RESEARCH IN PROTOZOOLOGY

In Four Volumes

EDITED BY

TZE-TUAN CHEN

VOLUME 1

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PREFACE

IT HAS been a quarter of a century since the publication of *Protozoa in Biological Research* by Calkins & Summers. Some indication of the extent of the progress made during that period is given by a comparison of the chapter on cytoplasmic inclusions in the 1941 review with the chapter on cytoplasmic organelles and inclusions in the first volume of this review; this extent indicates a need for a contemporaneous review.

We hope that this four-volume work will serve as a source of reference for students, protozoologists, and biologists in general. It covers the entire field of protozoology, including morphology, physiology, genetics, reproduction, movement, respiration, morphogenesis, nutrition and growth, immunology, ecology, effects of radiation, parasitology, taxonomy, and others.

Knowledge of protozoa is more important than is generally realized. Developments in this field are important because they contribute to developments in medicine, public health, physiology, experimental biology, biochemistry, etc.

The authors tried to avoid overlapping; the little that does occur appears valuable for its presentation of diverse points of view.

The editor wishes to express his deep gratitude for the unfailing cooperation of the many eminent protozoologists who have contributed to this review. He is especially grateful to Dr. D. H. Wenrich for his valuable suggestions, and to Dr. Ruth S. Lynch for her great help in editing the manuscripts. He is also indebted to the Board of Consultants, consisting of Drs. William Balamuth, A. C. Giese, R. F. Kimball, Norman D. Levine, William Trager, and D. H. Wenrich, who have helped the editor in making certain decisions on matters of importance.

Los Angeles, California T. T. CHEN

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CYTOPLASMIC ORGANELLES AND INCLUSIONS OF PROTOZOA

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I. INTRODUCTION†

This chapter is concerned with the heterogeneous complex of organelle systems and inclusion bodies which are suspended in the cytoplasmic matrix. Their study commenced in Delft, Holland when Anton van Leeuwenhoek (1632–1723) used his simple light microscope to discover unicellular organisms[207] which von Siebold later named Protozoa.[317] Refinement of the microscope and development of cytological techniques was closely followed by knowledge of the detailed structure of all cells. The abundance and variety of the cytoplasmic inclusions in the Protozoa indicated their unique value for the study of cytoplasmic organization. The structure of organelles has been stressed; comparatively little is known of the related physiology. An attempt, however, will be made, wherever possible, to correlate the two. The guiding principle of dynamic morphology is to understand that "What we are dealing with from the molecule all the way up—is not what is present but what structure does it have that gives it opportunity to operate, to act."[345]

II. LAMELLAR SYSTEMS

One of the most impressive features of the organelles of both unicellular and multicellular organisms is the frequent occurrence of membranous elements. Presumably the function of these membranes is to increase the active surface area.[108, 283]

A. Mitochondria

When the common presence of mitochondria in metazoan cells was established, a search commenced for these organelles in unicellular forms. In 1910 Fauré-Frémiet[92] published his monograph on mitochondria in Protozoa. He believed mitochondria to be universal, self-perpetuating entities of cytoplasm. He noted that they were usually randomly distributed but at times showed a tendency to aggregate next to the plasma membrane. ([93], see also [172]) Their variety in both size and shape was revealed by light microscopy.[2]

In sectioned material Palade[247] found a limiting membrane and an internal sometimes granular matrix, and a unique internal structure: ". . . a system

† Some of the work reported here was supported by Research Grant GM 08776 from the U.S. Public Health Service.

of internal ridges (*cristae mitochondriales*) that protude from the inside surface of the membrane toward the interior of the organelle; these ridges are disposed in series, within which they appear to be parallel to one another and more or less regularly spaced."[246] Following Palade's description, Sjöstrand (cf.[321]) proposed that their internal structure is comprised of double membranes not necessarily continuous with the limiting membrane of the organelle. Wolken and Palade [363, 364] were among the first to deal with the fine structure of protozoan mitochondria. Later investigations have identified mitochondria in almost all major groups of Protista.[34, 56, 84, 97, 148, 180, 240, 269, 313, 342, 355] Elements comparable to the "cristae mitochondriales" or "double membranes" have often been described as finger-like projections (microvilli) protruding into the interior of the mitochondrion from the inner limiting membrane. In *Paramecium multimicronucleatum* and *Tetrahymena pyriformis*, the microvilli, which are sometimes branched, pursue a sinuous course (Figs. 1 and 2, M) within the organelle and terminate in the mitrochondrial matrix without making a second contact with the inner membrane.[85, 312] In other organisms, the microvilli may open directly in the cytoplasmic matrix. (cf. [292]) In the ciliate, *Opalina ranarum*,† the mitochondria have a more or less tubular internal structure and correspond to what have been called Zeller bodies.[238, 239] In certain forms the internal structure resembles that found in metazoan cells,[8, 9] (compare Fig. 9, M, with Fig. 3, M′). The fairly numerous mitochondria of *Chilomonas* show considerable variation in shape; some are highly branched.[9] In *Spirostomum* along with more typical forms are some atypical ones, their interiors consisting of stacks of parallel membranes.[276] In the heliozoan *Actinosphaerium nucleofilum*[13] and in *Toxoplasma*,[116, 342] mitochondria sometimes display a tubular internal structure although typically the interiors are filled with vesicular units. These vesicular mitochondria bear some structural resemblance to multivesicular bodies.

In certain Protozoa the microvilli of the mitochondria are more complex. In *Pelomyxa carolinensis* they form a zigzag pattern[256] and show bulbous enlargements at the inflection points on the undulation and in addition a densely packed fibrillar material in the stroma.

In many protozoan groups differences in organelle fine structure occur in relation to physiological diversity. Of special significance is the complete absence of typical mitochondria in *Plasmodium berghei*.[295] Instead, a concentric double membrane, derived from an invagination of the plasma membrane, is located near the periphery of the organism (Fig. 3, CM). This system shares certain morphological features with mitochondria of the spermatocytes of *Helix promatia*[20] and Rudzinska and Trager[295] suggest that it may represent mitochondria. In some bacteria and certain members of

† Certain investigators classify the opalinids as flagellates. The reader is referred to Pitelka[262] for literature on this subject.

the genus *Bacillus*,[99, 109, 222, 344] a double membrane system exists which is also derived from the plasma membrane by invagination. Significantly, the entire cytochrome and succinic dehydrogenase systems of certain bacteria reside in the plasma membrane.[222, 314] These findings suggest that the plasma membrane in these forms, presumably enzymatically altered, is performing the function of mitochondria. Although the "dorsal cisternae" of *Giardi muris* at times appear to contain a homogeneous material (Fig. 4, DC), might it not be that they may harbor certain enzyme systems unique to this organism or comparable to those of others?

In certain parasitic flagellates,[6, 7, 12, 14, 147, 149] and ciliates,[234] mitochondria are either not well developed or entirely absent. Since these organisms are generally obligate anerobes, their energy is derived through fermentation.[175] In *Plasmodium cathermerium* mitochondria are present in oocysts and sporozoites[77] but are absent in merozoites.[225] While mitochondria are virtually absent in the trophic form of the gregarine *Gregarina rigida*,[23] mitochondria with tubular interiors are found at the periphery of the cephalont of the epimerite embedded in the host cell (Beams and Anderson, unpublished). There is pressing need for a systematic study of mitochondria in this group of organisms. Such a study should be rewarding especially in view of the complicated life cycle and the possibility of organelle differentiation and "dedifferentiation".

The literature on changes in mitochondrial morphology induced by certain physiological and pathological conditions has been reviewed by Rouiller;[292] a few examples will be cited here. It is well known that encystment occurs regularly in some unicellular forms either during reproduction or under adverse environmental conditions.[173] So far, Vickerman's work[337] is the only study to emphasize the change in mitochondrial structure in Sarcodina during encystment. In the active form of limax amoeba, *Acanthameba*, he found filamentous mitochondria showing tubular cristae enmeshed in a honeycomb-matrix and usually also a small, dense intracristal spherical body. At encystment the intracristal body decreases in size and becomes vesicular, the organelle assuming a spherical shape and decreasing in overall diameter. This altered structure is thought to represent a state of minimal metabolic activity. Beers[26] found, in *Didinum nasutum*, that mitochondria disappear in the later stages of encystment. To my knowledge no modern cytological techniques have been applied to an investigation of this group during encystment. Bak and Elliott[17] studied mitochondrial changes during the growth cycle of *Tetrahymena pyriformis* and during logarithmic growth found elongated mitochondria usually in close association with the inner plasma membrane. During the stationary growth phase many of the mitochondria moved deep into the endoplasm and became oval. Ten days later, dense intramitochondrial bodies appeared. As these bodies increased in size and filled the entire mitochondrial matrix the cristae were completely lost. The reaction of the dense intramitochondrial bodies to Sudan black B and

oil red O led Bak and Elliott[17] to suggest that they were composed of lipid. In non-dividing *Tetrahymena* (strain W) Roth and Minick[289] found the tubules composing the mitochondria to be closely packed; in individuals in the early stages of macronuclear division, the tubules are loosely packed and usually contain a "central dense mass".

The origin of mitochondria is not yet settled; whether they are formed *de novo* or from pre-existing mitochondria is moot. Gey *et al.*[119] have proposed that they are derived from vesicles brought into the cytoplasm by pinocytosis. As previously noted, Rudzinska and Trager[295] suggested that in *Plasmodium berghei* the mitochondria may be derived from the plasma membrane. Such ideas are certainly worthy of further investigation, since there is some evidence that mitochondria may be derived from the plasma membrane in metazoan cells.[283] Wohlfarth-Botterman (354, see also 356) has suggested that mitochondria arise mainly from free vesicular units in the cytoplasm which enlarge and develop a tubular interior. Some years ago, Joyet-Lavergne[184] and Calkins[42] thought mitochondria were derived from the nucleus. (see also[162]) Ehret and Powers[81] suggested their origin from the macronucleus in *Paramecium* and Brandt and Pappas[34] presented evidence suggesting derivation from the nuclear envelope in *Amoeba*. Increase of mitochondria by division is an old concept; some investigators have presented pictorial evidence highly suggestive of such reproductive mechanisms.[171, 216, 354] But a convincing demonstration of the origin of mitochondria is still wanting.

Working with the yeast *Torulopisi utilis*, Linnane and his associates studied its cytology and enzymology under both aerobiasis and anaerobiasis.[210]

Under aerobiasis the mitochondria had the characteristic cristae; isolated mitochondria showed electron transport, oxidative phosphorylation, and participation in the Krebs cycle. Under anaerobiasis no mitochondria were found. But in the cytoplasm numerous membranes displayed two geometrical patterns: one a multimembrane system similar to that demonstrated by Rudzinska and Trager[295] for *P. berghei*, and the other showing characteristic features of an endoplasmic reticulum. Their particulate fraction showed both succinic and $NADH_2$ dehydrogenase activity, both known to be present in aerobic cells.[142] Particles derived from fractions of anaerobically grown cells, unlike those obtained from aerobically grown cells, contain no detectable cytochromes. However, when cultures of anaerobically grown *T. utilis* were aerated, the reticular membranes rearranged themselves in parallel arrays which subsequently "... fuse and infold to form primitive mitochondria containing few cristae." The authors further noted that during this rearranging process, cytochromes were synthesized.

A new morphological definition of mitochondria emerged with electron microscopy. The one so aptly constructed by Rouiller[292] is useful. He states, "A mitochondrion is morphologically defined as a cell organelle bounded by a double membrane within which are membranous structures either

villous or vesicular, a ground substance or granular matrix and occasionally, very dense granulations." Mitochondria are also being defined biochemically. Many synonyms attached to this organelle in the past have been retired.

The establishment of the reality of mitochondria was followed by a lengthy controversy concerning their function. Most of their physiology and microchemistry derives from experiments on metazoan cells.[29, 163, 208] Their respiratory function has been known for many years;[192] today we casually speak of them as "the powerhouse of cells".[319] Only meager information has been derived from experiments on unicellular organisms. Protozoan mitochondria, like those of metazoan cells, can be stained with Janus green B, and give a positive reaction with tetrazolium.[98] Holter[165] demonstrated that certain large granules of centrifuged amoebae contain enzymes such as succinic dehydrogenase, acid phosphatase and protease. He suggested that this granular component was chemically similar to mitochondria of metazoan cells. Klein and Neff[195] isolated large volumes of mitochondria from *Acanthomoeba* by differential centrifuging and concluded that these organelles have many properties similar to those found in mammalian tissue.

Mitochondria may release their contents into the general cytoplasm through gaps in their limiting membrane.[354] Furthermore, Powers, *et al.*[270] and Rouiller[292] suggest that the mitochondrial tubules of *Paramecium* and *Stentor* appear to be directly connected with the cytoplasm. Chandra[53] has put forth the thesis (with evidence gathered from mammalian tissue) that the inner membrane of these organelles is not a distinct entity, but an integral part of what is essentially a single unit. The structure proposed by Chandra[53] would make the intramitochondrial substance accessible to the cytoplasm through intermembranous channels. Chandra suggests that such a structure facilitates the swelling of this organelle either by an "... unfolding of the cristae or sliding of the two membranes or by both these processes occurring simultaneously."

Precisely what the relationship is between the cristae, tubules, and microvilli in mitochondria is not known. Perhaps this inner framework is favorable for *in situ* functioning of the large number of enzymes involved in the many complex biochemical reactions of mitochondria.[319] Green and his associates[142, 143] have developed techniques expressly designed for reconstructing the finer details of mitochondrial structure and function and have obtained a small mitochondrial fragment to which they have applied the term electron transport particle. This unit of variable size can carry out the reactions of the citric acid cycle as long as it retains a well-defined double membrane; but when the outer membranous element is removed this capacity is lost. Under certain conditions an electron transport particle can be obtained which oxidizes both succinate and $NADH_2$. Accordingly, the authors suggest that a mitochondrion may be thought of as a polymer of repeating units of electron transport particles. Experimental designs such as those constructed by Green and his group[143] should be applicable to those

unicellular forms in which mitochondria are one of the most noticeable components of the cytoplasm.

As one reads the literature concerning mitochondria one must agree with the statement made by E. V. Cowdry[62] at a symposium held in 1953 on the *Structure and Function of Mitochondria*: "... it is really surprising how much older workers learned about mitochondria: (a) that they were basic components of cells as characteristic of the cytoplasm as chromatin is of the nucleus; (b) that they provide a very extensive surface area of interaction with the cytoplasm; (c) that they are of lipoprotein composition and (d) that they are centers of constructive metabolism, veritable little manufacturing plants of numerous products essential to life."

B. Kinetoplast

The kinetoplast, typically described as a rod-shaped body, is found in the cytoplasm of the Trypanosomidae and the Bodonidae.[19, 51, 69, 178, 196, 197, 198, 201, 206, 211, 214, 316, 348] It lies just anterior to the kinetosome of the flagellum and perpendicular to the long axis of the organism (Fig. 5, KP). Contrary to earlier opinion, it has no direct anatomical connection with the kinetosome of the flagellum (Fig. 5, KS). In preparations stained with Giemsa or iron hematoxylin it appears as a dense homogeneous and spherical or ovoid body (see insert, Fig. 5, KP). In 1924 Bresslau and Scremin[38] demonstrated that the kinetoplast was Feulgen positive, an observation many times confirmed. Under close examination, Feulgen preparations show the kinetoplast to consist of two components: (a) a Feulgen-positive central area and (b) a Feulgen-negative peripheral portion.[60] Cosgrove and Anderson[60] found that the stainability of the kinetoplast decreased but was not abolished after prolonged treatment of the organisms with deoxyribonuclease.

The mitochondrial nature of the kinetoplast has been repeatedly pointed out. In 1925 Causey[49] called attention to similarity in staining properties of the kinetoplast and mitochondria in *Leishmania braziliensis* and concluded that the kinetoplast was a mitochondrion. The observations made by him and others using light optics have been confirmed with electron optics. Both kinetoplast and mitochondrion present a submicroscopic structure of transversely oriented double membranes or cristae (Fig. 5, C).[54, 57, 170, 226, 261, 273, 274, 275, 280, 281, 326] In *Trypanosoma mega*[326] the double membranes show considerable variation, some being concentrically arranged as in *Plasmodium berghei* demonstrated by Rudzinska and Trager.[295] In addition to the finely granular kinetoplast matrix there are transversely oriented dense fibrils 25 Å in diameter, similar to macromolecular units of DNA in the nucleoplasm of bacteria (Fig. 5, DF). In *Trypanosoma lewisi* the fibrillar material appears as a bundle but in *Bodo* it is rather dispersed.[261,280]

Steinert *et al.*[323] and Steinert and Steinert[328] have used tritiated thymidine and autoradiographic methods to investigate the cyclic DNA synthesis in *T. mega*. These authors suggest a possible genetic function of the

kinetoplast. (see also [324, 325]) They further suggest[326] that the close synchrony of DNA synthesis in the nucleus and kinetoplast provides evidence which supports the old binucleate theory of Hartmann.[155, 331] This suggestion has been challenged by Ris[281] who states: "Since the flagellate nucleus contains typical chromosomes with 100 Å thick fibrils and the kinetoplast bacteria-type nucleoplasm with 25 Å fibrils, it (the kinetoplast) cannot be a secondary nucleus."

Rudzinska, D'Alesandro and Trager[297, 298] have recorded some interesting changes in the kinetoplast of *Leishmania donovani*. D'Alesandro[64] found that *L. donovani*, freshly removed from hamster spleen, differed antigenically from leptomonads. This observation led the group to wonder if there were any detectable fine structural differences among the various stages. They made observations on the intracellular forms of *L. donovani*, freed from host cells, and leptomonads (Leishmania forms become leptomonads when incubated at 28°C. for up to 21 hours). They found that in Leishmanias the kinetoplast is a regularly shaped elongate organelle with one to several cristae and a dense fibrillar band within the matrix. After incubation for five and a half hours at 28°C. the kinetoplast was large, irregular in shape and the fibrillar band less dense. Frequently, the kinetoplast was extended into the typical mitochondrial structure observed by other investigators.

The kinetoplast may be altered in its fine structure or entirely lost from the organism. Occasionally this follows the use of certain drugs.[229, 260, 333, 336] Hoare[160] has presented evidence of the spontaneous occurrence of strains of *Trypanosoma evansi* devoid of the kinetoplast; such strains may be maintained for a considerable length of time. He envisions the perpetuation of akinetoplastic forms to be due to the failure of the kinetoplast to divide and regards the phenomenon as a mutation of plasmogenes. Once the organism loses its kinetoplast it does not arise *de novo*. Werbitzki[348] produced an akinetoplastic strain of a trypanosome with acridine dyes and suggested that this structure is apparently not essential to the life of the organism. Muehlpfordt[229] noted that akinetoplastic forms of trypanosomes can be obtained by treating the organisms with the drug trypaflavin. However, the kinetoplast reappears when the treatment is stopped. In *Schizotrypanum cruzi*, the kinetoplast is not removed by trypaflavin.[229, see also 336]

The function and origin of the kinetoplast is unknown. Ris[281] stated that, "Whether it is a mitochondrion-like endosymbiote in addition to the regular mitochondria of the flagellate or the 'mother mitochondrion' of the cell which buds off smaller mitochondria must be determined from a study of akinetoplastic strains of Trypanosomes."

C. Chloroplast

Certain cytoplasmic organelles are unique to specific groups of Protozoa. One of these, the chloroplast, which contains chlorophyll, is a dominant cytoplasmic organelle in some unicellular organisms; among them are

certain members of the Euglenoidina.[181] Those organisms are readily cultured and are prime experimental material for chloroplast studies.[36, 37, 70, 139, 140, 141, 150, 194, 235, 271, 304, 305] Even within a given species chloroplasts vary greatly in size, shape, and number. From observations made with polarization optics investigators were able to deduce that this organelle has an orderly arranged lamellar pattern.(cf. [110]) These earlier findings were substantiated and extended by the morphological work of Wolken and Palade[363, 364] who were able to show, in light-adapted *Euglena gracilis* var. *bacillaris*, that the interior of the chloroplast contains a regular pattern of approximately twenty-one highly oriented dense lamellar layers. Each layer has a thickness of 250 Å.(see also [360]) In 1957 Sager and Palade[303] investigated the normal green strain of *Chlamydomonas reinhardii* and suggested that the basic structural units of chloroplasts are discs, each of which is constructed of a pair of membranes joined at their edges to form flat closed vesicles. Gibbs[120] has presented a rather detailed analysis of the chloroplast of *E. gracilis* var. *bacillaris* and *E. gracilis* strain Z. She reported that these chloroplasts consist of from ten to forty-five moderately dense bands (Fig. 7), each band varying in width from 25 μ to 210 μ. A band (lamella of other authors) is defined by Gibbs as consisting "... of a variable number of closely appressed discs.... In a section perpendicular to the plane of the discs, a band which is interpreted as three appressed discs appears as a thin dark lamella, a light space, a thick dark lamella, a light space, a thick dark lamella, a light space and finally another thin dark lamella." It has been suggested that chlorophyll is a component of the lamellae.[301, 365] The entire organelle complex is surrounded by a double membrane envelope which is sometimes continuous with the outer nuclear envelope.[123]

The previous anatomical studies concerned themselves with chloroplasts of flagellates grown in light. If similar organisms are grown in a dark environment, interesting changes are observable in the substructure of the organelle which presumably are concomitant with dark adaptation. Wolken and Palade[364] showed that in dark adapted Euglena, there is a disorganization of the bands along with a disappearance of chlorophyll. This effect is reversible, for when organisms are returned to light the original substructure is restored. There is good evidence to suggest that at the time the internal structure is being reorganized, chlorophyll is resynthesized.[301, 362] In the yellow strain of *Chlamydomonas* where chlorophyll is absent, there is no indication of any band organization.[302] In addition to the lamellar structures there are other components within the matrix of the organelle, namely granules and lipid bodies.[120, 302]

Non-green forms of *Euglena* can also be obtained by the treatment of the culture with certain drugs. It has been found that furadantin[221] and streptomycin[1, 272] can produce chlorosis of certain green euglenids.(see also [177]) Apparently a phenomenon similar to that which occurs when dark-adapted animals are returned to light occurs here, i.e., the animals regain

their green pigment when the drugs are withheld from the culture. One drug, the antihistamine pyribenzamine, has been found to produce a permanently colorless culture of *E. gracilis* var. *bacillaris*.[150] Permanently colorless organisms have also been obtained by ultraviolet irradiation.[305]

It has long been known that within the chloroplast of *E. gracilis* there exists another structure known as the pyrenoid which exhibits staining properties different from other portions of the organelle.[121, 178, 201] Gibbs[120] has shown that the pyrenoid is a differentiated region of the chloroplast matrix (Fig. 7, P), appearing as laminae separated from each other by fine lamellae directly continuous with the lamellae of the chloroplast. Each pyrenoid is rimmed on each side by a hemispherical shell of paramylon which appears structureless in electron micrographs (Fig. 7, PA). The pyrenoid of chloroplasts found in *Chlorogonium elongatum* consists of a dense finely granular substance.[154] Sager and Palade[303] found that the pyrenoid in *Chlamydomonas* is distinguished by "... a network of tubules embedded in a matrix of dense, finely granular material." These authors further noted that the matrix of the pyrenoid is continuous with that of the chloroplast and the tubules are connected with the discs of the surrounding chloroplast.

Epstein and Schiff[88] have studied the development of chloroplasts in *Euglena gracilis* by means of fluorescent and electron microscopy.(see also [87]) They suggest two methods of chloroplast formation: (a) by lamellae developing within the chloroplast and (b) by coalescence of proplastids. They further noted that under light conditions lamellae appear in the chloroplast at the rate of one about every six hours. Gibbs[122] presented evidence that chloroplast development in *Ochromonas danica* begins as a series of vesicles. These vesicles subsequently enlarge, arrange themselves linearly and finally fuse into discs. Some of the developmental patterns of chloroplasts in unicellular forms parallel those suggested by others for this organelle in higher plants.[161, 349]

Many studies have attempted to chemically characterize the chloroplast. It has been found that chlorophyll constitutes five to eight per cent of the organelle. Carotenoids, cytochromes *f* and b_3, lipids, proteins, and ribo- and deoxy-ribonucleic acids are also present.(cf. [362])

The replication of chloroplasts is apparently dependent upon synthesis of a specific DNA located within the organelle. Scher and Sagan[305] compared the incorporation of H^{-3} thymidine into normal green *E. gracilis* with that into temporary and permanent colorless organisms; the temporary colorless organisms are produced by dark adaptation and the permanent ones by ultraviolet irradiation. They found that only the permanently colorless *Euglena* failed to incorporate H^{-3} thymidine into cytoplasmic structures and suggested that this, "... presumably reflects the inability of these cytoplasmic structures to synthesize DNA as a consequence of ultraviolet irradiation." Excellent studies dealing with the presence of DNA in the chloroplast of *Chlamydomonas moewusii* have been published by Ris[280] and Ris and

Plaut.[282] These authors found that the chloroplast of *C. moewusii* contain one or more bodies of irregular shape which give a Feulgen-positive reaction of the same intensity as the nucleus. After deoxyribonuclease digestion the Feulgen reaction is either abolished or reduced markedly. When cells are stained with acridine orange, the chloroplasts show a bright yellowish-green fluorescence, indicative of the presence of DNA. When such cells are subjected to ribonuclease digestion followed by DNase digestion, the fluorescence of the organelle as well as of the nucleus disappears. Utilizing the fixative developed for bacteria by Ryter *et al.*[299] to preserve the 25 Å microfibrils which correspond to DNA macromolecules, Ris and Plaut were able to demonstrate areas within the chloroplast composed of these microfibrils. The areas containing DNA are surrounded by lamellae of the chloroplast. These findings would substantiate the claim that the chloroplast behaves as a genetic unit and need not be primarily under the control of the nucleus. From the results of their study, Ris and Plaut[282] drew attention to the striking structural and chemical similarity of a chloroplast and a blue-green alga, thereby recalling the old hypothesis of Famintzin[91] and Mereschowski[224] who suggested that chloroplasts originated from endosymbiotic blue-green algae.(see also [40, 318]) Ris and Plaut concluded: "This hypothesis also explains why the photosynthetic apparatus is associated with membrane systems which traverse freely the cytoplasm in blue-green algae but which in higher plants are incorporated into complex cell organelles, having a high degree of genetic individuality and containing just about every classified organelle found in free-living blue-green algae."

D. Golgi Complex

It was some years after the discovery of the Golgi material (1898) in metazoan cells[129] that cytologists attempted to demonstrate this system in unicellular organisms. In 1914 Hirschler[159] employing Golgi's technique, demonstrated osmiophilic rings and crescents in the cytoplasm of the gregarine, *Monocystis ascidae*. Some years later, Golgi bodies were found in other sporozoans by King and Gatenby[191] and Joyet-Lavergne.[185] The osmiophilic material demonstrated by these authors was found, often in a juxtanuclear position, in the early stages of the life cycle of these sporozoans. This osmiophilic substance was thought to be Golgi material since it was isomorphic with a material which gave a similar chemical reaction in ectodermal cells of developing embryos.[41] In the later stages of the life cycle of this group, the Golgi material is found dispersed throughout the cytoplasm. Duboscq and Grassé[74] presented evidence which suggested to them that the parabasal bodies of flagellates are homologous to the Golgi material found in germ cells of metazoans. (see also[3, 21, 39, 94, 130, 181, 190, 215, 322]) Reviews by Palay,[253] Pollister and Pollister[263] and Dalton[65] bring