transitions from ordered to disordered states which depend upon temperature and concentration. The determining of the kinds and distribution of the fatty acids in a variety of cells may be an approach for developing phylogenetic relationships as well as biochemical patterns in the evolution of the plant → animal. For a deeper understanding of the biological mechanism however, newer tools need to be developed to study the subtleties in the chemistry of the cell and its organelles in the living state.

Introduction

THE LIVING CELL is a dynamic system concerned with the processes of energy capture, transfer, and conversion—processes vital to growth, maintenance, and reproduction. Depending upon their environment and function, cells vary in shape, size, and internal organization. The cell has evolved organelles to carry on the life processes, for example, a nucleus, mitochondria, endoplasmic reticulum, ribosomes, golgi apparatus, and chromatophores (Fig. 1). More specialized differentiated organelles have also developed: the flagellum for cell movement; the photoreceptors, the chloroplasts for photosynthesis, and the retinal rods and cones for visual excitation; and other receptor organelles that respond to environmental stimuli. The cell, then, is a complex system of macromolecular structures enclosed within a semipermeable membrane.

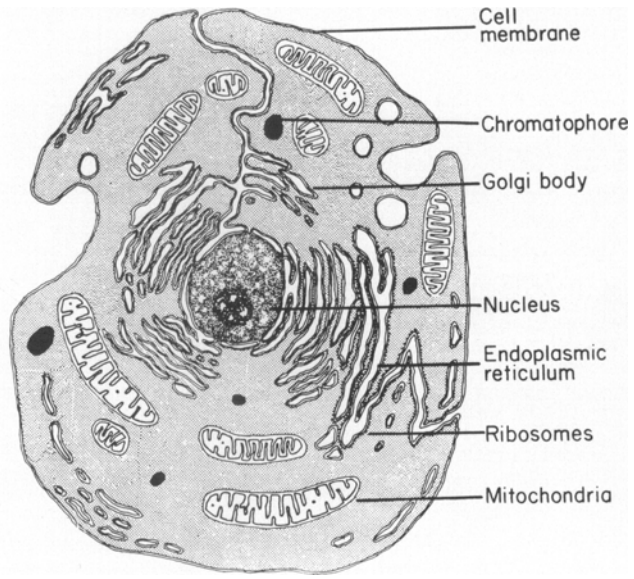


FIG. 1. Schematic animal cell.

The concept of a cell as the unit of life and the idea that the cell could not exist without a cell membrane go back to 1665 and the early work in microscopy of Hooke (7). However it was not until the development in the 1830's of the compound microscope and its application to biological tissues that it was possible to begin to resolve cell structures. A century of developments in optics was necessary for techniques of polarization, phase, and interference microscopy, applied to biology, to reveal structural information about the cell. This was followed by developments in electron microscopy in the 1940's and with newer methods of tissue preparation in the 1950's to begin to visualize the structure of the cell organelles in molecular dimensions. It is expected that newer analytical tools will be developed to study the chemistry of the cell in its dynamic living state so that the chemistry and molecular structure can be understood in terms of function. One advance in this direction is the development of the microspectrophotometer; with this instrument it is possible to study by spectral means the dynamic chemistry of the organelles within a living cell (18,20,21).

To explore the structure of the cell and its organelles, the protozoan algal flagellate *Euglena* has been chosen as a model. *Euglena gracilis* is a large cell, extending to a length of 70μ or more and to a diameter of 30μ (Fig. 2). When *Euglena* reaches a certain growth size, it simply divides into two cells. It possesses a complete complement of subcellular organelles: a nucleus, numerous mitochondria, endoplasmic reticulum, chromatophores, chloroplasts, lipid inclusions, vacuoles, golgi structures, eyespot, and a flagellum.

Euglena grown in light is green, utilizes light energy

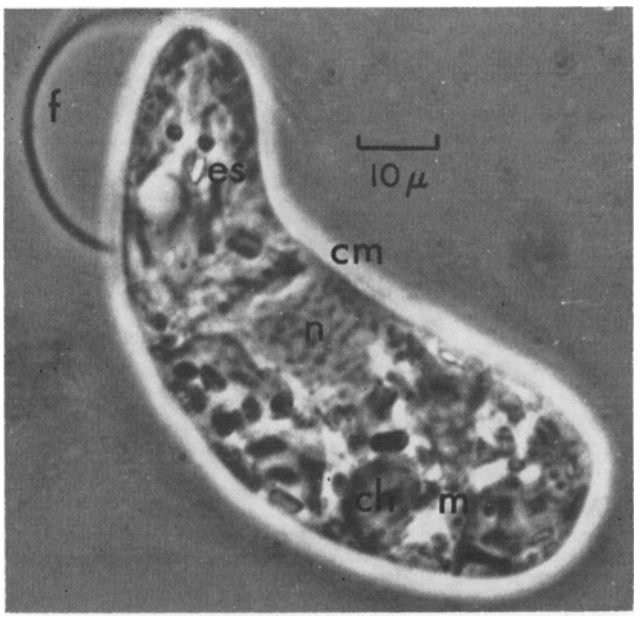


FIG. 2. *Euglena gracilis Z:* ch, chloroplasts; cm, cell membrane; es, eyespot; f, flagellum; m, mitochondria; n, nucleus.

via chlorophyll in its chloroplasts, and photosynthesizes like a plant (Fig. 3a); when grown in the dark, it loses its ability to synthesize chlorophyll to build chloroplasts and can no longer photosynthesize (Fig. 4a). In the absence of light *Euglena* must obtain its energy by chemosynthesis, which is typical of all animal cells. This is a reversible system in *Euglena,* providing that mutations do not occur. *Euglena* mutants can be produced by growth at temperatures above 33C, by pressure, and by drugs; these mutants are like the dark-grown cells in their chemistry; they can no longer synthesize chlorophyll, build

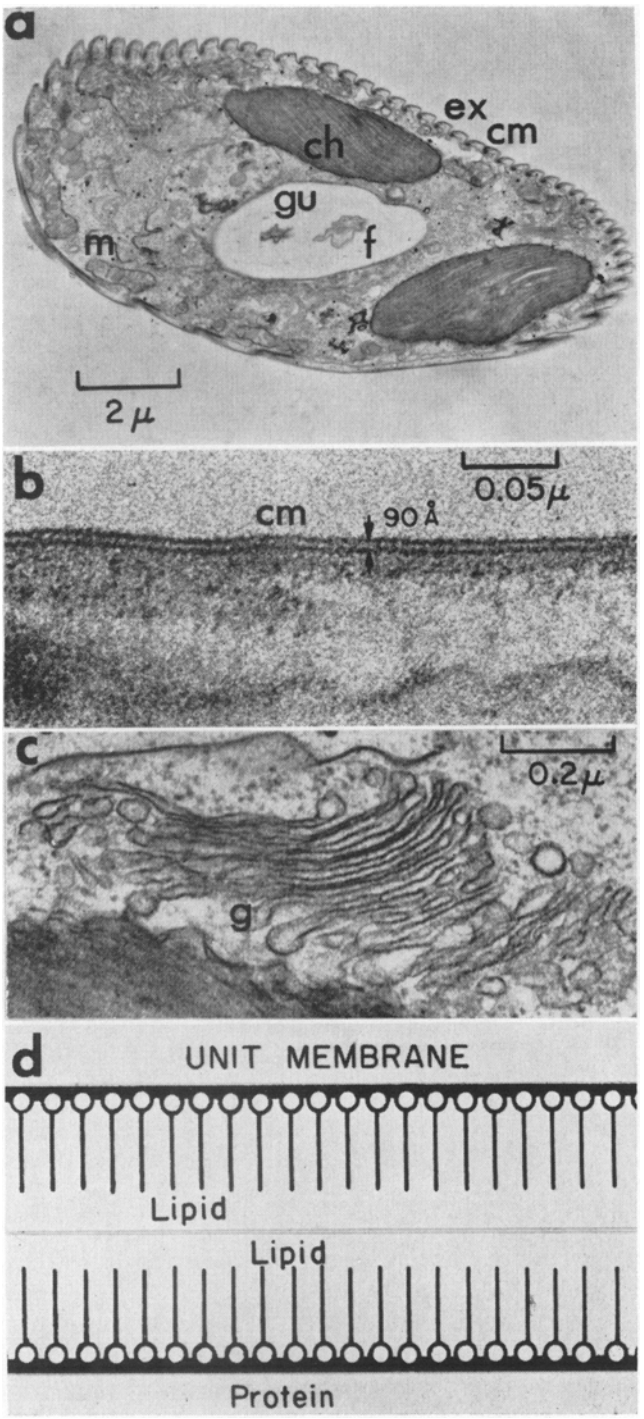


FIG. 3a. Section of *Euglena:* ch, chloroplasts; cm, cell membrane; ex, exoskeleton; f, flagellum; gu, gullet; m, mitochondria. b. *Euglena* cell membrane, cm (double membrane, 90Å). c. Golgi apparatus. d. Schematic unit membrane, lipid-protein.

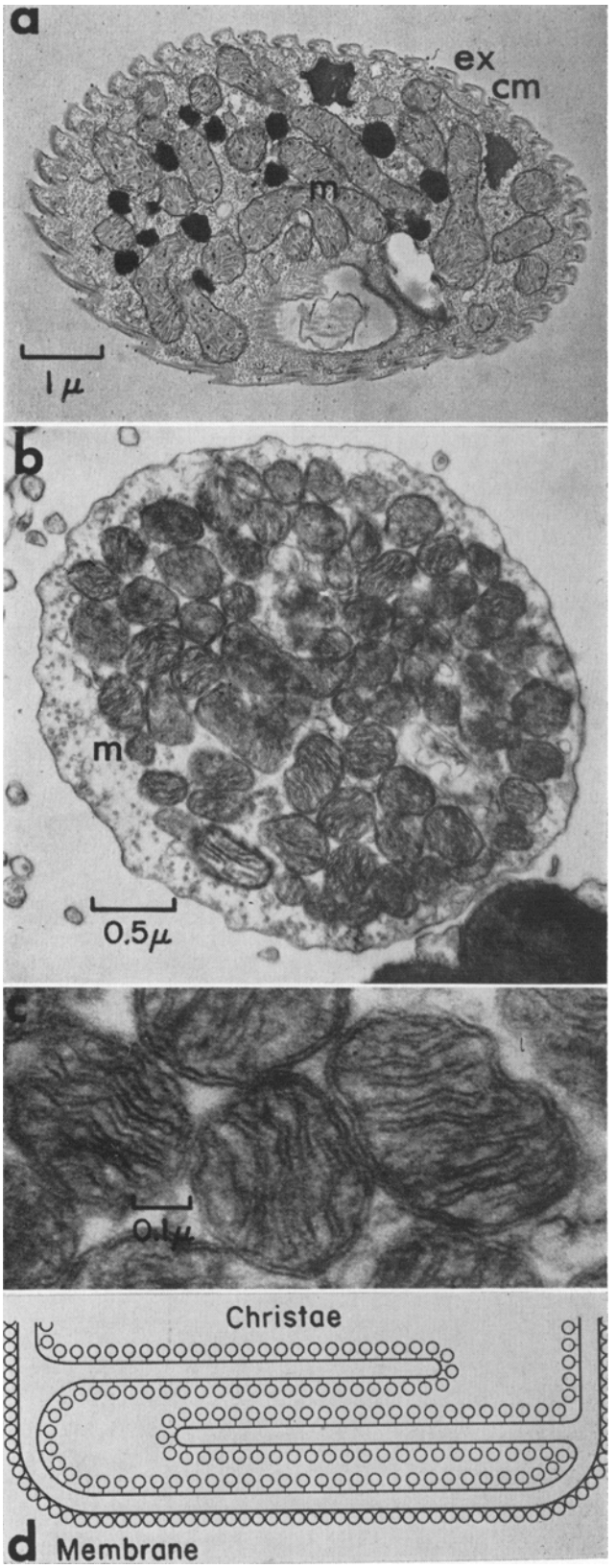


FIG. 4a. *Euglena,* dark-grown section: cm, cell membrane; ex, exoskeleton (Note numerous mitochondria, m; compare with light-grown, Figure 3a). b. Section of inner segment of frog retinal rod. Compare this with dark-grown *Euglena* cross-section, Figure 4a. c. Enlargement of mitochondria (from Figure 4b) to show inner and outer membranes. d. Schematic mitochondrion, showing inner and outer membranes. Note its structural relationship to Figure 3d.)

chloroplasts, or photosynthesize. The uniqueness of *Euglena,* then, is that it has the properties of both a plant and an animal and its behavior and chemistry

TABLE I
Analysis of *Euglena* Cells in Various Environments[a]

Product	Light-grown	Dark-grown	Temperature (HB) mutant light-grown
	%	%	%
Ash	10.0	8.0	7.5
Fat	13.7	9.1	7.8
α-linolenic acid[b]	7.8	0.6	
Protein	69.3	36.3	48.1
Carbohydrates	7.0	46.6	36.6
Water	77.1	71.4	77.7

[a] Taken from Wolken (21), page 102.
[b] From Erwin and Bloch (5).

depend upon its environment (Table 1). For example, *Euglena gracilis* synthesizes linoleic and linolenic acid as well as longer-chain polyunsaturated acids. Korn (9) determined the structure of 51 fatty acids of *Euglena*. This diverse pattern contains all the fatty acids typical of both higher plants and higher animals. These light ⟷ dark adaptations bring about plant ⟷ animal changes in the chemistry and structure of *Euglena* cells (21).

Organization of the Cell

The cell membrane is described as a double membrane of lipids (mostly phospholipids) and proteins. Its molecular structure, as schematized by Danielli and Davson (4), is depicted in Fig. 3d and is referred to as the unit membrane. Recent studies by x-ray diffraction and electron microscopy indicate a unit of the order of 90 Å (6,13,15). This is observed in the electron micrograph of the cell membrane of *Euglena* (Fig. 3b). The molecular structure and dynamics of the cell membrane, as currently viewed, are summarized by active researchers on membrane structure in "Biological Membranes: Recent Progress" (11). How the cell-membrane molecular structure functions in allowing for the differential diffusion of ions and the exchange of gases still remains an unresolved and important problem for research.

Enclosed within the cell membrane is the cell fluid, the cytoplasm. The cytoplasm contains organelles, systems of complicated membranous structure. One such organization of membranes is the endoplasmic reticulum (Fig. 1), to which the ribosomes for protein synthesis are attached. Closely associated with the endoplasmic reticulum is another cluster of membranes (lamellae) with numerous vesicles, the golgi apparatus (Fig. 3c).

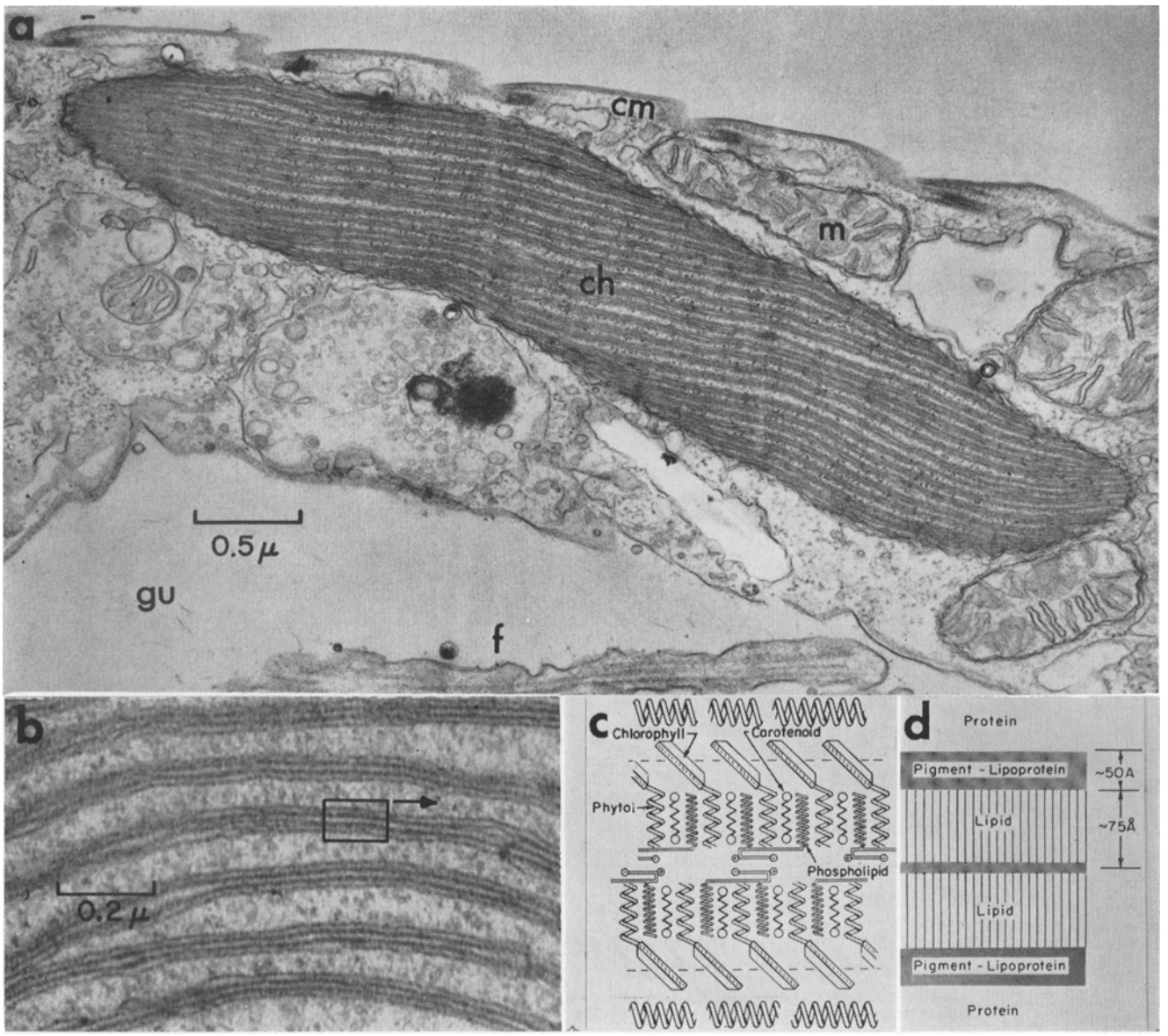


FIG. 5a. *Euglena* chloroplast. b. Enlarged area in the chloroplast. c. Conceptual model of molecular packing in the lamellae (from M. Calvin, Brookhaven Symposium No. 11, p. 160, 1959). d. Thickness of layers. (Note relationship to Figures 3d and 4d.)

Of the cytoplasmic organelles, the most numerous are the mitochondria (Fig. 4). Mitochondria in *Euglena* are not particularly different structurally from the mitochondria found in all plant and animal cells (Fig. 4a and Fig. 4b). Lipids, mainly phospholipids, make up 30% to 40% of the dry weight of mitochondria; of these, chemical analysis shows that phosphatidylethanolamine, phosphatidylcholine (lecithin), and cholesterol are the major phospholipids. Although mitochondria contain the enzymatic system for the biosynthesis of fatty acids, they do not possess all the enzymes required for assembly of the complex phosphatides (10).

Structural studies by electron microscopy indicate that mitochondria possess two membranes, a limiting membrane and an inner membrane (Figs. 4c and 4d). A system of ridges protrudes from the inside surface of the inner membrane. These ridges have been designated as *cristae*. It has been suggested that the oxidative enzymes of the mitochondria are built into these *cristae*.

The chloroplasts are *Euglena's* photoreceptors for photosynthesis. There are indications that a chemical and structural relationship exists between the mitochondria and the chloroplasts. In *Euglena* the chloroplasts are large structures from more than 1μ in diameter to 7μ or more in length (Fig. 3a and 5). Electron microscopy shows that the chloroplast has an internal fine structure of lamellae (Fig. 5b). These appear as double membranes, the total thickness of which is of the order of 250 Å; each membrane is of the order of 50 to 100 Å in thickness (Fig. 5). These electron-dense membranes are associated with the lipids, and the less dense interspaces with aqueous proteins, dissolved salts, and water-soluble enzymes. These assumptions are based on staining and chemical reactions of fixing agents (e.g., osmium tetroxide) within these structures. From spectroscopy an estimate of the chlorophyll concentration in a single chloroplast can be made, e.g., there are 1.3×10^9 chlorophyll molecules per *Euglena* chloroplast (21). It is believed that these chlorophyll molecules reside on the surfaces of the lamellae for efficient capture of light energy (17,19).

From chemical analysis of the pigments, chlorophyll and carotenoids, and from the study of the geometry of the chloroplast, a structural molecular model was proposed (16,19) in which the chlorophylls are arranged in a monomolecular layer with space for the necessary carotenoid molecules. This kind of molecular packing not only provides space for all of the chlorophyll and carotenoid molecules in the monolayer but would permit energetic interaction between these molecules (Fig. 5c and 8a). Such a highly ordered molecular structure for the chloroplast greatly increases its surface area to provide not only for the pigments but also reacting sites for enzymes, and close association with other necessary molecules.

Lipids make up a considerable part of the chloroplasts. Benson (2) showed that mono- and digalactosyl glycerides are the major chloroplast lipids. The galactosyl glycerides contain unusually high concentrations of polyunsaturated acids and account for the major portion of these acids in chloroplasts. The major phospholipid of photosynthetic *Euglena* is phosphatidylserine whereas dark-grown cells contain primarily phosphatidylethanolamine (8). Also, α-linolenic acid seems to be localized in the chloroplast and when *Euglena* is dark-grown, α-linolenic acid disappears as does the chloroplast structure (3). The percentage of total fats increases from 9.1% for dark-grown *Euglena* to 13.7%, for light-grown photosynthesizing *Euglena* cells (Table 1). This suggests the question: is this change in lipids related to the synthesis of chlorophyll, to the development of the chloroplast, and to photosynthesis? It is interesting to note that the heat-treated (HB) mutant, which is incapable of resuming photosynthesis when placed in the light, contains only 7.8% total fat.

Erwin and Bloch (5) investigated α-linolenate content of light- and dark-grown *Euglena gracilis*. They found that the α-linolenic acid of dark-grown cells increased from 0.6% to 7.8% when the cells were light-grown for 28 hours (less than two generations). Upon analysis, 85% of the α-linolenic acid was found in the chloroplast fraction. In *Euglena* the galactolipids and the unsaturated fatty acids disappear when the organism is grown in darkness (8,14), and they reappear when these dark-grown cells are adapted to light again. Therefore there seems to be a correlation between the photosynthetic activity and the concentration of α-linolenic acid. One of the conclusions reached was that organisms which derive energy from photosynthesis and photophosphorylation seem to require α-linolenic acid whereas γ-linolenic acid, arachidonic acid, and C_{20} polynoic acids appear to be necessary for organisms that depend on respiration and oxidative phosphorylation (5). Also, when the lipids are extracted from chloroplasts, their photochemistry is inhibited, suggesting that the lipids may be more than a structural part of the chloroplasts.

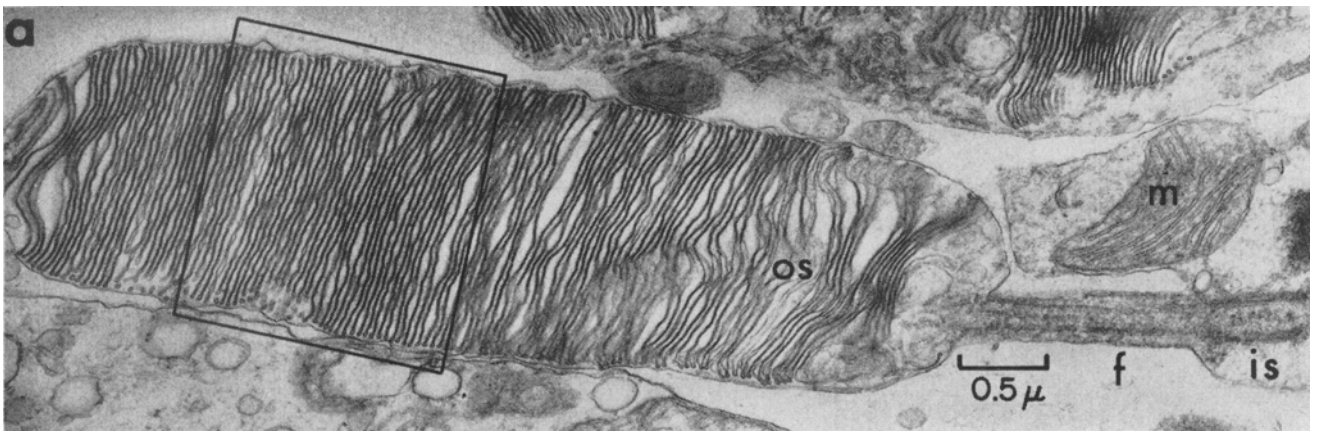
Discussion

The subcellular organelles that are visualized by using *Euglena* as a model cell all appear to have a basic molecular architecture of double-layered lamellae. This is observed for the cell membrane (Fig. 3b), the golgi structure (Fig. 3c), the mitochondria (Fig. 4), and the chloroplasts (Fig. 5). Lamellae with similar dimensions are observed for the visual photoreceptors, the retinal rods and cones (Fig. 6); for myelin structure (Fig. 7); and for other membranes (21).

Besides chlorophyll in the chloroplasts, all photoreceptors contain carotenoids: C_{40}—as in β-carotene, C_{20}—retinal (the aldehyde of Vitamin A) in the retinal rods and cones of the eye. These polyenes are considered part of the lipids of these structures. Whether these pigment molecules have other functions than light capture is not known; however it has been hypothesized that they may stabilize the lamallae of the photoreceptors (17,19). For most efficient light-capture the pigment molecules most likely would be oriented as monomolecular layers on the surfaces of the lamellae of the chloroplast and retinal rod outer segments as schematized in Fig. 8.

Arguments as to the number of lamellae membranes and their molecular dimensions exist because of the nature of cell fixation necessary for electron microscopy. Therefore the question of which fixative, osmium tetroxide (OsO_4) or permanganate ($KMnO_4$), gives the most reliable results still remains. The subtle chemical differences between these and other cell fixatives are yet to be resolved. All of these subcellular organelles contain high concentrations of lipids, and it is believed that osmium stains the polar surfaces of oriented lipid molecules.

The structure of the cell organelles, as observed with electron microscopy, may be associated with a unique property of lipids, that is, their capacity to form



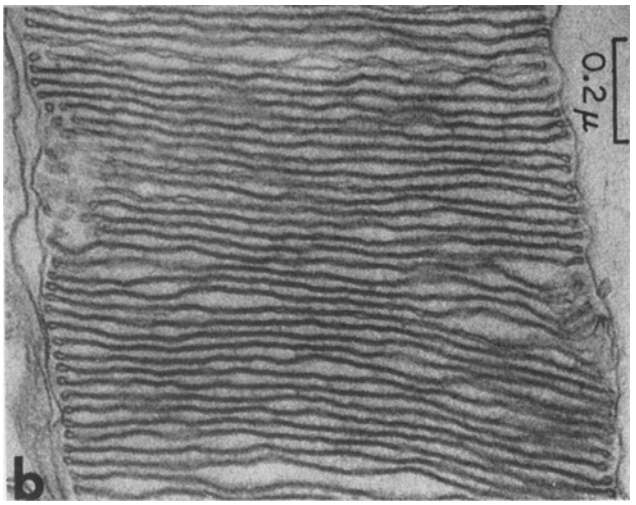


FIG. 6a. Retinal rod from eye of cattle: is, inner segment; os, outer segment. Note cilium, f (flagellum), that connects outer and inner segments. b. Enlarged area of outer segment to show structure of lamellae.

fairly uniform thin layers. This is attributable to the presence of hydrophilic, water-soluble groups at one end of the molecule and to hydrophobic, fat-soluble groups at the other end. This allows the lipids to form molecular sheets, which can serve to separate one part of a reaction from another. These folded sheets or lamellae greatly maximize the surface area and minimize the organelle volume. The ordered structure of the organelle membranes exhibits properties of liquid crystals for the organelles are temperature- and concentration-dependent (1).

It has been suggested that temperature variation alone, within the physiological range, can alter the physical state of fatty acids and phospholipids. Luzzati and Husson (12) and Stoeckenius (15) have studied the phase changes which occur in brain phospholipids in vitro. They found that lipids may exist in both an expanded lamellar form and a condensed hexagonal form and that the transition from the lamellar to hexagonal forms occurs near 37C. Characteristic x-ray diffraction patterns indicated a condensation owing to the breakdown of ordered structure as rising temperature increased kinetic activity and hence disorder. It is of interest then that the chloroplasts of *Euglena* are permanently removed at a temperature above 33C within a generation. The result is a mutation, heat-bleached (HB) *Euglenas* (Fig. 4 and Table I). It does not seem unreasonable that the temperature sensitivity of the synthesis of polyunsaturated acids interferes with the development of the chloroplast structure.

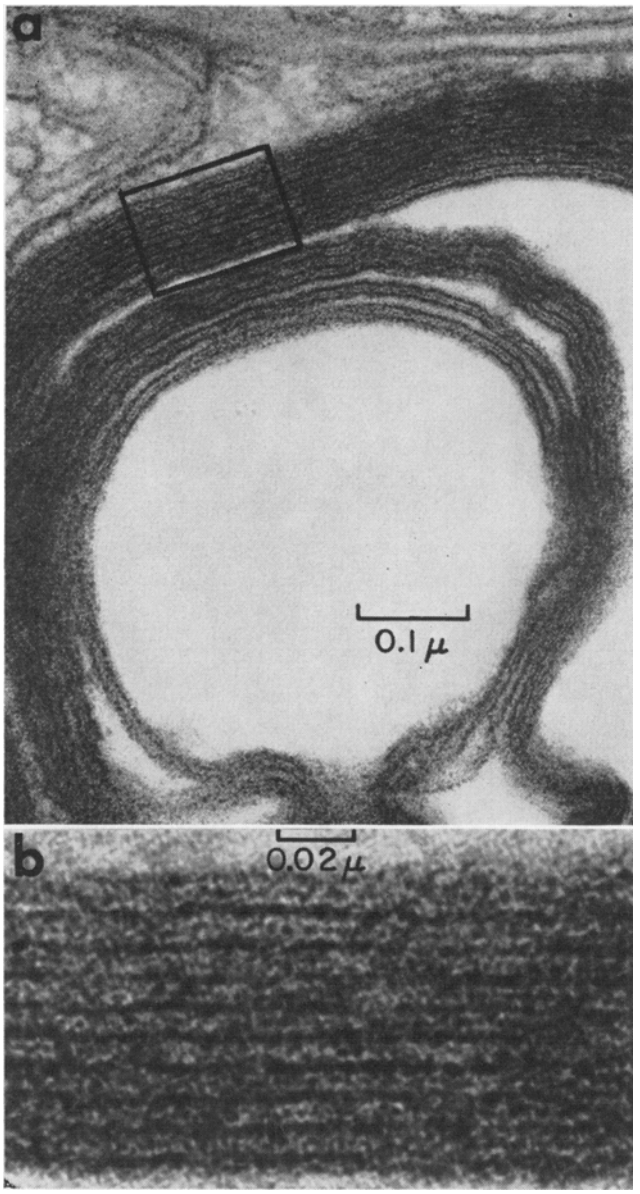


FIG. 7a. Myelin structure. b. Enlargement of small area to show spacings. (Note similarity to the cell membrane, cm, Figure 3b; to golgi structure, Figure 3c; and to the mitochondria, m, Figure 4c.)

ACKNOWLEDGMENT

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REFERENCES

1. Bangham, A. D., Physical Structure and Behaviour of Lipids and Lipid Enzymes in "Advances in Lipid Research," edited by R. Poaletti

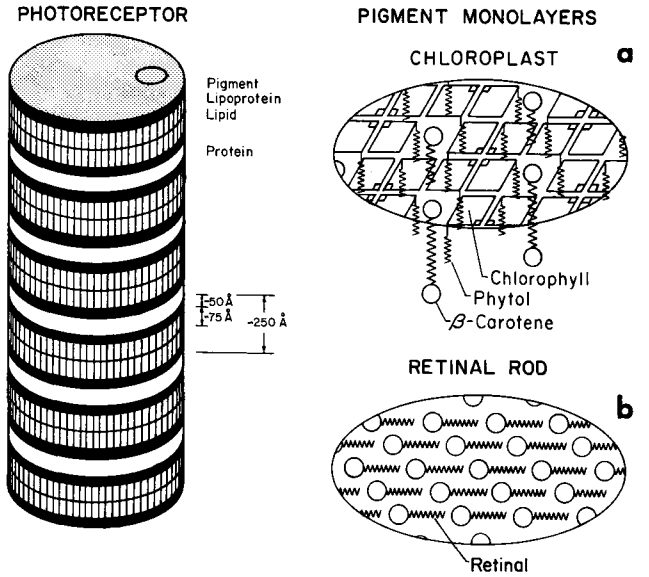

FIG. 8. Schematic model to show packing of the pigment molecules as a monolayer in or on the lamellae of the photoreceptors, for a) the chloroplast and b) the retinal rod outer segment.

and D. Kritchevsky, Academic Press, New York, New York, 1963.
2. Benson, A. A., Ann. Rev. Plant Physiol. *15*, 1 (1964).
3. Bloch, Lipid Patterns in the Evolution of Organisms in "Evolving Genes and Proteins," edited by V. Bryson and H. Vogel, Academic Press, New York, New York, 1965.
4. Danielli, J. F., and H. Davson, J. Cell. Comp. Physiol. *5*, 495 (1935).
5. Erwin, J., and K. Bloch, Biochem. Z. *338*, 496 (1963).
6. Finian, J. B., "Chemical Ultrastructure in Living Tissues," Charles C Thomas, publisher, Springfield, Ill., 1961.
7. Hooke, R., in R. T. Gunther's "Early Science in Oxford," Oxford University Press, Oxford, England, 1938.
8. Hulanicka, D., J. Erwin and K. Bloch, J. Biol. Chem. *239*, 2778 (1964).
9. Korn, E. D., J. Lipid Res. *5*, 352 (1964).
10. Lehninger, A. L., "The Mitochondrion," W. A. Benjamin Inc., New York, New York, 1964.
11. Loewenstein, W. R., ed., "Biological Membranes: Recent Progress," Ann. N. Y. Acad. Sci. *137*, 403–1048 (1966).
12. Luzzati, V., and F. Husson, J. Cell. Biol. *12*, 207 (1962).
13. Robertson, J. D., in "Biological Membranes: Recent Progress," Ann. N. Y. Acad. Sci. *137*(2), 421–440 (1966).
14. Rosenberg, A., Biochemistry *2*, 1148 (1963).
15. Stoeckenius, W., J. Cell Biol. *12*, 221 (1962).
16. Wolken, J. J., and F. A. Schwertz, J. Gen. Physiol. *37*, 111 (1953).
17. Wolken, J. J., J. Theoret. Biol. *3*, 192 (1962).
18. Wolken, J. J., and G. K. Strother, Applied Optics *2*, 899 (1963).
19. Wolken, J. J., JAOCS *43*, 271 (1966).
20. Wolken, J. J., "Vision: Biophysics and Biochemistry of the Retinal Photoreceptors," Charles C Thomas, publisher, Springfield, Ill., 1966.
21. Wolken, J. J., "Euglena: An Organism for Biochemical and Biophysical Studies," 2nd ed., Appleton-Century-Crofts, New York, New York, 1967.

Discussion

DR. NORTON: Is it just my imagination, or were the two central areas thicker than the two outer ones?

DR. WOLKEN: Yes, in each lamella of the *Euglena* chloroplast there appears to be four areas, two of which are quite dense and two of lesser density. However, identification of kinds of molecules and their precise location within these areas is most difficult to determine, for much more needs to be learned about the fixation of tissue cells and application of new microanalytical methods for identifying the chemical composition of these cell organelles.

DR. TIEN: With regard to Dr. Wolken's comment concerning the possibility of constructing a membrane system with visual pigments, I should like to describe very briefly what we have done earlier. At one time we (with Rudin and Mueller) extracted the rhodopsin from the retinal tissues of various species (frogs, cows, and bees). Since we were not sure whether the bleached rhodopsin would work, we also had the frog's and bee's eyes dark-adapted over a period of time before extracting rhodopsin. We thought if we could form a bilayer membrane containing visual pigments such as rhodopsin, and if we were to stimulate the membrane with a light of high intensity, it might show some photosensitivity phenomena such as photovoltaic effects. Since all these experiments were done in the dark, we did not observe anything that was unusual. Perhaps the experimental set-up was inadequate for investigating these phenomena. We hope to make a new attempt shortly.

DR. WOLKEN: In my discussion, I did not go into the problem of visual excitation, it has a history of its own. But since the question was raised I would like to indicate that all visual pigments "rhodopsins" contain *retinal* (the aldehyde of Vitamin A complexed to a protein or lipoprotein, *opsin*). Retinal exists in a specific geometric configuration *11-cis* (or neo b), which is stable in the dark. Light then uncouples the *11-cis* to the *all-trans* retinal from the complex. The photochemistry of the visual pigments has been beautifully worked out by Wald, Hubbard, Dartnall and their associates.

There is some experimental work to show that rhodopsins exhibit photoconductivity and that the outer segments of the rod exhibit properties of a solid-state system. However, it is far from conclusive.

DR. THOMPSON: Did you place microelectrodes?

DR. WOLKEN: No, we have not been able to insert a microelectrode in the chloroplast of a plant cell or in the *Euglena*. However, there have been some interesting experimental results in getting the early receptor potential by probing with a microelectrode in the visual photoreceptors, the retinal rods. It is too soon to know, but there is hope of relating the potential change with light excitation and the chemistry, by combining studies of the electrophysiology using microelectrodes and following simultaneously the photochemistry by observing the spectral shifts on light excitation using microspectrophotometry.

Vitamin A dissolved in an organic solvent has an absorption spectrum with a maximum about 328 mμ. Vitamin A aldehyde (retinal) has an absorption about 375 mμ when it is complexed with a protein, as in rhodopsin, the shift in absorption maximum is to around 500 mμ. Chlorophyll when adsorbed or complexed with a protein, as in *chloroplastin* also shifts in absorption maximum further in the red part of the spectrum.

DR. LLOYD M. SMITH (University of California, Davis, Calif.): I would be interested to know from your studies if these oil droplets or chromatophores are similar to the chylomicra of plasma?

DR. WOLKEN: Yes.

DR. SMITH: Are these globules naked or are they surrounded with either a bimolecular membrane or a monomolecular membrane?

DR. WOLKEN: Well, that is a tough question. We do know that the pigment is spread on these oil globules and they are various colors. In fact, in more primitive creatures who are not as highly developed as us, they are very near their photosensitive areas and, in birds, lizards, and snakes these oil globules act as screen pigment granules. Believe it or not, one of the first patents for color photography used this principle and was developed by two Frenchmen, Lumieré and Lumieré.