UCRL-16531

# University of California

# Ernest O. Lawrence Radiation Laboratory

CHLOROPLAST STRUCTURE

## DISCLAIMER

UCRL-16531

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

# CHLOROPLAST STRUCTURE

Roderic B. Park

November 1965

## II The Chloroplast as a Complete Photosynthetic System

There is abundant evidence that the materials within a chloroplast are fully competent to perform the entire photosynthetic process. The process begins with absorption of light by chlorophyll or an accessory pigment, transfer of this energy to an energy trap where quantum conversion takes place, and the subsequent use of chemical energy formed at the trap for the process of $CO_2$ fixation into carbohydrate. Evidence that all these reactions occur within the chloroplast comes both from in vivo and in vitro experiments which are reviewed below.

Among the first experiments directly demonstrating the association of photosynthesis functions with chloroplasts are those of Engelmann (1). Englemann observed a number of fresh water algae under the light microscope in the presence of bacteria which were chemotactic along a positive dissolved oxygen gradient. Some of his most elegant experiments were performed with the green alga Spirogyra. A figure from Englemann's 1894 paper is reproduced in Fig. 1. When the entire filament of Spirogyra was illuminated the bacteria congregated all along the spiral chloroplast. If only portions of the filament were illuminated, bacteria congregated only where the small beam of light impinged upon the chloroplast. This result had two implications. The first and most obvious was that a chloroplast must be illuminated in order to produce oxygen. The second implication was that oxygen was produced very close to the site of light absorption by chlorophyll. Prior to this time it might have been assumed that oxygen evolution was a reaction quite remote to the site of light absorption.

A second series of experiments relevant to the demonstration of complete photosynthetic capacity by chloroplast were those of Robin Hill (2,3) in 1937 and 1939. Hill showed that chloroplasts isolated in isotonic buffer from leaves and centrifically concentrated evolved oxygen in the presence of light when supplied with an added electron exceptor such as ferric ion. This reaction, called the Hill reaction, is shown below.

$$2H_2O + 4Fe^{+++} \xrightarrow[\text{chloroplasts}]{\text{light}} O_2 + 4H^+ + 4Fe^{++}$$

Photosynthesis differs from the Hill reaction in that carbon dioxide rather than an artificial oxidant such as ferric ion is the electron acceptor. Hill was not able to demonstrate that $CO_2$ could serve as an electron acceptor in isolated chloroplasts, and such an experiment awaited the development of more elaborate biochemical techniques. Hill showed, however, that the light reactions of photosynthesis leading to the oxidation of water and the reduction of some added acceptor occured within isolated chloroplasts.

Arnon et al. (4) in 1954 were the first workers to demonstrate conclusively that carbon dioxide could be reduced by isolated chloroplasts into intermediates of the carbon cycle of photosynthesis. In their work they demonstrated qualitatively though not quantitively that chloroplasts contained the entire photosynthetic apparatus leading from light absorption to fixation of carbon dioxide. However, one obvious deficiency existed, and continues to exist, in the acceptance of the chloroplast as the sole site of photosynthesis in vivo. This deficiency is that rates of photosynthesis in isolated chloroplast on a unit chlorophyll basis seldom exceed 5% of the in vivo rate (5). In vivo rates of

photosynthesis in both higher plants and algae can be as great as 200 μM $CO_2$ fixed/hr/mg chlorophyll (6). The rate of $CO_2$ fixation in isolated chloroplasts seldom exceeds 10 μM/hr/mg chlorophyll. This discrepancy may be in large part due to the damage which occurs to chloroplast during the isolation process. Recent work by Spencer and Unt (7) shows that chloroplasts which are carefully isolated so as to retain their external membrane systems and stroma protein possess considerably higher $CO_2$ fixation capacities than chloroplasts isolated by ordinary procedures. Work such as that by Spencer and Wildman (8) and Spencer and Unt (7) may eventually lead to isolation procedures which yield chloroplast capable of in vivo photosynthetic rates. Only then will we have conclusive proof that the chloroplast is fully competent to account for all aspects of photosynthesis as observed in intact systems.

Once the chloroplast was qualitatively accepted as a totally competent photosynthesis organelle, interest in distribution of photosynthetic function among chloroplast substructures increased. The remainder of this chapter is concerned with these studies of structure and distribution of function in mature chloroplasts. Aspects of chloroplast development are considered in the the chapter by Bogarad.

## III Structure of Chloroplasts as Revealed by Light Microscopy

Chloroplasts are easily viewed in the in vivo state by light microscopy. As mentioned earlier, these organelles may assume bizzare shapes, especially in some algae. In many plants, however, the chloroplast appears as a green saucer shaped body 5-10 microns in diameter. In green algae and some bryophytes the chloroplast contains an organized body called the pyrenoid, which is often surrounded by starch plates or lipid reserves. Chlorophyll as observed by light microscopy in the chloroplasts of algae and bryophytes appears uniformly distributed. In higher plants, however, the chloroplast from top view is seen to consist of a green field filled with small (0.2-1 μ) totally absorbing bodies called grana. The green field in which the grana lie is referred to as the stroma region of the chloroplast. Side views of the chloroplast show that the grana regions are interconnected by material indistinguishable from the grana themselves. These general observations were summarized by Heitz (9) in 1936. Two of his photographs illustrating these aspects of chloroplast morphology are reproduced in Fig. 2. Higher plant chloroplasts may be viewed by fluorescence microscopy using blue actinic light and observing the red fluorescence of chlorophyll. The chlorophyll fluorescence is seen to reside primarily in the grana stacks. Spencer and Wildman (8) have interpreted this to mean that the chlorophyll is localized in the grana regions of the chloroplast. However, we know from electron microscopy that not all membranes within the chloroplast are in the grana stacks, but that many are membranes which run between grana stacks. Do these intergrana membranes contain chlorophyll? It is doubtful that fluorescence obser-

vations of whole chloroplasts will give us this answer since the electron micrographs show that the membrane concentration in the grana stack is much larger than the membrane concentration in the stroma. A similar ratio of fluorescence intensities might obscure fluorescence from the intergrana areas. Also, as mentioned in the next section, certain higher plant cells contain only large non-granal membranes and no grana membranes, though they appear to be photosynthetic. Obviously in these systems chlorophyll is distributed in the large membrane system of the chloroplast.

Some recent experiments by Lintilhac and Park (10) support the arguments that chlorophyll is uniformly distributed throughout the internal membrane system. Chloroplast internal membranes placed on an electron microscope grid were observed by both fluorescence and electron microscopy (see Fig. 3). All the membranes, both small and large thylakoids, (11) are seen to contain chlorophyll. Direct observations of this sort are directly contrary to the conclusions of Spencer and Wildman.

The light microscope has been used to study both dichroism and birefringence in chloroplasts. Since dichroism is considered in the chapter by Butler, we are only concerned with birefringence here. Menke (12) and Frey-Wyssling (13) both studied chloroplast birefringence in media of varying refractive index. In this way they could differentiate between intrinsic and form birefringence. Form birefringence was interpreted as resulting from a layered system (12,13) within the chloroplast. Frey-Wyssling proposed a model consisting of alternate layers of protein and lipid to account for the form birefringence. This model of layered structures was to a large extent realized with

the application of electron microscopy to chloroplast structure.

Chloroplast structure and function are closely allied. For this reason it is important that the biochemist is aware of the morphological status of the chloroplasts with which he works. Initial studies by Kahn and von Wettstein, (14) Spencer and Wildman (8) and Spencer and Unt (7) show that chloroplasts isolated in 0.4 molar sucrose buffered with tris or phosphate tend to be of two types. The first type retains its outer membrane and refractal jacket of strona protein around the grana membrane and is called Class I chloroplast. The second type of chloroplast becomes ruptured during the isolation procedure and loses its outer membrane and stroma material. The latter type is referred to as a Class II chloroplast. The biochemical assays by Spencer and Unt show that Class I chloroplasts retain to the greatest extent the properties of in vivo chloroplasts - that is, comparatively high rates of $CO_2$ fixation (10 μM/hr/mg chlorophyll), low rates of Hill reaction due to coupled phosphorylation, and ability to form pseudopodia when resuspended in appropriate media. Heitz had shown that chloroplast pseudopodia formation was a widespread and normal occurence in plant cells. A drawing of this phenomenon taken from Heitz's paper appears in Fig. 4, in which the pseudopodia are shown extending into the cytoplasm. These observations of Heitz have been extended by Spencer and Wildman (8) and by Wildman et al. (15). Interestingly enough, the relatively high rates of $CO_2$ fixation in Class I chloroplasts are attainable with no added cofactors. Thus it would seem that the integrity of the outer membrane has retained these cofactors in the chloroplasts, a situation that does not occur in other biochemical preparations. In conclusion, the light microscope is and continues to be a very valuable tool

for studying the chloroplast in its in vivo environment. Light microscopy of chloroplast preparations is also a useful tool for the biochemist who wishes better to understand the photosynthetic capacities of his material.

## IV Structure of Chloroplasts as Revealed by Electron Microscopy

The electron microscope was first used to study chloroplast structure in 1940 (16). This early micrograph from Ruskas Laboratory showed that an isolated, dried chloroplast appeared to contain a number of internal membranes. The development of shadowing techniques by Williams and Wyckoff (17) in 1945 opened the way for the early ultrastructural investigations of chloroplast morphology by Granick and Porter, (18) Frey-Wyssling and Mühlethaler (19) and Steinmann (20). These early studies were made on shadowed preparations of isolated chloroplasts. The shadow technique shadowed that the lamellar system of the chloroplast was a series of membranes which were piled upon one another much like a stack of coins. Steinmann (20) in 1952 showed the existence of these membranes in the first thin sections of chloroplasts, and confirmed the conclusions obtained from observation of shadowed preparations. Thus, the predictions from light microscopy (12, 13) were to a large extent confirmed. The following 15 years have produced an enormous number of electron micrographs of chloroplast material, which have been subjected to various fixation and preparative procedures. In general, the results from sectioning are summarized in 3 micrographs, shown in Figs. 5-7, in which green alga, a red alga and a higher plant chloroplast are compared. Each chloroplast is surrounded by a double membrane system. A high magnification picture of Chlorella double membrane shows that whereas the external membrane has the same morphological characteristics from $KMnO_4$ staining as the plasma membrane, the internal membrane of the double membrane appears identical in staining characteristics to the internal membranes of the chloroplast. In all the chloroplasts the internal membrane system is embedded in a matrix called the

stroma. The internal membranes are actually closed, flattened sacks which have been termed thylakoids by Menke (11) and compartments by Weier (21). The thylakoids of algae are much larger, in general, than those of higher plants. The thylakoids of red algae are separated by 325 Å particles which may contain the accessory (phycoerythrin) pigment system (22). The thylakoids of green algae are appressed in groups of two, three, or four, giving the structures seen in the Chlorella cross-section. In any higher plant chloroplasts, on the other hand, small thylakoids are stacked to make grana structures, whereas the larger connecting membrane systems termed the large thylakoids by Menke (11) and frets by Weier (21) are much less frequent than they are in the algae. A summary of nomenclature given to these membranes by Weier and Menke is given in Fig. 8. The degree of natural variation within a single plant is most graphically shown by the morphology of plastids in the neighboring mesophyll and bundle sheath cells of many monocots. Such a micrograph is shown in Fig. 9. The mesophyll cells are seen to contain the same kind of chloroplast as shown for a typical higher plant chloroplast, in Fig. 7. The adjacent bundle sheath cells, on the other hand, contain plastids which are indistinguishable from those present in certain algae. Thus, a higher plant appears to have a genetic capacity to produce a considerable variation in the detailed membrane system inside a chloroplast. Thompson and Weier (23) have shown that the nutritional status of bean plants can markedly affect the membrane arrangement within the chloroplast. Under conditions of low phosphate, the plastids of bean plants tend to assume the morphology of the bundle sheath cell plastids of monocots.

The gross morphology of a chloroplast may also be seen by the freeze-etch technique developed in Frey-Wyssling's Laboratory (24). The freeze-

etching of chloroplasts possibly gives the most accurate description of chloroplast ultrastructure yet obtained by the electron microscope, since the number of artifacts under conditions of rapid freezing are minimal (25). A micrograph from an isolated spinach chloroplast is presented in Fig. 10. It is seen in the isolated chloroplast that the thylakoids are somewhat swollen and that the protein and ribosomes in the stroma are quite evident. On the other hand, in in vivo material, the membranes and stroma material are so tightly appressed that the individual character of the stroma proteins is not so evident. This swelling which occurs during isolation may account for the loss of some of the photosynthetic activity of isolated chloroplasts. The swollen and shrunken states seen by freeze-etching may also explain volume and scattering changes observed in isolated chloroplasts as a consequence of osmotic changes, pH changes, and illumination. Tangential views of the internal membranes, evident in the upper portions of Fig. 10, show substructure within the chlorophyll containing membrane. Evidence for the relationship of chlorophyll to these substructures is presented in the next section.

In summary, then, electron microscopy has shown that the chloroplast consists of two phases, a thylakoid phase which we shall see contains the chlorophyll, and a stroma phase which is the site of carbon cycle enzymes and other synthetic capacities of the chloroplast. Assignments of functions to these structures is considered next.

## V Distribution of Function Within Chloroplasts

The work of Trebst et al. (26) and Park and Pon (27) demonstrates that the light reactions of photosynthesis and the associated electron transport reactions leading from the oxidation of water to the reduction of ferredoxin occur within the internal membrane system of chloroplasts, while the $CO_2$ fixation reactions of the carbon cycle occur within the stroma regions of the chloroplast. It should be added at this point that enzymatic systems other than $CO_2$ fixation systems are present in the stroma, the most notable being specific chloroplast ribosomes (28,29) and an apparent ability to synthesize protein (30). Since these interesting capacities are not directly related to photosynthesis and chlorophyll, they are not considered here. If chloroplasts isolated in isotonic media are subjected to a hypotonic environment, the plastids are seen to swell and the stroma material leaks from the plastid. Centrifugation of this preparation yields a green precipitate and a soluble protein supernatant, and it is found that the protein is approximately equally distributed between the two phases (27). The green precipitate consists of membranes which are about 10% chlorophyll by weight and the supernatant which consists of the soluble stroma material. That both are needed for the photosynthetic process is shown in Fig. 11, in which two dimensional chromatograms of the $CO_2$ fixation products of membranes alone, and the two mixed together, are shown. There is about a fifty fold enhancement of $CO_2$ fixation capacity when the two systems are mixed together. These results are diagramatically presented in Fig. 12, in which the distribution of photosynthetic function between the membrane phase of the chloroplast and the stroma portion of the chloroplast are presented.

Since it is the internal membrane system of the chloroplast which contains chlorophyll and performs the quantum conversion act of photosynthesis, it is to the detailed description and analysis of these internal membranes that the remainder of this chapter will be devoted.

## A. Internal Membrane Systems of Chloroplasts

As already mentioned, chloroplast internal membranes are approximately 10% chlorophyll by weight. When illuminated, these membranes bring about the oxidation of water to produce oxygen gas and electron transport with accompanying phosphorylation to the level of a reducing agent which will reduce the soluble cofactor, spinach ferredoxin. The kinds of information available about these membranes are their chemical composition and the properties of membrane proteins, their enzymology and their morphology as seen in the light and electron microscopes and by X-ray diffraction. One of the most interesting and frustrating aspects of studying these membranes is the process of correlating these three factors, the composition, the enzymology and the morphology, into a unified and consistent picture. We shall first discuss morphological features of these membranes, then the chemical composition and enzymology and finally we shall sttempt to correlate these various kinds of information.

### 1. Morphology

Our knowledge of chloroplast internal membrane substructure comes from several sorts of experiments. These involve electron and light microscopy and the process of X-ray diffraction. The electron microscope techniques are those of staining and section preparation, heavy metal shadowing, negative staining, and freeze-etching. These will be considered separately.

A typical example of sectioned internal membrane systems of chloroplasts was shown in Figs. 5-7. If electron microscopy following potassium permangenate staining is done at high magnifications, a 75-90 Å periodicity is seen along the membranes (31,32,33). This periodicity has been described in both higher and lower plants. Such a periodicity is also beautifully evident in the preparations of Kahn and von Wettstein,(14) although these authors do not comment upon it. These experiments, then, would tend to confirm models which have been advanced by Sjöstrand (34) and others, which suggests that membranes are built from micellular subunits. Such periodicity, however, could be artifact caused by lipid micelle formation during fixation.

Heavy metal shadowing of chloroplast internal membranes was first shown by Steinmann (20) to reveal a substructure on the membrane surface. This substructure consisted of a granularity with about a 200 Å periodicity. Following this, Steinmann and Frey-Wyssling (35) demonstrated similar structures in other plants and Park and Pon (27,36) continued these studies with spinach chloroplasts. At times this substructure becomes very highly organized to give a paracrystalline array such as that shown in Fig. 13 (37). The fact remains that membranes occur in spinach which are apparently competent in quantum conversion and electron transport and yet contain no structure whatsoever, as seen by heavy metal shadowing. On the other hand, there is evidence that the most efficient membranes, in terms of quantum conversion, are the highly structured ones which appear in spinach under short/day and perhaps some unknown additional conditions (38). The main subunits seen in Fig. 13 measure 185 by 155 by 100 Å. These units are termed quantasomes, and

we have suggested that they may be the smallest units involved in photosynthetic conversion (37). It is also evident in the micrograph in Fig. 13 that a quantasome consists of subunits which are present on about 75-90 Å periodicities. These subunits may correspond to the subunits seen in the histological work utilizing $KMnO_4$ fixation. Work with Pharbitis by Park (39) indicates that the quantasome structure is evident in this plant on the external portion of the thylakoid, whereas the internal portion of the thylakoid as viewed after sonication consists of 90 Å particles distributed along the surface. This is shown in Fig. 14, and would indicate that the thylakoid membrane has two sides, a granular side with about a 90 Å periodicity which corresponds to the internal regions of the thylakoid, and a large particle surface which corresponds to the external surface of the thylakoid and the quantasome structure. As we shall see, this small particle surface and large particles are evident in both tissues in the freeze-etch process. Again, it is not possible to exclude that these structures may in part result from micelle formation or other artifact during preparation of the specimen for microscopy.

Negative staining of internal membranes of spinach chloroplast has been performed by Park (40) and Oda et al. (41) and Bronchart (42). These studies show that there is occasionally a 100 Å particle attached to the internal membrane system in well preserved areas. However, the identification of such particles is in doubt since they appear to be indistinguishable from the size and structural morphology of the $CO_2$ fixation enzyme of photosynthesis which is located in the stroma. Both the particles described by Oda et al. (41) and the colorless enzyme (carboxydismutase, see Fig. 15) are 100 to 110 Å in diameter and contain

an electron dense central core. It is not yet clear that Oda's membranes were washed completely free from fraction I protein. The less well preserved areas of negative stained membranes do show substructure (42) which may be related to the subunits seen in freeze-etching.

The freeze-etch technique has been applied by Moor (25) and by Park and Branton (43) to spinach chloroplasts. One such picture of a chloroplast cross-section was shown earlier in Fig. 10. A different kind of information is obtained if one observes the internal membranes of chloroplasts in tangential view rather than in cross-section. A typical example from the work of Park and Branton is shown in Fig. 16. In this picture there are surfaces of small particles, surfaces with few or no particles and surfaces with large particles. This view is typical whether one is looking at intact algal cells, intact higher plant cells, or isolated higher plant chloroplasts or chloroplast fragments. No other preparative technique gives such a detailed and consistent picture of membrane substructure. It is apparent from work with swollen, isolated plastids that the small particle surface may correspond to the internal, small particle surface seen in the Pharbitis thylakoid. Breakage then occurs stepwise down through a single membrane, yielding various layers within a 100 Å thick membrane. The large particles (150 Å) are located within the membrane and are exposed when the surrounding material is removed by breakage.

Low angle X-ray scattering experiments by Kreutz (44) have shown that there is a 37 Å periodicity along the thylakoid membrane. Menke has interpreted these experiments to mean that the membrane itself consists of a bimolecular leaflet of lipid covered on one side by protein in a way consistent with the Danielli-Davson model (45).

2. Chemical composition

The chemical composition of a single quantasome may be calculated from the size of the quantasome, its density, and a knowledge of membrane chemical composition. Such an analysis is given in Table I. It is seen from the data in Table I that the quantasome is sufficiently large to contain at least one of each of the components of the electron transport pathway of photosynthesis. The proteins in these membranes have been studied by Criddle and Park (57) and Biggins and Park (58). They are similar to the "structural protein" of mitochondria. Eighty percent of the protein recovered in a detergent solubilized preparation (58) gave ~~off~~ a molecular weight of 20,000 - 40,000. The schlieren peak was heterogeneous and contained both cytochromes $b_6$ and f. Whether or not the quantasome corresponds to the photosynthetic unit of Emerson and Arnold (59) is less certain. Recent work by Isawa and Good (60) suggests on the basis of inhibitor evidence that the oxygen evolving, photosynthetic unit may be considerably larger than the quantasome. It may be that the number of electron transport chains in photosynthesis considerably exceeds the number of oxygen evolution sites and that perhaps a number of quantasomes are attached to one oxygen-evolving site. These possibilities have been discussed by Park (39). It may be concluded, at any rate, that the photosynthetic unit is considerably larger than one of the 75-90 Å units as seen by histological techniques, heavy metal shadowing in Pharbitis, and freeze-etching. On the other hand, a particle the size of the quantasome which is seen sometimes by heavy metal shadowing and invariably by the freeze-etch procedure may correspond to a quantum conversion site in the membrane. One appealing thought that arises from knowledge of membrane breakage concerns experiments in which

plastids have been broken into particles containing different chlorophyll a to b ratios by the process of freezing or thawing, or use of detergents. Knowledge that under freeze-etch conditions the membrane may break down the center rather than on either outer surface is an indication that similar breakages may occur during biochemical preparations. Thus, systems one and two of photosynthesis might be on opposite sides of the membrane, unable to transfer excitation energy, as shown by Sauer and Park (61).

The view of membrane structure presented in Fig. 16 must be correlated with a chemical composition and enzymological data presented in Table I. The question of how lipid and protein are localized within the membrane is only partially answered. A view of an acetone extracted membrane is shown in Fig. 17, and it is seen that lipid removal from the membrane yields a series of particles corresponding to quantasome size but in much greater relief (39). In many places it is possible to peer right down through the membrane to the plastic film background. Certain places in the membrane show a 90 Å periodicity. Thus, it appears that lipid and protein alternate with 90 Å periodicity along the membrane and that lipid may be regarded as wrapped around a protein matrix. Such a model would be consistent with the biochemical experiments of Sastry and Kates (62) who have shown that the lipid of chloroplast membranes is readily accessible for attack by lipases and galactosidases from Phaseolus multriflorus enzyme preparations. Bamberger and Park (63) approached this problem by partial enzymatic digestion of membranes and study of the freeze-etched residues. These studies suggested that chlorophyll is mainly associated with the large particles and their embedding matrix, as seen in freeze-etching. The smooth surface on which

the large particles and embedding matrix lie appears to be composed of galactolipid. In general, however, a conclusive localization of substances including chlorophyll within the membrane awaits future investigations.

In conclusion, we know a considerable amount about the chemical composition and morphology of the internal membrane system of the chloroplast. The greatest gap in our knowledge falls in the area which lies between solution chemistry, from which we know the chemical composition, and present electron microscope techniques, from which we know the morphology. It is the micromorphology of associations of discrete molecules within the membrane which will finally help us to explain not only the in vivo environment of chlorophyll molecules, but the entire photosynthetic, quantum conversion and electron transport process. We must count on the ingenuity of investigators in the future to solve this problem.

TABLE I

The composition of the quantasome based on its volume and density and on the chemical compositon of chloroplast internal membranes. See Park and Pon (36), Lichtenthaler and Park (46) and Park and Biggins (37) for original references.

| Lipid[1] (Composition in moles per mole of quantasome) | | |
|---|---|---|
| 230 Chlorophylls | | |
| 160 chlorophyll a | 143,000 | 206,400 |
| 70 chlorophyll b | 63,400 | |
| 48 Carotenoids | | 27,400 |
| 14 B-carotene | 7,600 | |
| 22 lutein | 12,600 | |
| 6 violaxanthin | 3,600 | |
| 6 neoaxanthin | 3,600 | |
| 46 Quinone compounds | | 31,800 |
| 16 plastoquinone A | 12,000 | |
| 8 plastoquinone B | 9,000 | |
| 6 plastoquinone C | 3,000 | |
| 8-10 a-tocopherol | 3,800 | |
| 4 a-tocopherylquinone | 2,000 | |
| 4 vitamin $K_1$ | | |
| 116 Phospholipids[2] (phosphatidylglycerols) | | 90,800 |
| 114 Digalactosyldiglyceride | | 134,000 |
| 346 Monogalactosyldiglyceride | | 268,000 |
| 48 Sulfolipid | | 41,000 |
| ? Sterols | | 15,000 |
| Unidentified lipids | | 175,000 |

TABLE I (continued)

| | | |
|---|---|---|
| | TOTAL | 928,000 |
| Protein | | |
| 9,380 nitrogen atoms as protein | | 928,000 |
| 2 manganese | | 110 |
| 12 iron including 1 as cytochrome $b_6$ and 1 as cytochrome f[3] | | 672 |
| 6 copper | | 218 |
| | TOTAL | 930,000 |
| Total lipid plus protein[4] | | 1,920,000 |

1- The fatty acid contribution to the molecular weight was determined from the analyses of Wolf et al. (47) and Debuch (48).

2- The 116 phospholipids include 14 molecules of glycerophosphoryl inositol, 52 of glycerophosphoryl glycerol, 6 of glycerophosphoryl ethanolamine, 42 of glycerophosphoryl choline, and 2 of glycerophosphate.

3- Lundegardh (49,50) reports the existence of cytochrome $b_3$ as well as cytochromes $b_6$ and f in chloroplasts. Other components of the electron transport chain which exist in a ratio of close to 1 per quantasome are plastocyanin (1 plastocyanin/300 chlorophylls, Katoh et al., 51,52 ) , ferredoxin (1 ferredoxin/400 chlorophylls, Tagawa and Arnon, 53), and P-700 (1 P-700/400 chlorophylls, Kok and Hoch, 54).

4- Amino acid analyses by Weber (55) show an enrichment in amino acids with non-polar side chains similar to the amino acid analysis of structural protein of mitochondria (56).

## Figure Captions

Fig. 1. Localization of photosynthetic $O_2$ production in Spirogyra after Engelmann (1).

Fig. 2. Top view and side view of a chloroplast as seen by light microscopy. The dark regions within the chloroplasts are grana. From data of Heitz (9).

Fig. 3. Comparison of a) fluorescence, and b) electron micrographs of the same chloroplast membrane specimen on formvar film (10).

Fig. 4. Drawing of pseudopodia formation by chloroplasts, from Heitz (9).

Fig. 5. Thin section of a $KMnO_4$ fixed Chlorella pyrenoidosa cell. The cup shaped chloroplast contains a large pyrenoid.

Fig. 6. A thin section of glutaraldehyde osmium fixed Porphyridium cruentum. Courtesy of Drs. E. Gmaitt and S. F. Conti.

Fig. 7. Thin section of $KMnO_4$ fixed Spinacea oleracea chloroplast.

Fig. 8. Nomenclature of chloroplast internal membrane systems used by Weir (21) and Menke (11).

Fig. 9. Variation of chloroplast internal membrane structure in two adjacent cells (bundle sheath cell and mesophyll cell) in sugar cane leaf. Courtesy of Professor W. M. Laetsch.

Fig. 10. Freeze-etch preparation of an isolated spinach chloroplast.

Fig. 11. Chromatograms of the products of $C^{14}O_2$ fixation by a) chloroplast internal membranes, b) chloroplast stroma, and c) internal membranes and stroma. From Park and Pon (27).

Fig. 12. A schematic presentation of the distribution of photosynthetic function between chloroplast internal membranes and stroma.

Fig. 13. Paracrystalline quantasome array in spinach chloroplast internal membrane. Four subunits are seen per quantasome. From Park and Biggins (37).

Fig. 14. Shadowed preparation of ruptured Pharbitis thylakoids (39).

Fig. 15. Carboxydismutase as seen by phosphotungstic acid negative staining. The proteins (550,000 MW) measure 80 x 110 Å and have an electron dense central core.

Fig. 16. A tangential view of chloroplast internal membranes by freeze-etching (43).

Fig. 17. A spinach chloroplast thylakoid membrane after acetone extraction (39).

References

1. Th.W. Engelmann, Archiv für die Gesammte Physiologie des Menschen und des Theire von Pflüger 57, 375 (1894).

2. R. Hill, Nature 139, 881 (1937).

3. R. Hill, Proc. Roy. Soc. London 127B, 192 (1939).

4. D.I. Arnon, M.B. Allen, and F.R. Whatley, Nature 174, 394 (1954).

5. R. Gee, G. Josh, R.F. Bils, and P. Saltman, Plant Physiol. 40, 89 (1965).

6. J.A. Bassham, Advances in Enzymol. 25, 39 (1963).

7. D. Spencer and H. Unt, Aust. J. Biol. Sci. 18, 197 (1965).

8. D. Spencer and S.G. Wildman, Aust. J. Biol. Sci. 15, 599 (1962).

9. E. Heitz, Planta 26, 134 (1936).

10. P. Lintilhac and R.B. Park, submitted to J. Cell. Biol.

11. W. Menke, Experientia 16, 537 (1960).

12. W. Menke, Protoplasma 22, 56 (1934).

13. A. Frey-Wyssling, Protoplasma 29, 279 (1937).

14. A. Kahn and D. von Wettstein, J. Ultrastructure Res. 5, 557 (1961).

15. S.G. Wildman, T. Hongladarom, and S.I. Honda, Science 138, 434 (1962).

16. G.A. Kausche and H. Ruska, Naturwissenschaften 28, 303 (1940).

17. R.C. Williams and R. Wyckoff, Proc. Soc. Exp. Biol. + Med. 58, 265 (1945). ~~Exp. Cell Research 4, 188 (1953).~~

18. S. Granick and K.R. Porter, Am. J. Botany 34, 545 (1947).

19. A. Frey-Wyssling and K. Mühlethaler, Vierteljahrsschr. naturforsch. Ges. Zürich 94, 179 (1949).

20. E. Steinmann, Experientia 8, 300 (1952).

21. T.E. Weier, R.C. Stocking, C.E. Bracker, and E.B. Risley, Am. J. Botany 52, 339 (1965).

22. E. Gantt and S.F. Conti, J. Cell Biol. 26, 365 (1965).

23. W.W. Thomson and T.E. Weier, Am. J. Botany 49, 1047 (1962).

24. H. Moor, K. Mühlethaler, H. Waldner, and A. Frey-Wyssling, J. Biophys. Biochem. Cytology 10, 1 (1961).

25. H. Moor, Z. zellforsch u. microskop. Anat. 62, 546 (1964).

26. A.V. Trebst, H.Y. Tsujimoto, and D.I. Arnon, Nature 182, 351 (1958).

27. R.B. Park and N.G. Pon, J. Mol. Biol. 3, 1 (1961).

28. J.W. Lyttleton, Exp. Cell Research 26, 312 (1962).

29. A.B. Jacobsen, H. Swift, and L. Bogorad, J. Cell Biol. 17, 557 (1963).

30. R.I.B. Francki, N.K. Boardman, and S.G. Wildman, Biochemistry 4, 865 (1965).

31. S. Murakami, Y. Morimura, and A. Takamiya, special issue of Plant and Cell Physiol., Tokyo, 65 (1963).

32. H.R. Hohl and A. Hepton, J. Ultrastructure Research 12, 542 (1965).

33. T.E. Weier, A.H.P. Engelbrecht, A. Harrison, and E.B. Risley, J. Ultrastructure Research 13, 92 (1965).

34. F.S. Sjöstrand, J. Ultrastructure Research 9, 340 (1963).

35. A. Frey-Wyssling and E. Steinmann, Vierteljahrsschr. naturforsch. Ges. Zürich 98, 20 (1953).

36. R.B. Park and N.G. Pon, J. Mol. Biol. 6, 105 (1963).

37. R.B. Park and J. Biggins, Science 144, 1009 (1964).

38. R.B. Park and S. Drury, Proceedings of conference "Croissance et Vieillissement des Chloroplastes" held at Gorsem, Belgium in September 1965 (In press).

39. R.B. Park, J. Cell. Biol. 27, 151 (1965). ~~In press (1965b)~~.

40. R.B. Park, in "The General Physiology of Cell Specialization" (D. Mazia and A. Tyler, ed.), p. 219. McGraw-Hill Book Company, Inc., New York, 1963.

41. T. Oda and H. Huzisige, Exp. Cell Research 37, 481 (1965).

42. R. Bronchart, Compt. rend. Acad. Sci. (Paris) 260, 4564 (1965).

43. R.B. Park and D. Branton, manuscript in preparation (1965).

44. W. Kreutz, Z. Naturforsch. 19b, 441 (1964).

45. J.F. Danielli and H. Dauson, J. Cell Comp. Physiol. 5, 495 (1935).

46. H.K. Lichtenthaler and R.B. Park, Nature 198, 1070 (1963).

47. F.T. Wolf, J.G. Coniglio, and J.T. Davis, Plant Physiol. 37, 83 (1962).

48. H. Debuch, Experientia 18, 61 (1962).

49. H. Lundegardh, Physiol. Plantarum 15, 390 (1962).

50. H. Lundegardh, Physiol. Plantarum 18, 269 (1965).

51. S. Katoh, I. Suga, I. Shiratori, and A. Takamiya, Arch. Biochem. Biophys. 94, 136 (1961).

52. S. Katoh and A. Takamiya, Plant and Cell Physiol. 4, 335 (1963).

53. K. Tagawa and D.I. Arnon, Nature 195, 537 (1962).

54. B. Kok and G. Hoch, in "Light and Life" (W.D. McElroy and B. Glass, eds.), p. 397. Johns Hopkins Press, Baltimore, Maryland, 1961.

55. P. Weber, Z. Naturforsch. 18b, 1105 (1963).

56. R.S. Criddle, R.M. Bock, D.E. Green, and H. Tisdale, Biochemistry 1, 827 (1962).

57. R.S. Criddle and R.L. Park, Biochem. Biophys. Res. Comm. 17, 74 (1964).

58. J. Biggins and R.B. Park, Plant Physiol., In press (1965).

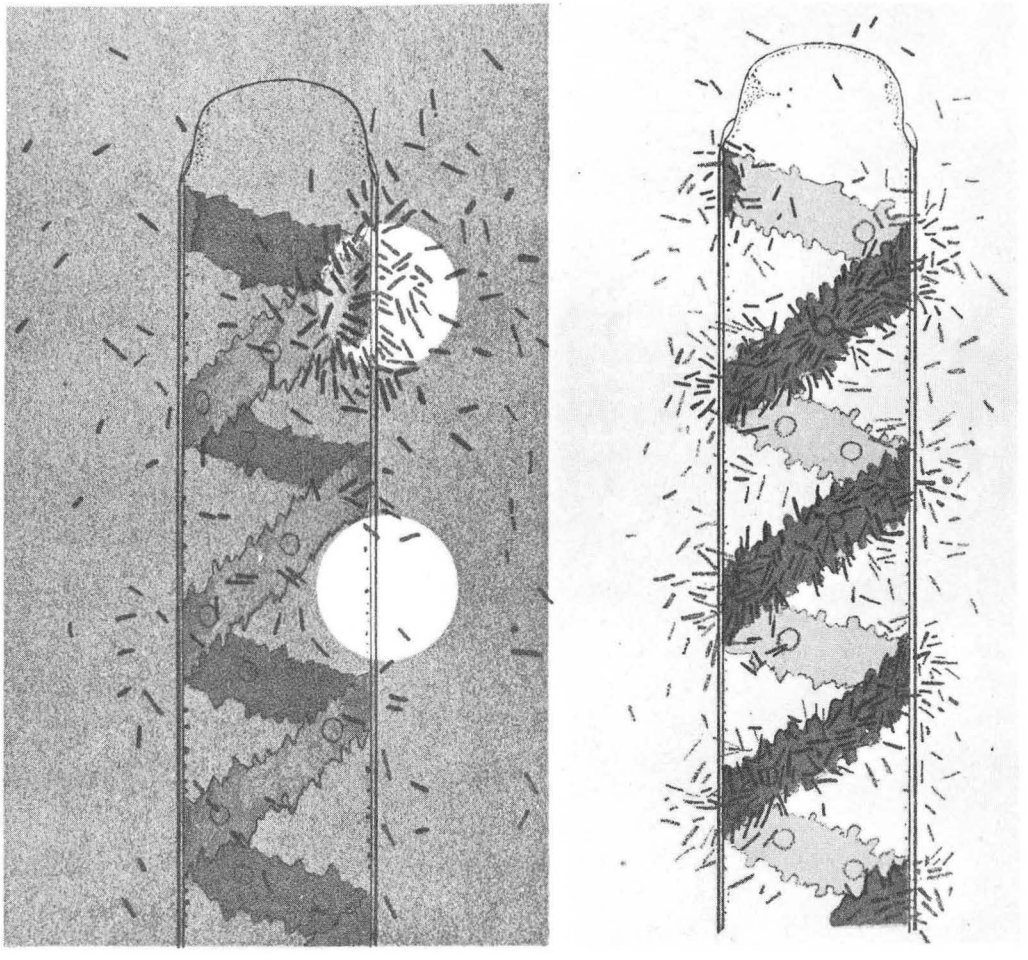
59. R. Emerson and W. Arnold, J. Gen. Physiol. 16, 191 (1932).

60. S. Izawa and N.E. Good, Biochim. Biophys. Acta 102, 20 (1965).

61. K. Sauer and R.B. Park, Biochemistry, In press (1965).

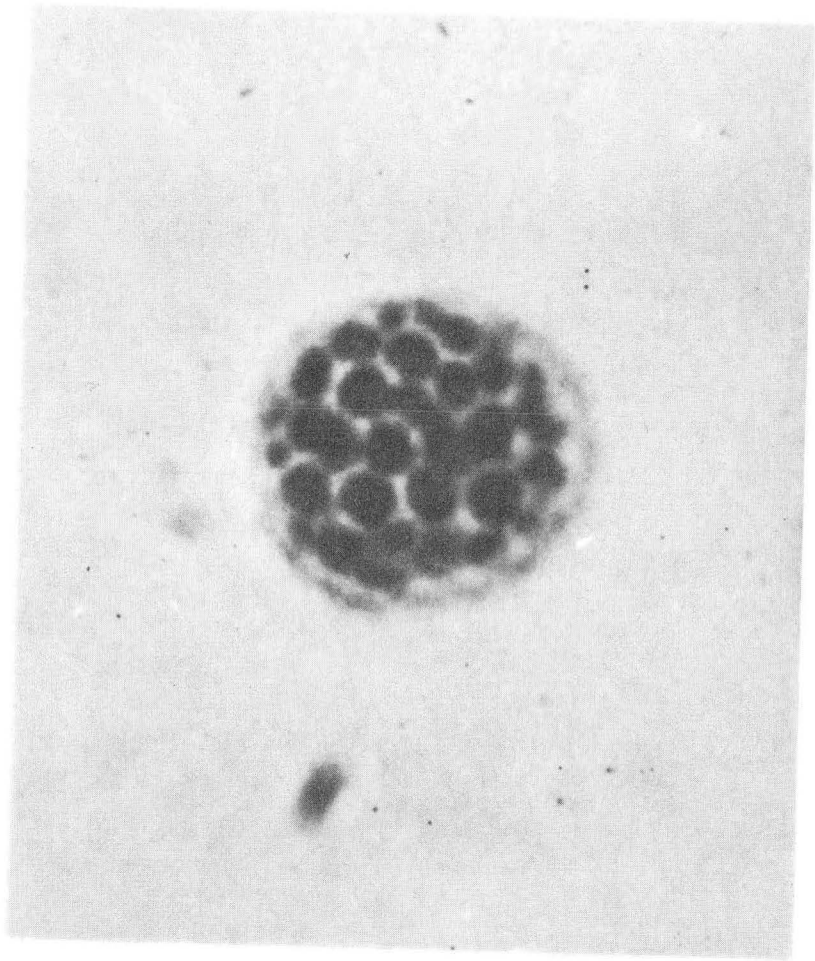
62. P.S. Sastry and M. Kates, Biochemistry 3, 1280 (1964).

63. E.S. Bamberger and R.B. Park, manuscript in preparation (1965).



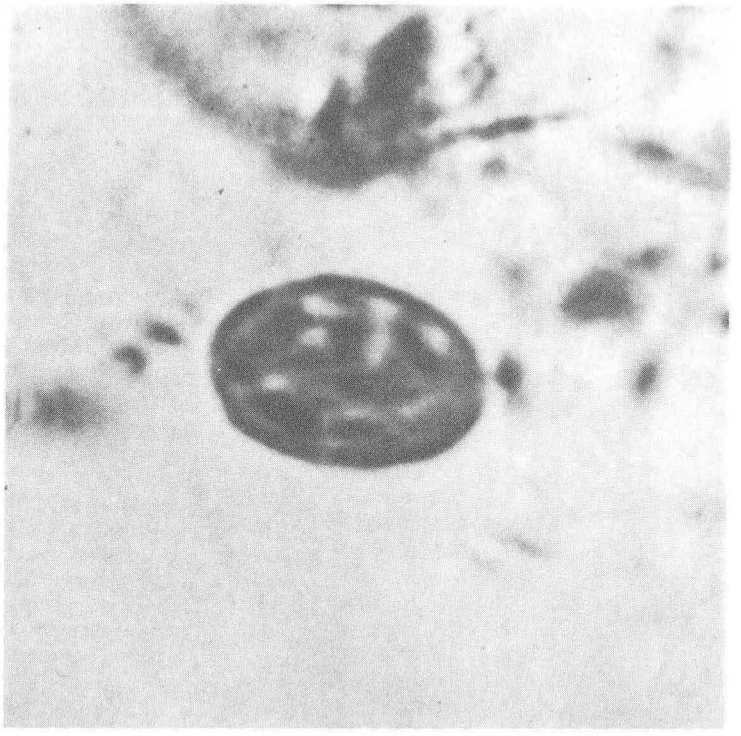
ZN-5532

Fig. 1



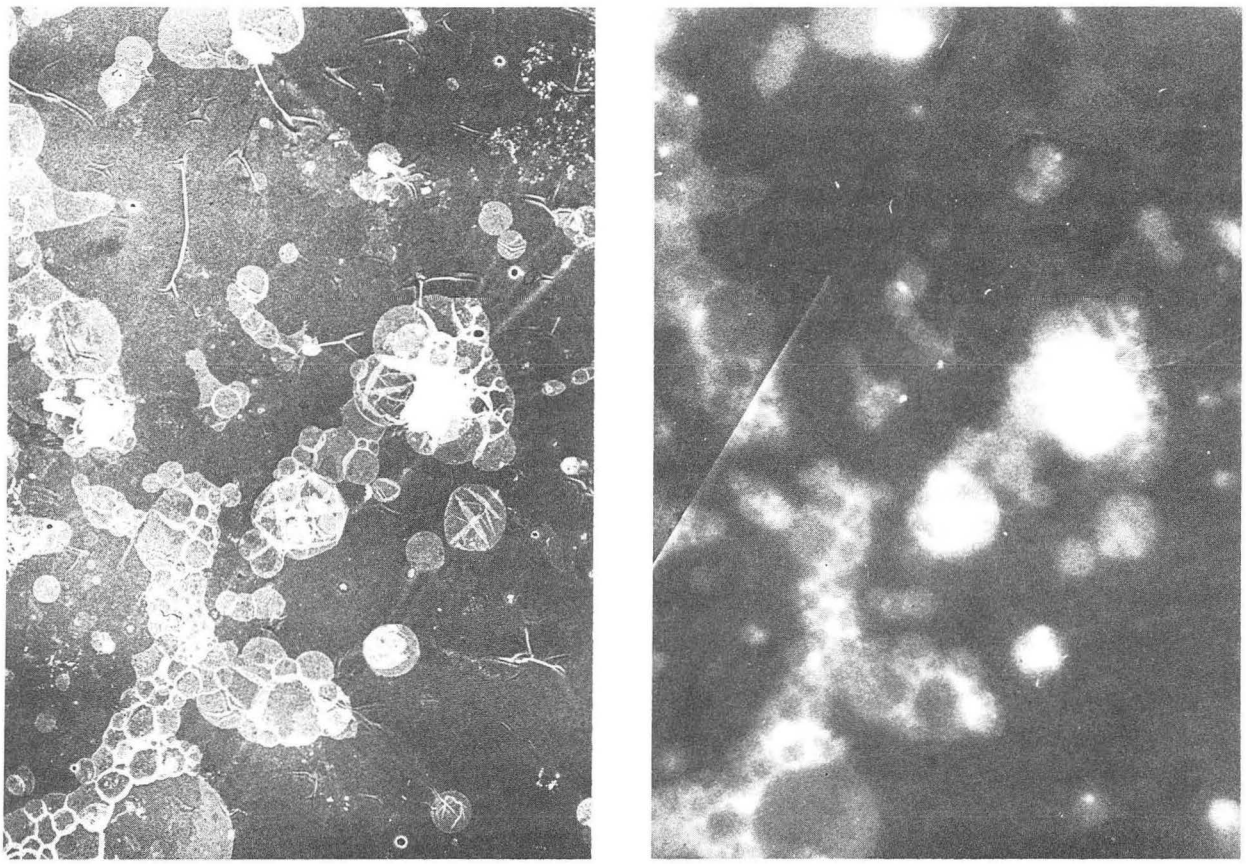
ZN-5527

Fig. 2a



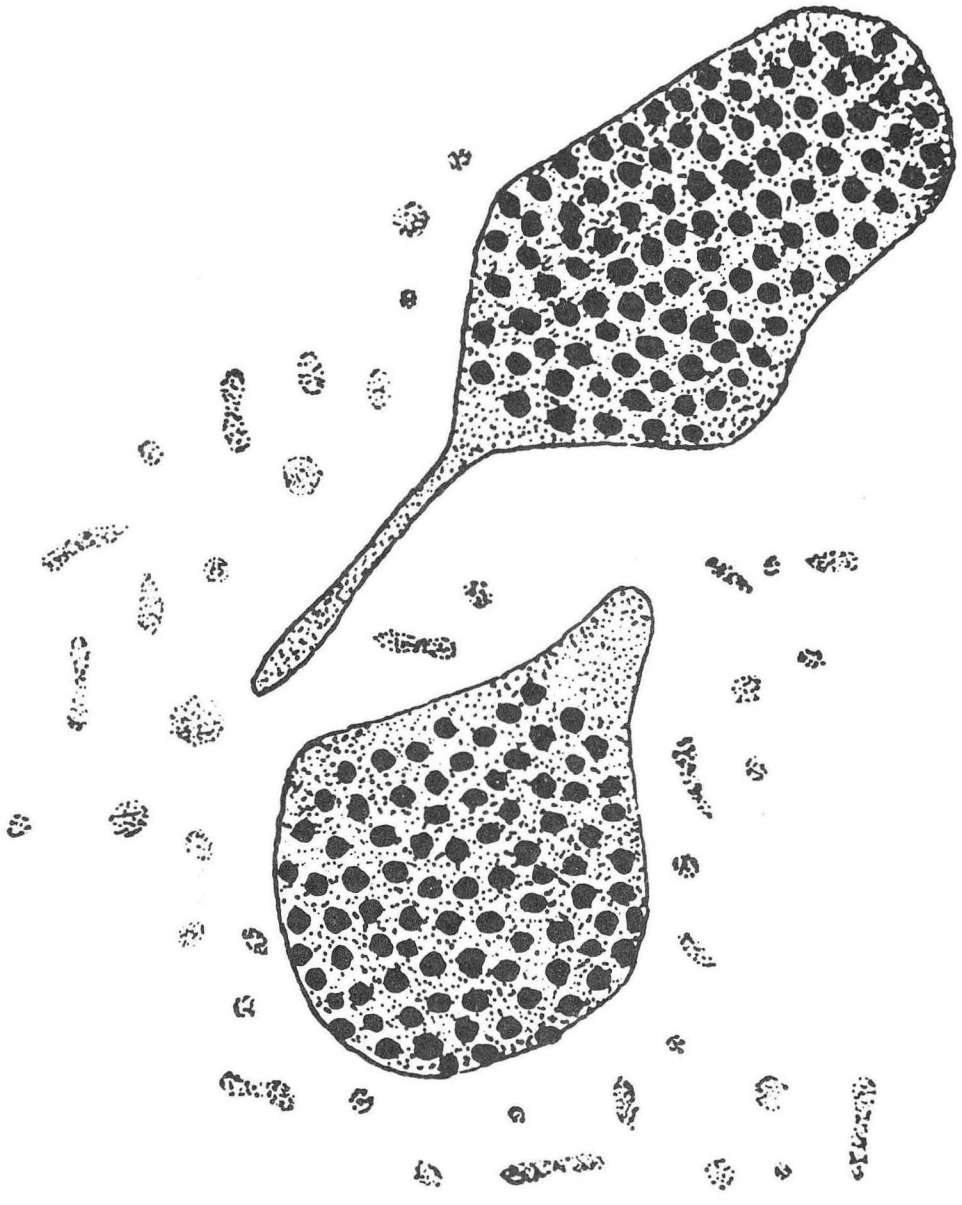
ZN-5526

Fig. 2b



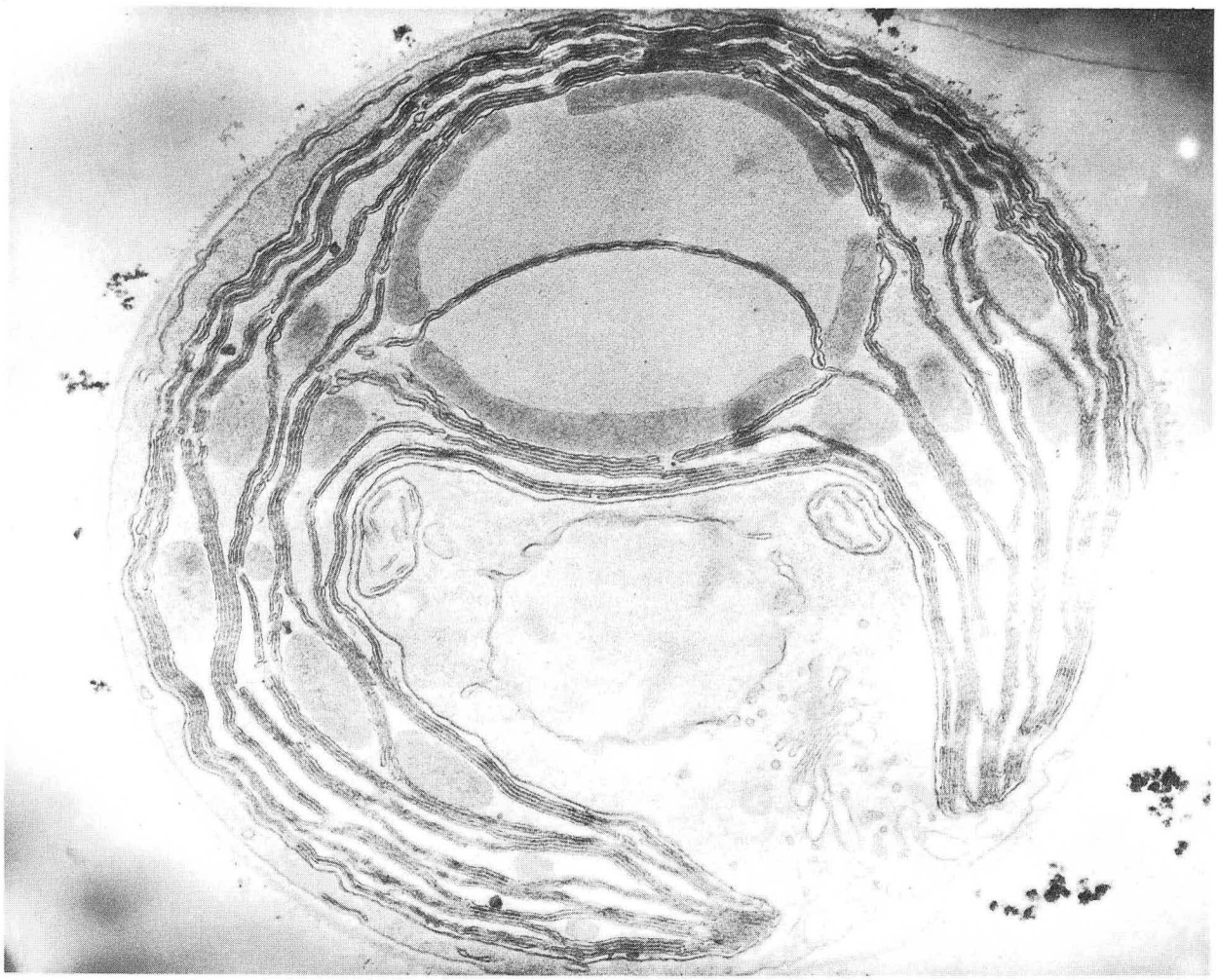
ZN-5537

Fig. 3



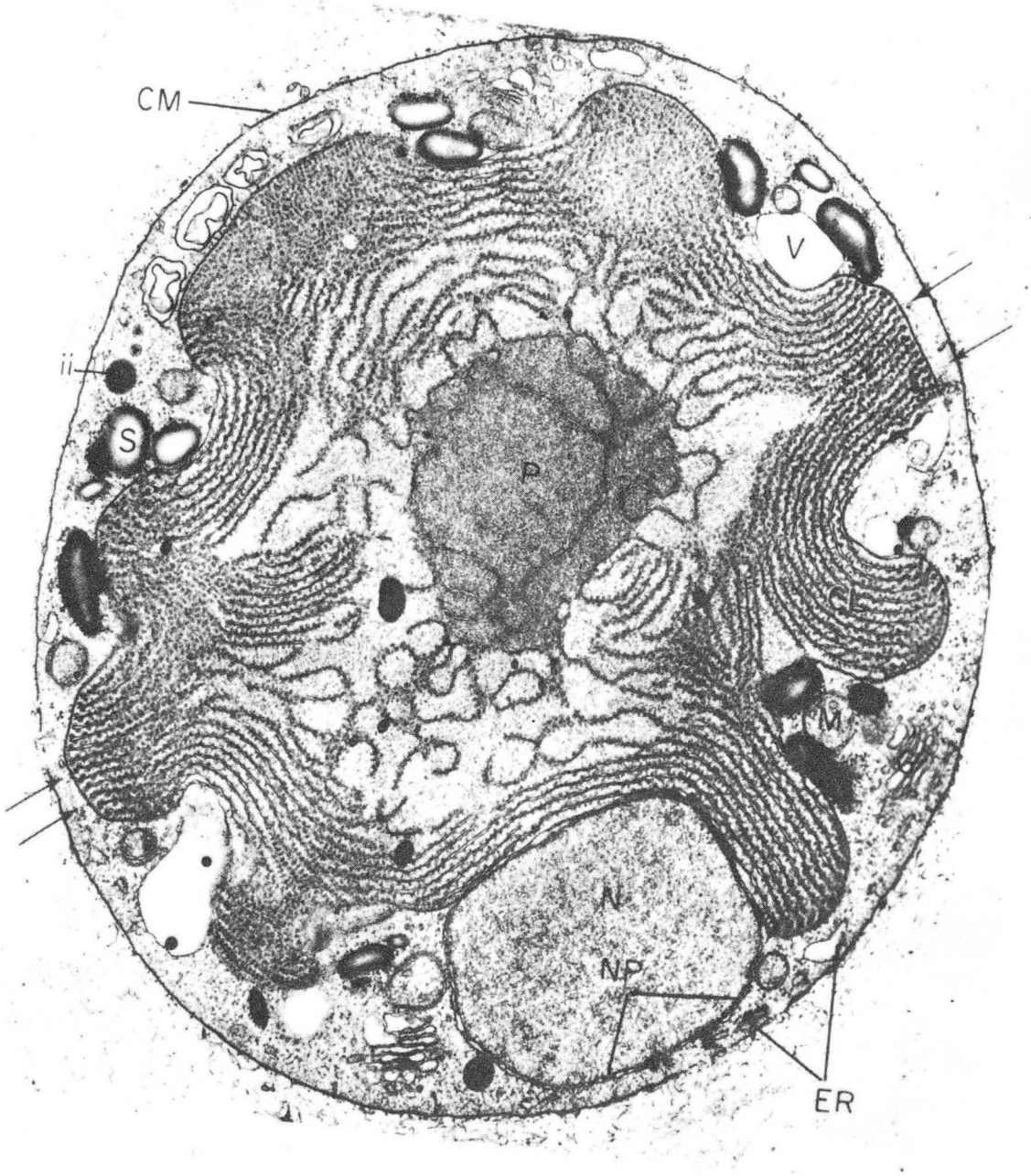
ZN-5525

Fig. 4



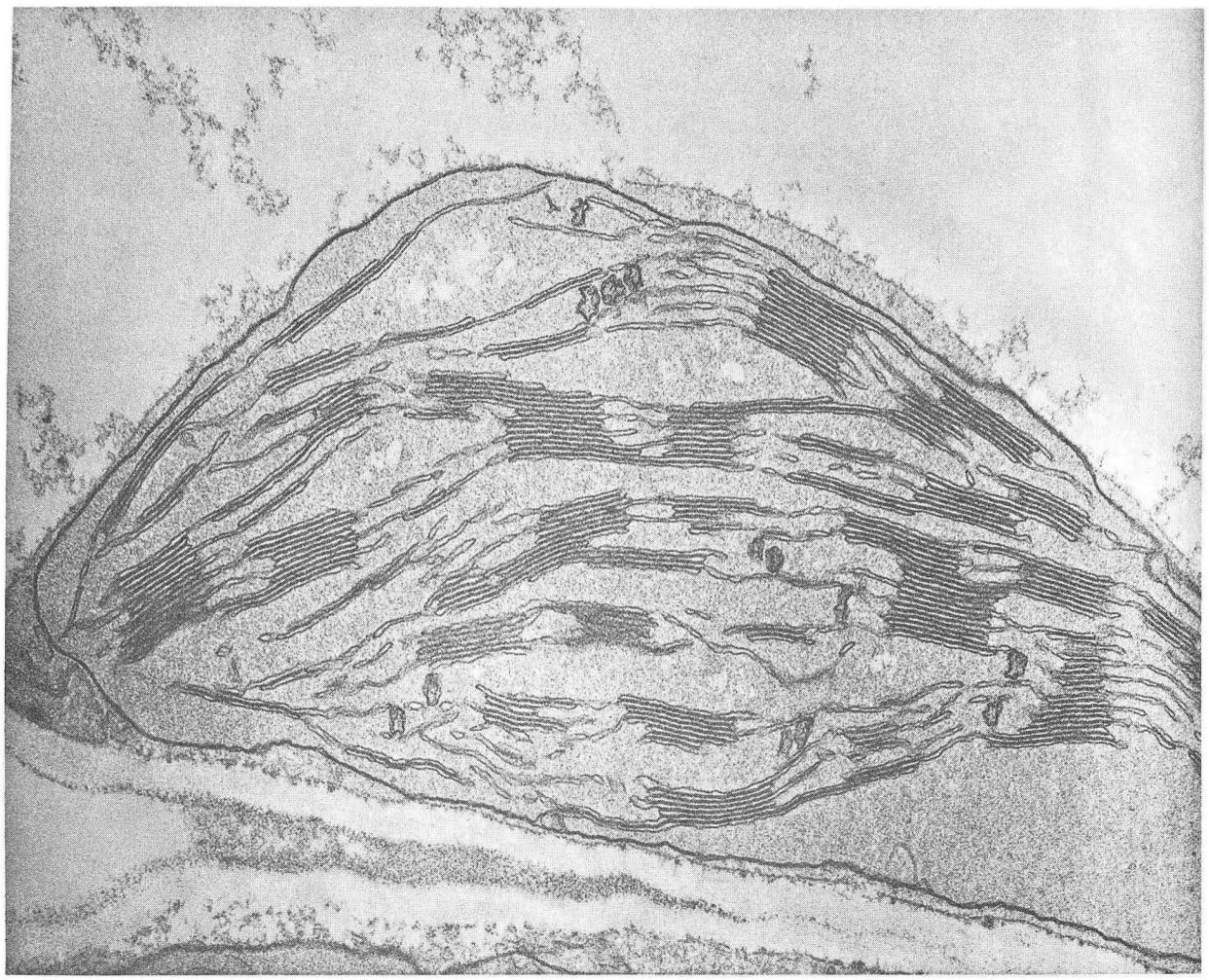
ZN-5528

Fig. 5




ZN-5538

Fig. 6



ZN-5535

Fig. 7

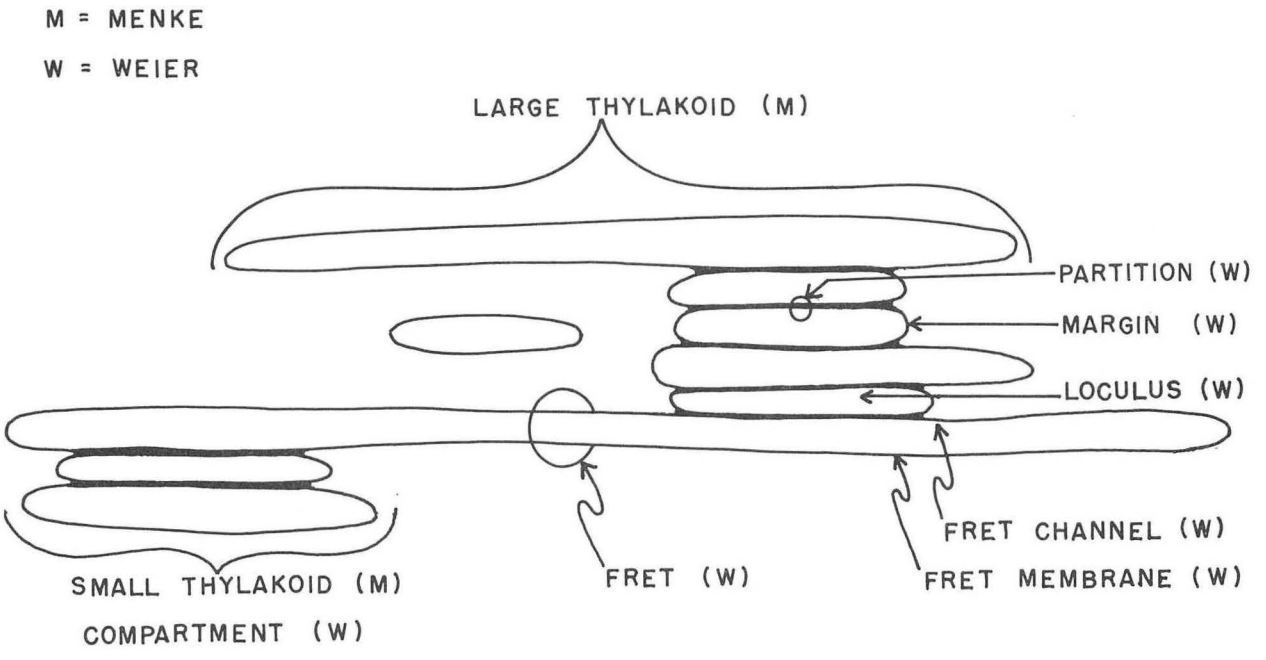
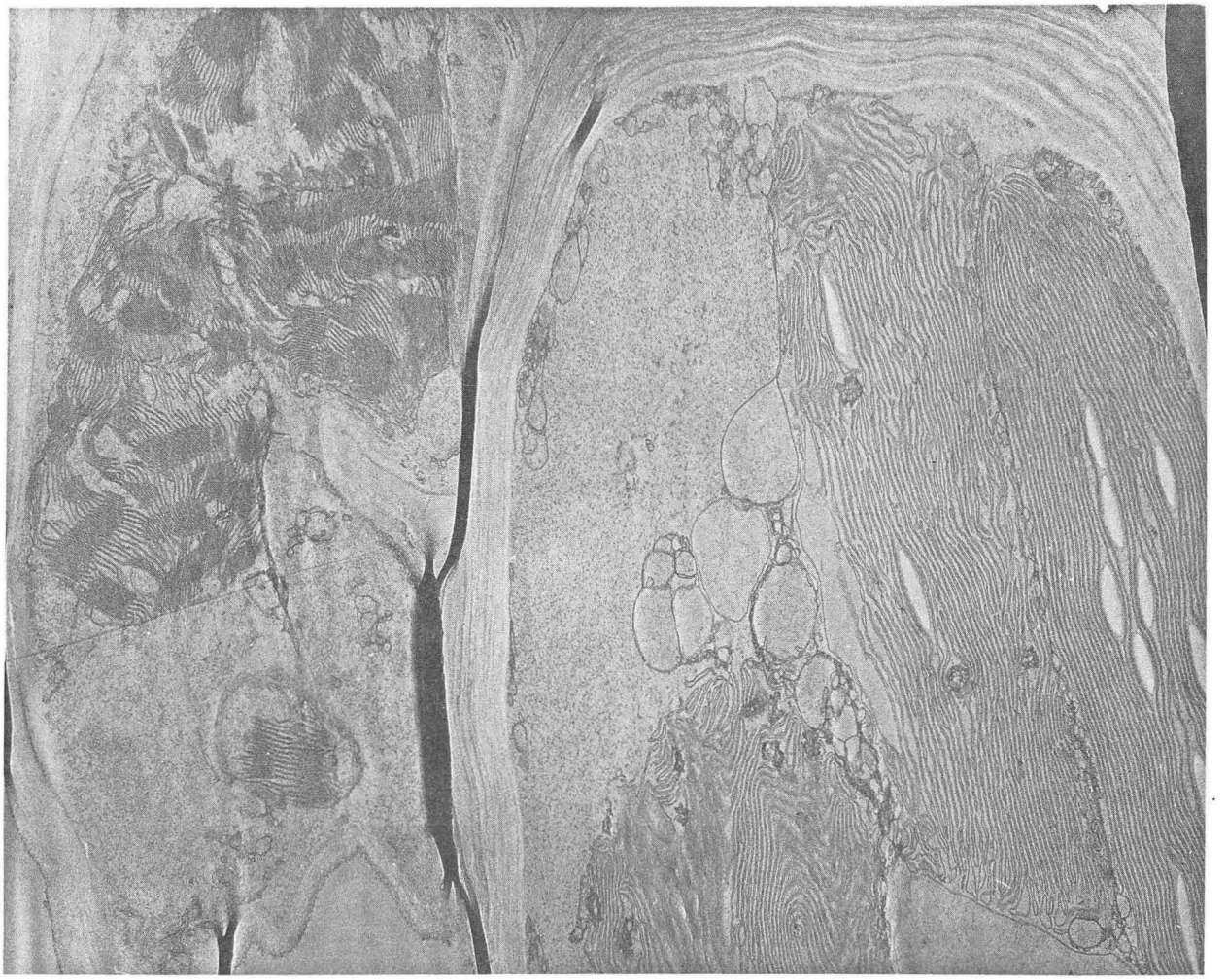

M = MENKE
W = WEIER
LARGE THYLAKOID (M)
PARTITION (W)
MARGIN (W)
LOCULUS (W)
FRET CHANNEL (W)
FRET MEMBRANE (W)
FRET (W)
SMALL THYLAKOID (M)
COMPARTMENT (W)
MUB-6944

Fig. 8

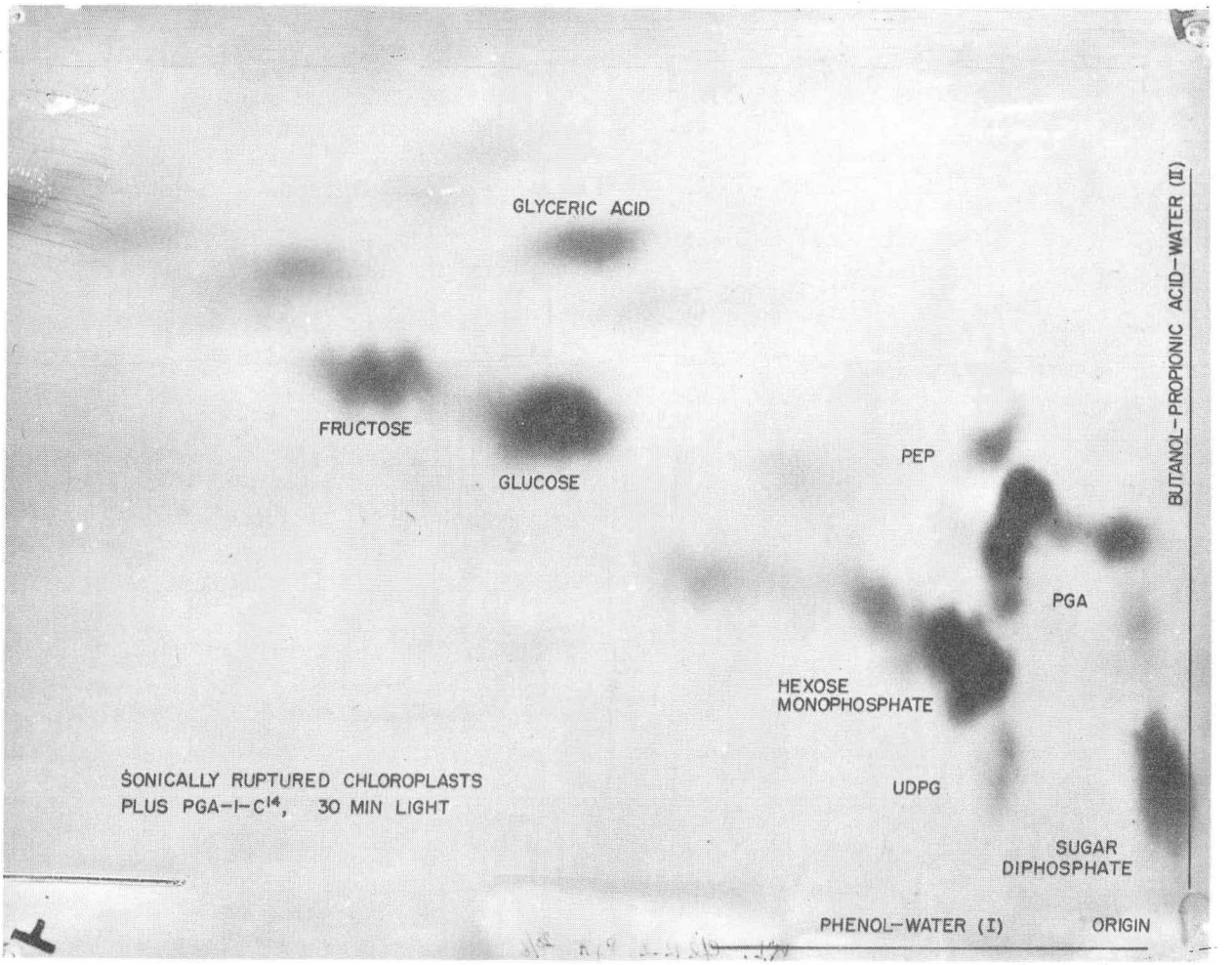


ZN-5533

Fig. 9

ZN-5534

Fig. 10




ZN-2307

Fig. 11

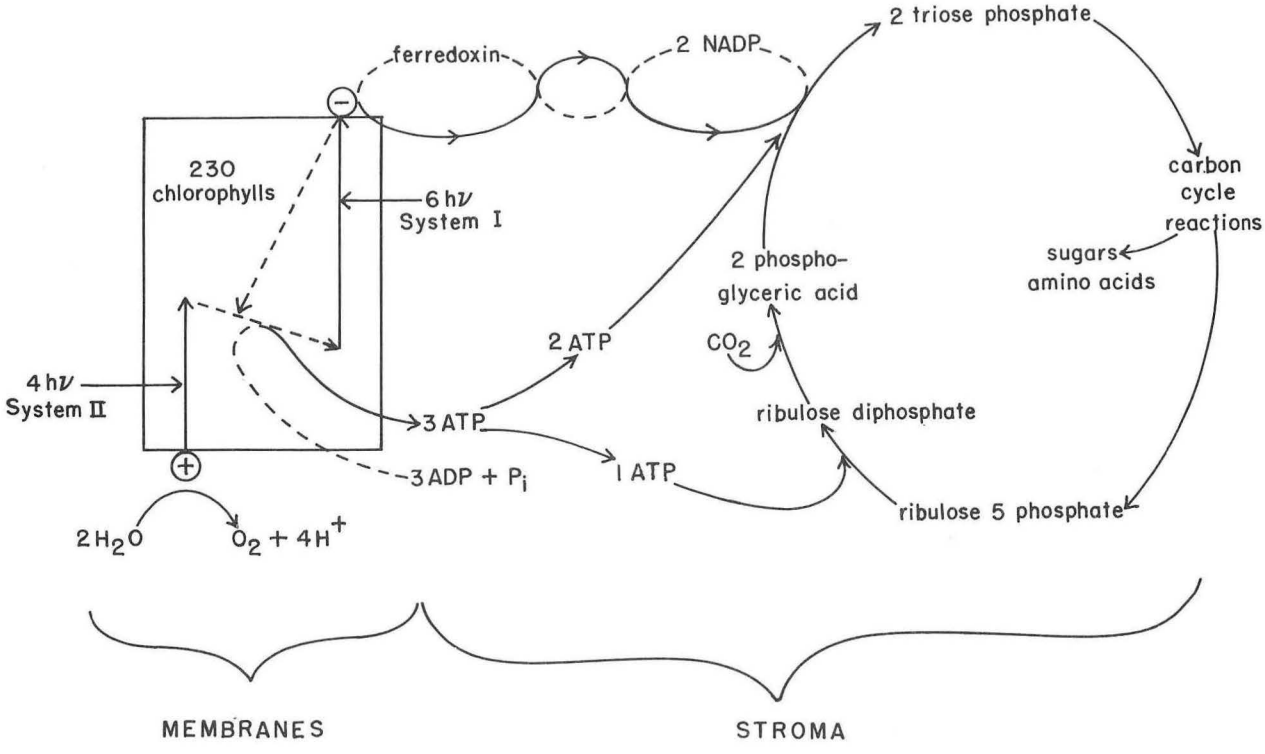
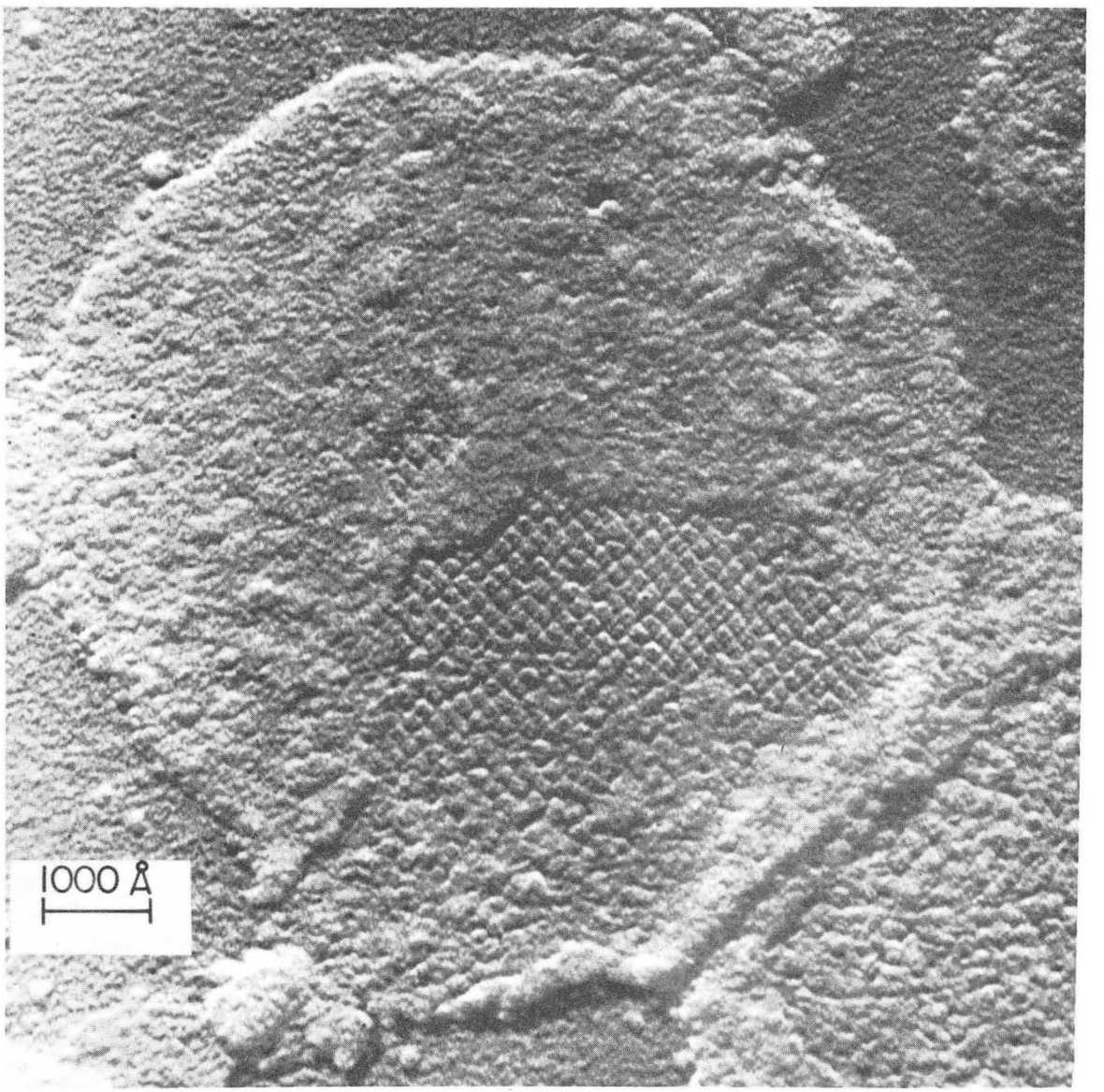
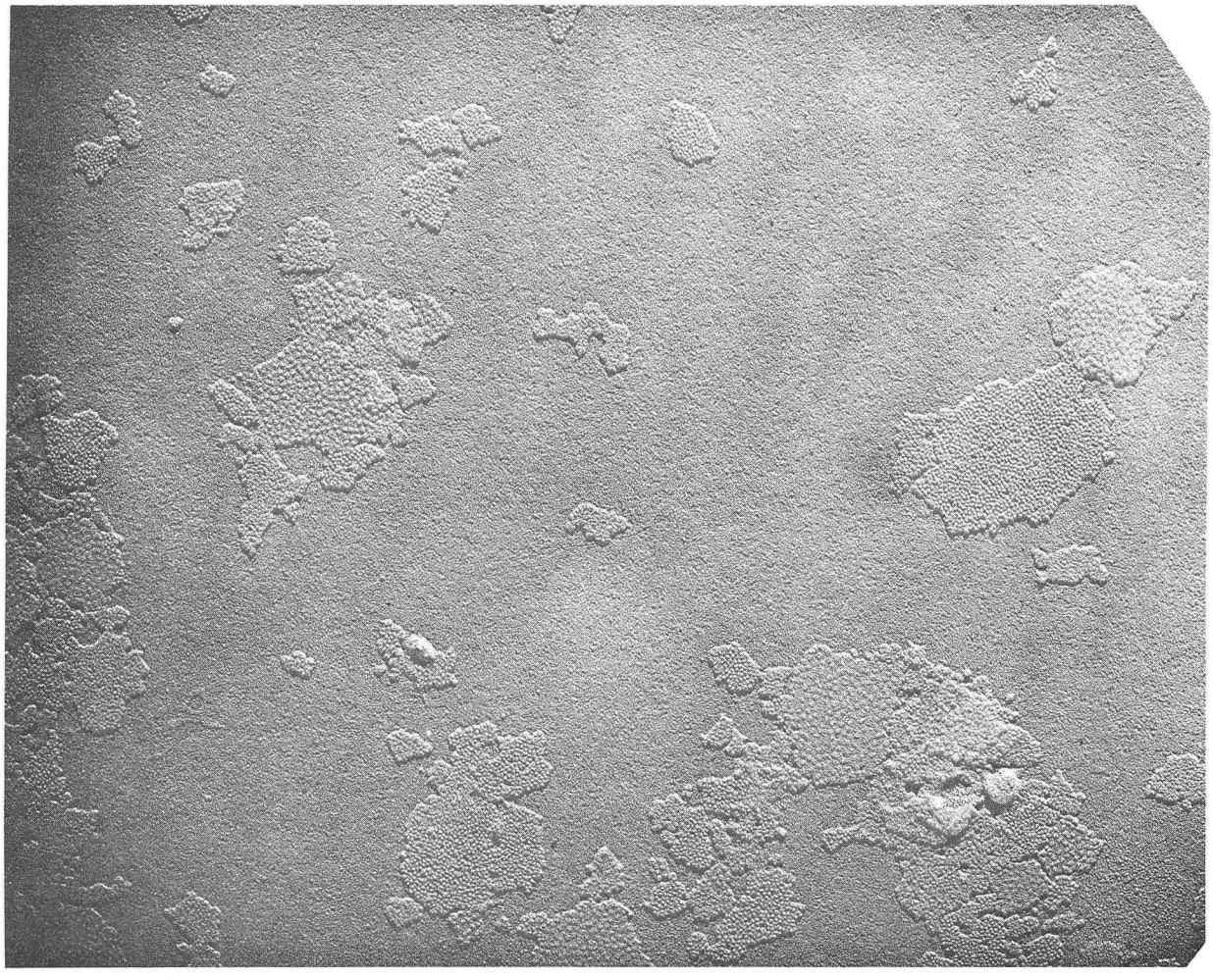

2 triose phosphate
ferredoxin
2 NADP
carbon
cycle
reactions
230
chlorophylls
6 hν
System I
sugars
amino acids
2 phospho-
glyceric acid
2 ATP
CO2
4 hν
System II
3 ATP
ribulose diphosphate
3 ADP + Pi
1 ATP
ribulose 5 phosphate
2H2O
O2 + 4H+
MEMBRANES
STROMA
MUB-6945

Fig. 12



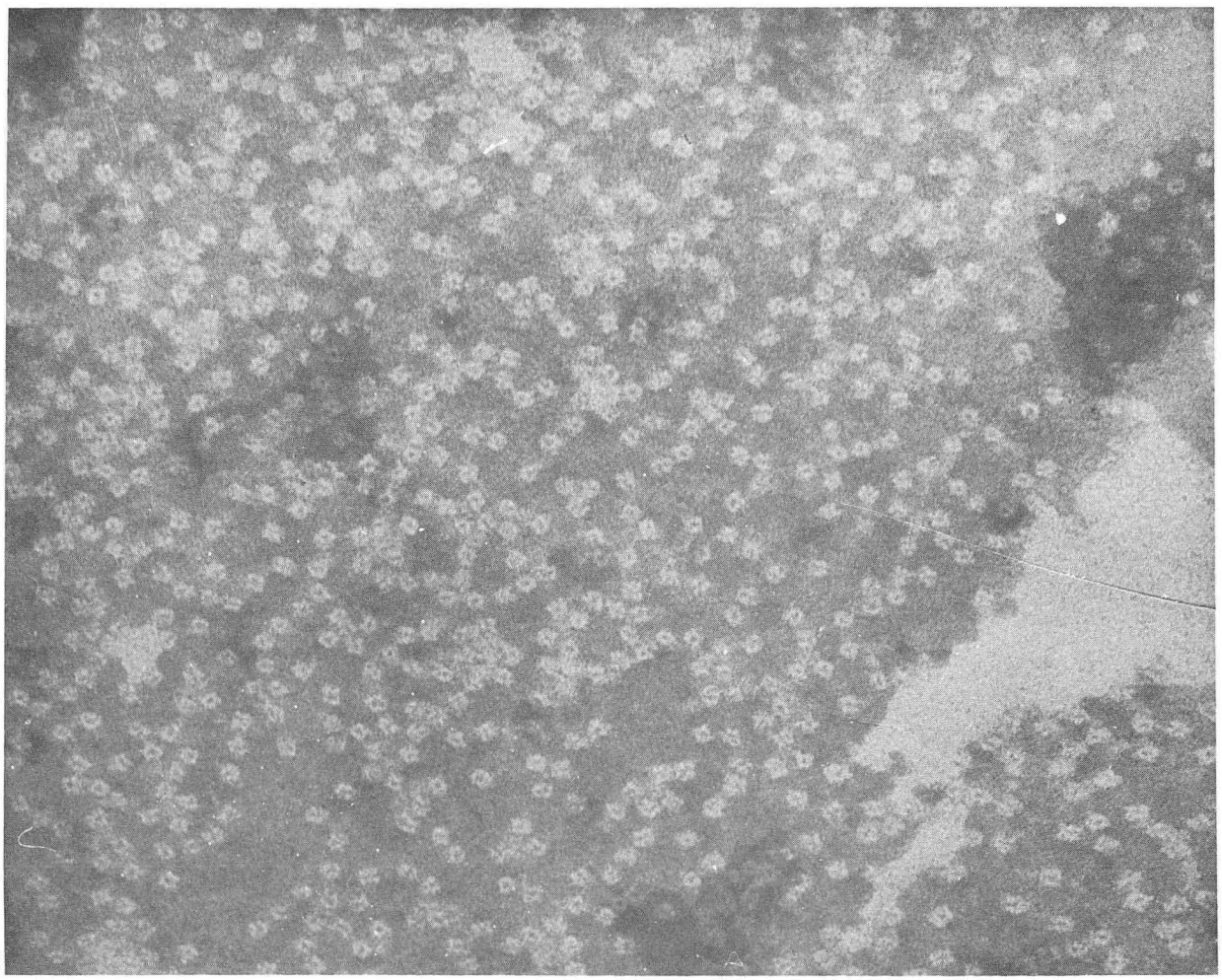

ZN-4304

Fig. 13



ZN-5530

Fig. 14

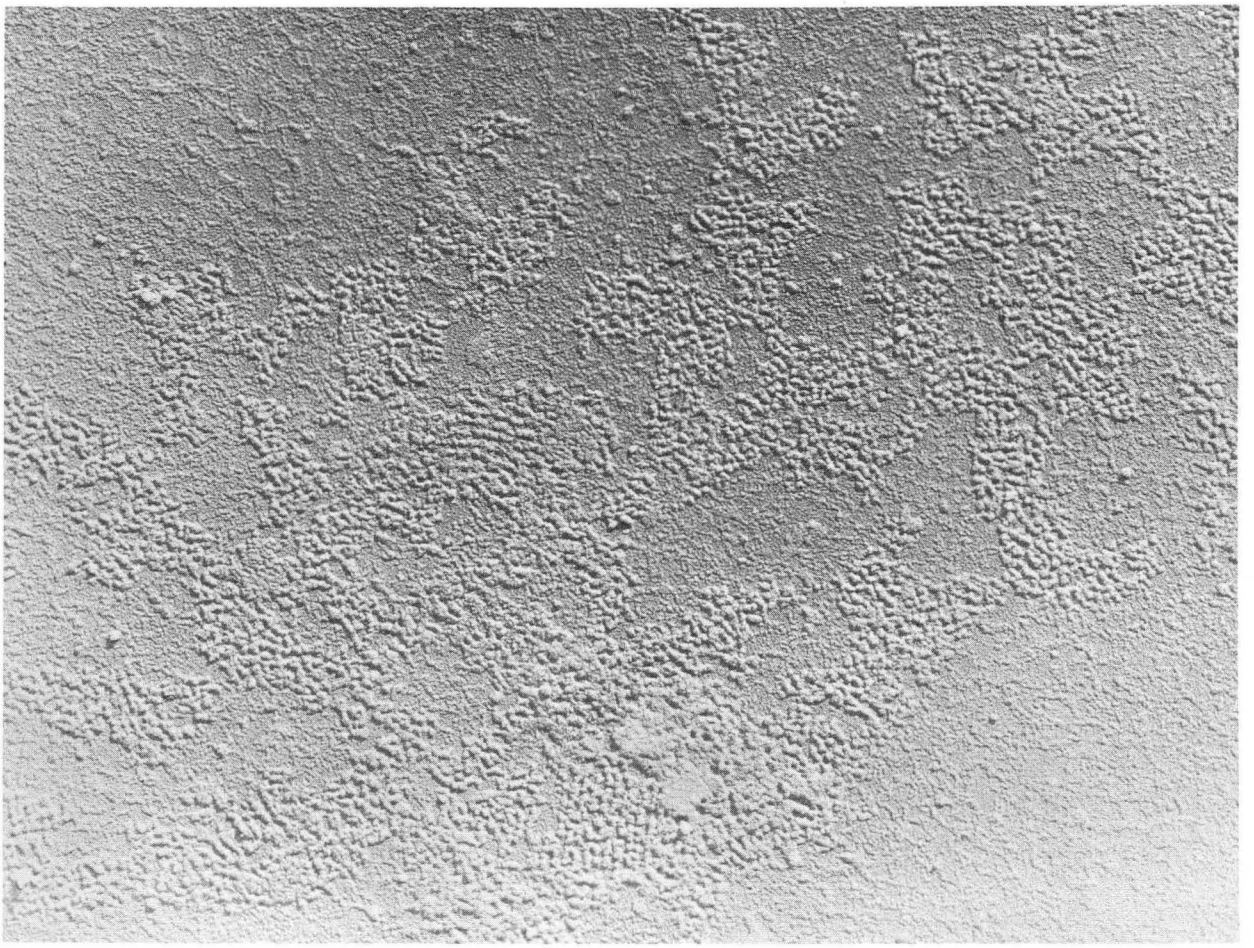


ZN-5529

Fig. 15

ZN-5531

Fig. 16



ZN-5536

Fig. 17