# DIFFERENTIATION OF THE CHLOROPLAST OF *ANTHOCEROS*

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ABSTRACT

It has been demonstrated that the following changes accompany differentiation in the plastid of the liverwort *Anthoceros eckloni*. The inner membrane of the plastid folds to give rise to small vesicles which grow and may fuse to form thylakoids, *i.e.* flat bags. The thylakoids may "pair" to produce the doublets (thick membranes) of the grana. The doublets may be produced also by the invagination of a thylakoid. In both cases, the doublets are produced only where outside-to-outside contact of thylakoid membranes occurs, which supports the thesis that the thylakoid membranes are polarized. The thylakoids also fold outwards, anastomose, and may fuse. This results in a complicated membrane system, for which an interpretation becomes very difficult. The starch is produced in the matrix, and the pyrenoid bodies are interpreted as specialized regions of the matrix. Younger plastids have grana, but the mature plastid has so many doublets that distinct grana cannot be recognized. This interpretation of the changes which occur during the differentiation of the plastid differs radically from those of Menke (1961) and Manton (1962) who studied this same genus, but is compatible with findings in algae and angiosperms.

It is almost generally agreed that the complicated membrane system of chloroplasts consists of a series of double membranes, each double membrane forming a closed "bag." These "bags" have also been called "thylakoids," "discs," or "flat vesicles" by different authors. The term *thylakoid*, coined by Menke, will be used here.

Manton (1962) does not agree that thylakoids are present in the chloroplasts of *Anthoceros*, and she interprets the membrane structure of the chloroplasts as manifestations of a single membrane.

If thylakoids are present, it will be useful, for greater clarity, to refer to the "outside" and the "inside" of the thylakoid membrane. Even if the membrane is not polarized, this designation will help to describe the topography of structures within the chloroplast. The thylakoids occur within the matrix of the chloroplast, so that the "outside" of the membranes is in contact with the matrix. The term *matrix* is used to designate that part of the stroma which is free of lamellae.

It is generally accepted that starch is formed in the matrix (*cf.* Menke, 1962) and not in the thylakoids, and that thylakoids may come into close contact, so forming the "thick membranes" of the grana. These thick membranes, called "doublets" by Manton, are double membranes, in contact outside-to-outside. The possibility that thylakoids may invaginate was demonstrated by Menke (1960) in *Oenothera*, and he suggested that this process, if continued so that the invagination reaches and fuses with the opposite side of the thylakoid, will result in the division of the thylakoid.

The chloroplast of *Anthoceros* has recently been studied by Menke (1961) and Manton (1962). Menke (1962) remarked that the chloroplast of

*Anthoceros* showed many exceptions to the general "Bauprinzip." The exceptions are: (*a*) The doublets are produced by the coming together of the opposite sides of a thylakoid. This means that there is an inside-to-inside association, compared to the reverse which is true for other plants. (*b*) Starch is formed within thylakoids, and not in the matrix. This means that the enzymes which are necessary for starch formation occur *within* the thylakoids and not in the matrix, which is apparently normally the case. (*c*) The parts of the compound pyrenoid are enclosed in membranes which are "Ausstülpungen" of the thylakoids. From this, it follows that the pyrenoid bodies, like the starch grains, occur *within* the thylakoids. Menke (1962) remarked: "In the perhaps polarized thylakoid membrane there seems to have occurred an interchange of inside and outside. One gets the impression that a reversal of morphological relationships has resulted here."

The fact that Manton (1962) discards the concept of thylakoids does not enable one to compare her results with those of Menke (1961), as regards the above-mentioned three points.

Because of the intercalary meristem at the base of the sporogonium of *Anthoceros*, this liverwort lends itself extremely well to studies involving changes which occur as cells mature. If longitudinal sections of the sporogonium are studied, one can observe rows of cells which become progressively older as the distance from the meristem increases.

In the present study of *Anthoceros eckloni*, an attempt has been made to find answers to the following questions:

(*a*) Are closed membrane systems (thylakoids) present in the chloroplasts of *Anthoceros* (Menke, 1961), or not (Manton, 1962)?

(*b*) If thylakoids are present, has a reversal of morphological relationships resulted here? This seemed to be a point of some importance, because many workers believe that thylakoids arise by invagination of the plastid membrane, and a reversal of polarity of the thylakoid membrane

*Abbreviations for Figures*

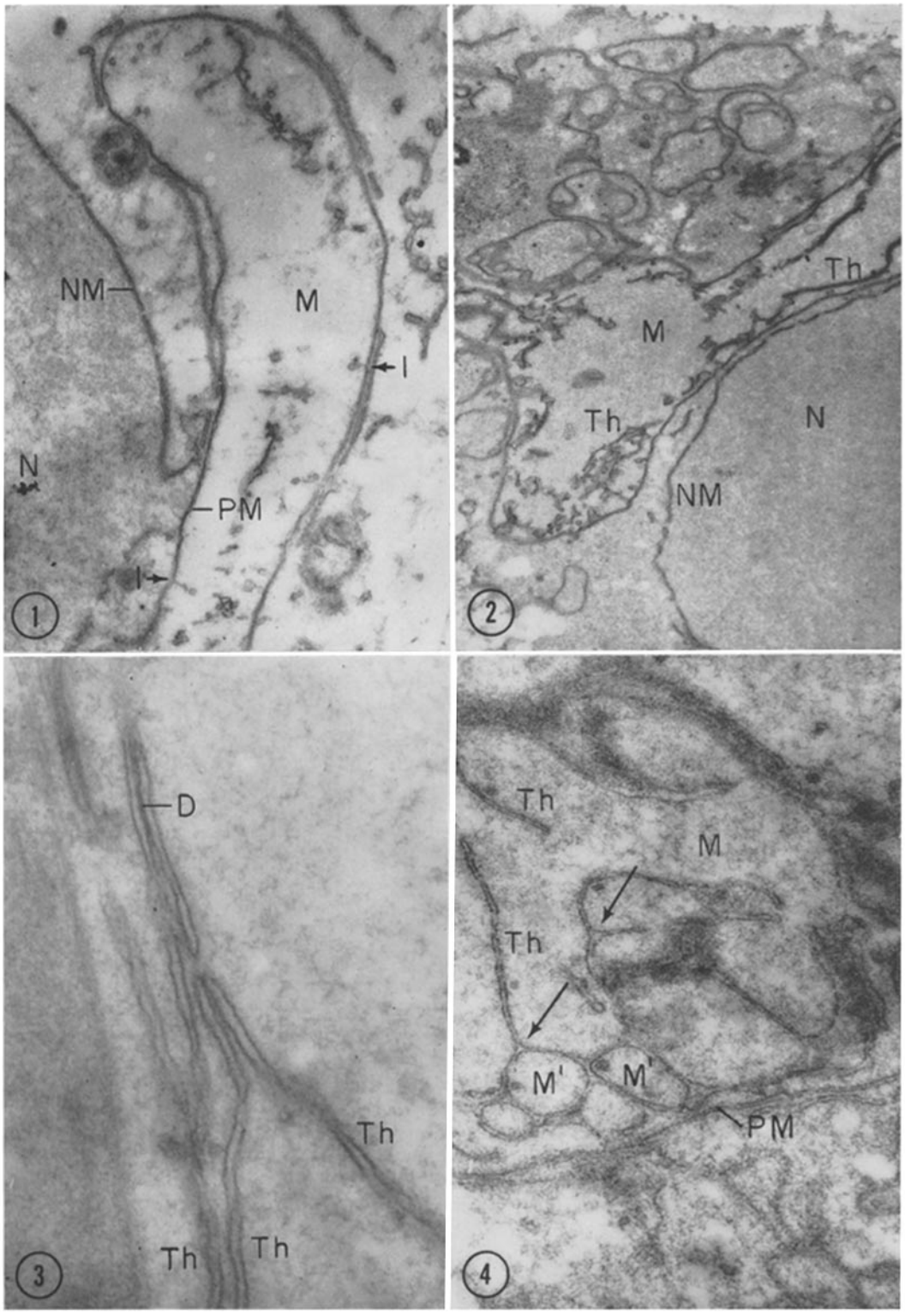
*C*, Cytoplasm
*D*, Doublet
*I*, Invagination of plastid membrane
*M*, Matrix of plastid
*N*, Nucleus
*NM*, Nuclear membrane
*P*, Pyrenoid
*PM*, Plastid membrane
*S*, Starch grain
*Th*, Thylakoid

FIGURE 1 Young plastid next to nucleus in meristematic region. Fixation in 1.5 per cent $KMnO_4$ for 1 hour; epoxy resin embedding. Nucleus appears to the left of the plastid, and a big opening can be seen in the nuclear membrane. Arrows indicate invaginations of inner plastid membrane; small vesicles occur in the matrix (*M*), at places clusters of these vesicles can be seen. × 18,000.

FIGURE 2 Plastid, cytoplasm, and nucleus in epidermal cell in meristematic region. Invaginations of inner plastid membrane can be seen; some thylakoids have an irregular shape; nuclear membrane with numerous pores. Flattened thylakoids (*Th*) are arranged parallel to the plastid membrane. Fixation in 1.5 per cent $KMnO_4$ for 1 hour; embedded in methacrylate. × 16,000.

FIGURE 3 Plastid in cell about 10 cells above the meristematic region, and, therefore, slightly "older" than the plastids illustrated in Figs. 1 and 2. Four different thylakoids can be seen, one cut obliquely. Where thylakoids come in contact, doublets (*D*) are formed. Fixation in 1.5 per cent $KMnO_4$ for 1 hour; Epoxy resin embedding. × 60,000.

FIGURE 4 Plastid in epidermal cell, 28 cells from the base of the meristem, and, therefore, more differentiated than the plastid illustrated in Fig. 3. Note the outfolding and anastomosing of the thylakoids indicated by arrows. Areas labeled $M^1$ are vesicle-like, enclosed regions of the matrix; appearance of these areas is identical to that of the matrix (*M*). Fixation in 1.5 per cent $KMnO_4$ for 1 hour. Embedded in methacrylate. × 60,000.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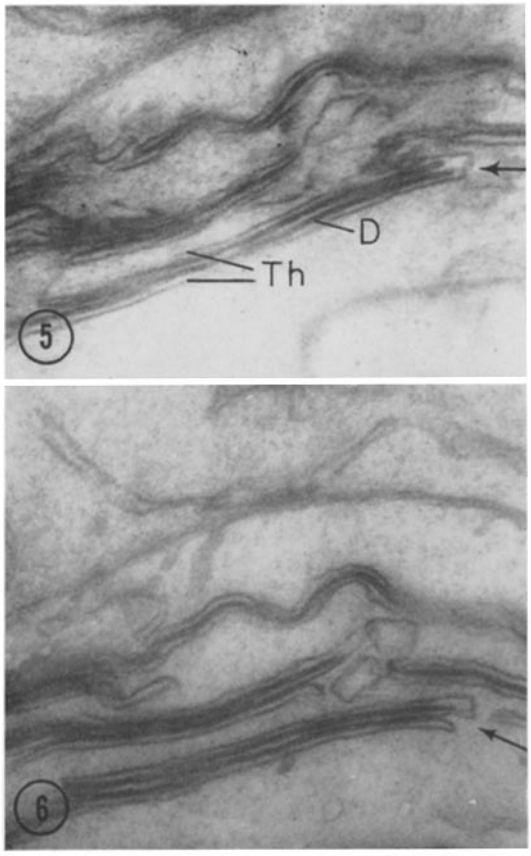
may, therefore, also involve a change in polarity of the plastid membrane.

(*c*) If thylakoids are present, and if they were misinterpreted by Menke (1961, 1962), his description of the developmental stages will have to be revised. "Ausstülpungen" of thylakoids may then prove to be invaginations, etc.

(*d*) Are doublets formed by an inside-to-inside contact of thylakoid membranes (Menke, 1961, 1962), or by outside-to-outside association, which is normally the case?

(*e*) Are grana present in the chloroplasts of *Anthoceros* (Menke, 1961), or are there no grana (Kaja, 1957)?




FIGURES 5 AND 6 Plastid in epidermal cell in consecutive sections. In Fig. 5, arrow points to a thylakoid in which two invaginations have almost reached the end of the thylakoid. In Fig. 6, the arrow points to the same region, which now appears different. The suggested interpretation is that the different appearance in Fig. 6 is due to a fusion of the lower invagination with the end of the thylakoid, the other invagination shows perhaps incomplete fusion.

Fixation in 1.5 $KMnO_4$ for 1 hour. Embedded in epoxy resin. × 60,000.

## MATERIAL AND METHOD

*Anthoceros eckloni* plants were collected in their natural habitat and fixed as soon as possible. $KMnO_4$ fixation at room temperature (between 1.5 per cent and 3 per cent for 30 to 60 minutes) was followed by embedding in methacrylate or epoxy resin.

Methacrylate embedding, even after very careful prepolymerization by either heat or ultraviolet irradiation, often resulted in bad polymerization damage. When a short fixation (5 minutes) with 1.5 per cent $KMnO_4$ at room temperature was followed by postfixation in osmium tetroxide, the tissue was less susceptible to damage. The epoxy resin embedding gave practically no polymerization damage but the blocks were difficult to cut and chatter occurred very commonly.

The microscope[1] used was a Siemens Elmiskop normally operated at 80 kv.

## INTERPRETATION OF MICROGRAPHS

In *Anthoceros eckloni* there is only one plastid per cell in the mature sporophyte. Plastids arise only by division of pre-existing plastids. This division of the plastid may take place at a stage when there is already a complicated system of membranes within the plastid. The nucleus is always close to the plastid.

Figs. 12 *a* to *f* summarize the developmental changes in the plastid of *Anthoceros eckloni* as interpreted by the present author.

Fig. 12 *a* represents the most undifferentiated plastids that have been found in rapidly growing sporophytes. The inner membrane of the plastid invaginates, and it is suggested that these projections become detached from the plastid membrane to form small vesicles (thylakoids).[2] Fig. 1 is a micrograph of such a plastid. Invaginations can be seen, marked by arrows. Small thylakoids occur relatively sparsely in the matrix of the

[1] The writer's thanks are due to Mr. J. W. Matthews and Mr. D. L. Allinson, who operated the instrument, for their assistance.

[2] A similar process of invagination of the inner plastid membrane has been reported by Mühlethaler and Frey-Wyssling (1959), Menke (1962), and other authors.

plastid. At places these small thylakoids occur in groups, giving the impression that they may fuse, so producing larger thylakoids. In this micrograph the nucleus can be seen to the left, and the plastid at this stage is in intimate contact with the nucleus, at places only the plastid membrane separating the two structures. This has been observed in several cases, and one wonders whether there is any physiological importance to this phenomenon.

Fig. 2 represents a plastid only slightly older than the one illustrated in Fig. 1. Invaginations of the inner plastid membrane can still be seen, but there are more thylakoids, and the flattened thylakoids become arranged parallel to the surface of the plastid. In less actively growing sporophytes this is indeed the most undifferentiated plastid that can be found.

The following processes now soon follow (illustrated in Fig. 12 *b*). Thylakoids come into close contact, forming doublets where outside-to-outside contact takes place. This is clearly illustrated in Fig. 3, where the doublets (*D*) are labeled. The thylakoids (*Th*) are now flat bags, and the smaller vesicles are practically absent. The thylakoids now also fold outwards, as illustrated to the left in Fig. 12 *b* and Fig. 4. The latter micrograph proved to be the key to the understanding of the membrane system of the chloroplast of *Anthoceros*. It is suggested that the outfoldings of the thylakoids anastomose with other thylakoids or with outfoldings of the same thylakoid. As a result, the anastomosing thylakoid system partly encloses matrix regions, which in section now may appear like vesicles. The areas labeled $M^1$ in Fig. 4 appear to be vesicles with limiting single membranes. Groups of such "vesicles" can be seen in Fig. 9. The areas labeled $M^1$ in Fig. 4 appear to have the same structure as the matrix (a finely granular background with isolated, larger, darker granules). There can be no doubt that these areas are partly or completely included by an anastomosing thylakoid, and represent part of the matrix.

Where two thylakoids have paired, a thin-thick-thin distribution of membranes can be seen, the thick membrane representing a doublet (see Fig. 7). The arrow in this micrograph points to a place of contact between the membranes of the two thylakoids. If fusion takes place here, and if this process is repeated involving the same membranes, a further vesicle-like matrix area will be partly enclosed by a thylakoid membrane. There is no direct evidence that this process does take place, but it is a further complication which should not be ignored. These processes could lead to a stage as illustrated in Fig. 12 *c*.

The doublets produced by pairing of thylakoids cannot terminate within a thylakoid, nor will *such* a doublet end as illustrated in Figs. 5 and 10 (indicated by arrows). Such endings always occur *within* thylakoids. This seems to be sufficient evidence to explain such doublet formation as infolding (or invagination) of the thylakoid membrane. This process is illustrated in Fig. 12 *d*. Whether the doublet is produced by pairing of thylakoids or by invagination, it always forms as a result of *outside-to-outside* contact of thylakoid membranes.

Fig. 10 is a micrograph of a chloroplast which is characterized by stacking of doublets to form grana or granum-like areas. The thylakoids are by now so extensive and anastomosed that it is impossible to state which process (pairing of thylakoids or invagination) is the main doublet-forming one.

Some changes that may take place as a result of growth are practically impossible to establish by studying micrographs. It is almost impossible to determine whether an invagination can reach the other end of the thylakoid and fuse with it and, in so doing, divide it into two, but this process is suggested in the interpretation of the following micrographs.

Figs. 5 and 6 are of serial sections. When the two micrographs are compared, one can see the ends of two invaginations in a thylakoid in Fig. 5, and what appears to be three different thylakoids in Fig. 6. This illustrates the extreme care that should be taken in the interpretation of these micrographs. A possible explanation is that the invaginations have grown from the left and that the lower invagination has reached, and fused with, the opposite side of the thylakoid, in the region from which Fig. 6 was prepared, but not as yet in the region above, which is represented in Fig. 5. The other invagination is interpreted as being in the process of fusing. This is, perhaps, the closest one can get with these techniques to illustrate that invaginations can fuse with the opposite side of a thylakoid, to give rise eventually to an increase in the number of thylakoids.

The over-all thickness of the doublets is 145 A, more or less. In older plastids (Fig. 8) the doublet appears to have a dark line in the middle separ-

ating the two lighter regions from each other, each about 50 A in thickness.

In Figs. 12 *d* to *f* the origin of the pyrenoid and position of the starch grains are illustrated, while invagination of thylakoids continues, and one thylakoid has become divided by an invagination. The matrix is stippled; areas of the matrix become specialized (indicated by denser stippling), and the starch grains appear in the matrix, in contact with a pyrenoid body. In Fig. 11 the contact between a starch grain and pyrenoid body is indicated by an arrow. It seems unlikey that starch is formed only in association with pyrenoid bodies, because starch is formed at an earlier stage before pyrenoid bodies are present (see Fig. 9). From this micrograph it is also clear that the starch is produced in the matrix.

In Fig. 12 *f* it can be seen that the pyrenoid body and starch grains *appear* as though they occur within a thylakoid.

The fully mature plastid is not illustrated, but it is characterized by numerous starch grains, pyrenoid bodies, the almost complete absence of the vesicle-like matrix regions, and an increase in the extent of doublets, with the result that to recognize different grana becomes impossible. What has become of the vesicle-like matrix regions? It is suggested that owing to starch formation and an increase in the number of lamellae, they have become flattened and, in doing so, have formed doublets (see area $M^1$, Fig. 12 *f*).

## DISCUSSION

Recent reviews have appeared on chloroplast structure and development (Menke, 1962, and Wolken, 1959), and, therefore, reference will be made only to publications that have a direct bearing on the present work.

There are indications that, in higher plants, plastids may arise *de novo* (Badenhuizen, 1962 *b*, and Mühlethaler and Frey-Wyssling, 1959). In *Anthoceros*, this does not occur; the plastids arise only by fission of pre-existing plastids in the sporogonium.

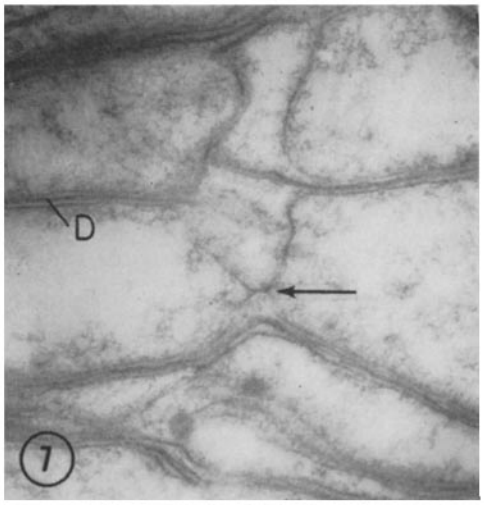
The present work indicates the presence of closed membrane systems (thylakoids) within the chloroplast of *Anthoceros*, and Manton's (1962) claim that the concept of thylakoids is incompatible with findings in this plant cannot be supported. Manton (1962) refers to a *single* membrane which folds to form clefts and doublets, but, as she discards the concept of thylakoids, it is impossible to distinguish the matrix from areas within thylakoids, or to refer to the "inside" or "outside" of the membrane.

If the matrix areas ($M^1$) are misinterpreted as thylakoids (and this can easily happen since they *appear* to be included by a single membrane), then it would appear as if "a reversal of morphological relationships has resulted here" (Menke, 1962). It is suggested that Menke (1961, 1962) misinterpreted these structures as thylakoids, which can very easily happen if early stages were not available. Menke (1961) in his Fig. 4, shows a thylakoid with a number of outgrowths marked by arrows. It is reproduced here, in Fig. 12 *g*, to enable comparisons to be made between his interpretation and the one given here. If it is borne in mind that thylakoids may invaginate (as demonstrated by Menke (1960) in *Oenothera*,

FIGURE 7 Plastid in cell above meristem. Typical thin-thick-thin distribution of membranes can be seen, due to pairing of thylakoids or formation of invaginations. The arrow indicates very close contact between two outfoldings of opposite thylakoids. Fixation in 1 per cent $KMnO_4$ for 5 minutes, followed by post-fixation in $OsO_4$ for 1 hour. Embedded in methacrylate. × 60,000.

FIGURE 8 Granum-like region of almost mature plastid. The doublets have a dark part in the middle and two less dense regions at the sides. Doublet is 145 A wide more or less, and the less dense part is 50 A, more or less. Fixation in 1.5 per cent $KMnO_4$ for 1 hour. Epoxy resin embedding. × 140,000.

FIGURE 9 Plastid in epidermal cell, 48 cells from the meristematic region. The plastid is characterized by stacking of membranes to form granum-like regions, but many vesicle-like, enclosed regions ($M^1$) can be seen. The plastid membrane still exhibits invagination, and starch grains ($S$) occur in the matrix ($M$). Fixation in 1.5 per cent $KMnO_4$ for 1 hour. Embedded in methacrylate. × 24,000.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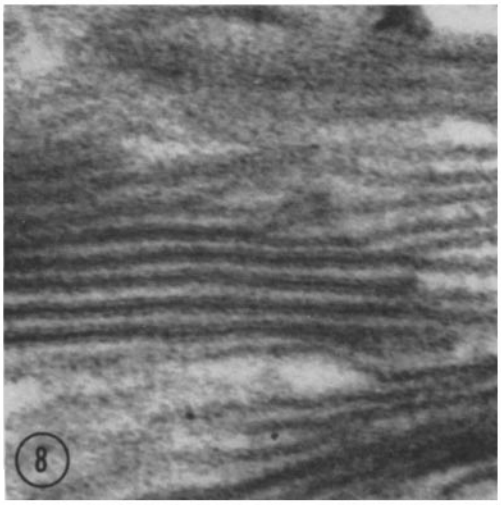
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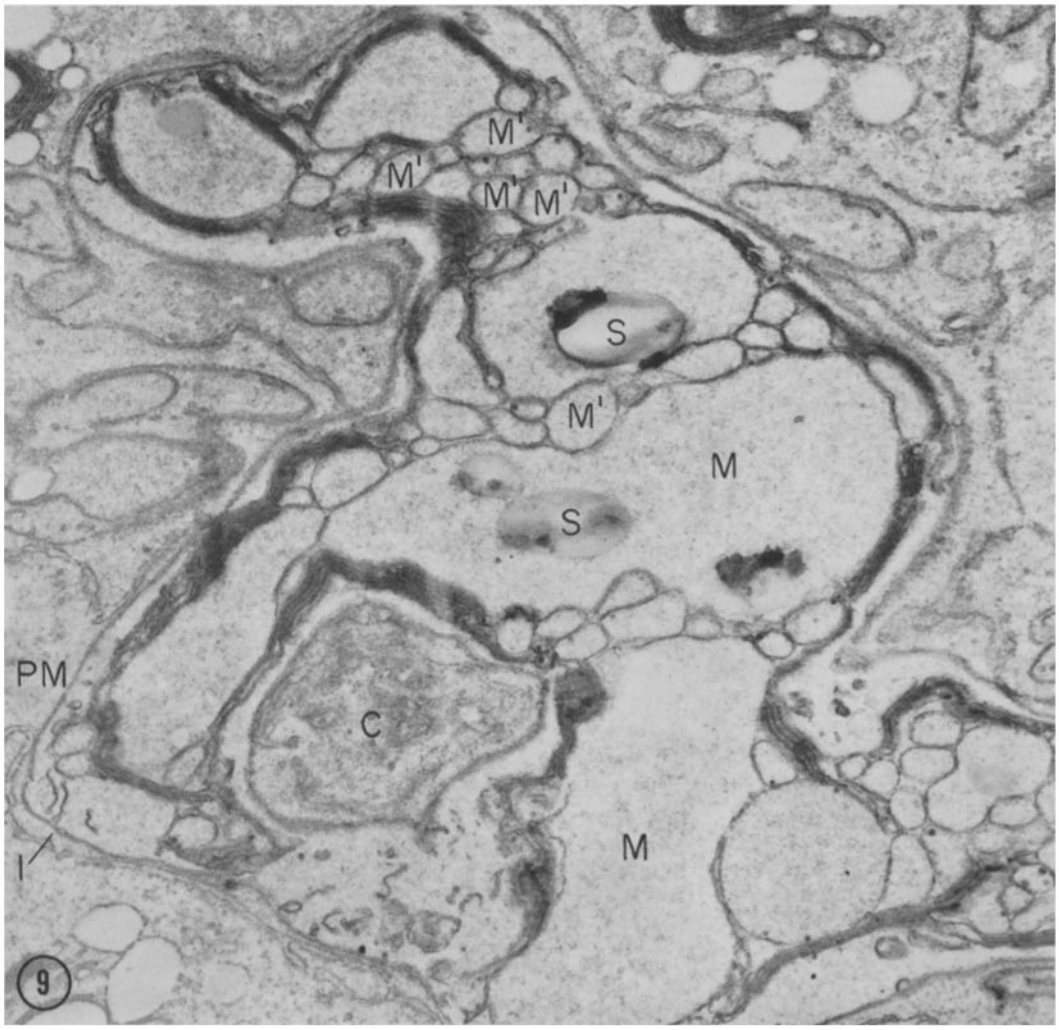
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and in *Anthoceros* in the present study), the structure here should be interpreted as follows: Area *A*, called a thylakoid by Menke, is a partly enclosed area between thylakoids. The thylakoids form doublets by pairing and invagination. The arrows which refer to outfoldings of the thylakoid, according to Menke, indicate invagination of thylakoids at some places and pairing of thylakoids at other places.

If doublets are produced by invagination and by pairing of thylakoids, they are formed only where an outside-to-outside contact of thylakoid membranes takes place, and not by an inside-to-inside association as claimed by Menke (1961). This brings the results of *Anthoceros* in agreement with those of other plants, and this feature points to the probability that the thylakoid membranes are polarized, and that this polarity determines how doublets are produced.

Starch occurs in the thylakoids, according to Menke (1961). If Menke has made a misinterpretation of thylakoids, it would follow that the site of starch grains will also be misinterpreted. From the present study it would appear that starch is produced in the matrix at a time when the latter is clearly recognizable. That the structures, which are called starch grains by Menke (1961), are not produced *within* thylakoids can be seen even in his own micrographs. In his Fig. 2 the structure labeled starch grain, at the bottom of the micrograph, is next to the plastid membrane (without an intervening membrane) and is thus in the matrix, and not in a thylakoid as claimed by Menke. But these bodies are dark and granular,

FIGURE 10 Chloroplast showing stacks of doublets in epidermal cell, 49 cells away from meristem. Many doublets have been formed by the process of invagination; a number of such doublets can be seen. Vesicle-like, enclosed matrix regions are present ($M^1$). Arrows point to tips of doublets formed by invagination of thylakoids.

Fixation in 1.5 per cent $KMnO_4$ for 1 hour. Methacrylate embedding. × 60,000.

FIGURE 11 Plastid with starch grains (*S*) and pyrenoid bodies (*P*). Polymerization damage resulted in swelling of thylakoids, and the vesicle-like, included matrix regions ($M^1$) are more pronounced and rounded. Arrow points to starch grain in contact with pyrenoid body. × 16,000.

FIGURE 12 *a* to *f* Diagrams to illustrate suggested interpretation of the developmental changes which occur in the plastids of *Anthoceros*.

FIGURE 12 *a* Inner plastid membrane invaginates (*I*) and produces vesicles (*Th*). (Compare micrographs 1 and 2).

FIGURE 12 *b* Pairing of thylakoids takes place to form doublets (*D*), *cf.* Fig. 3; thylakoids fold outwards (*E*), *cf.* Fig. 4, and at places the outfoldings come into contact with similar outfoldings of other thylakoids (*cf.* Fig. 7).

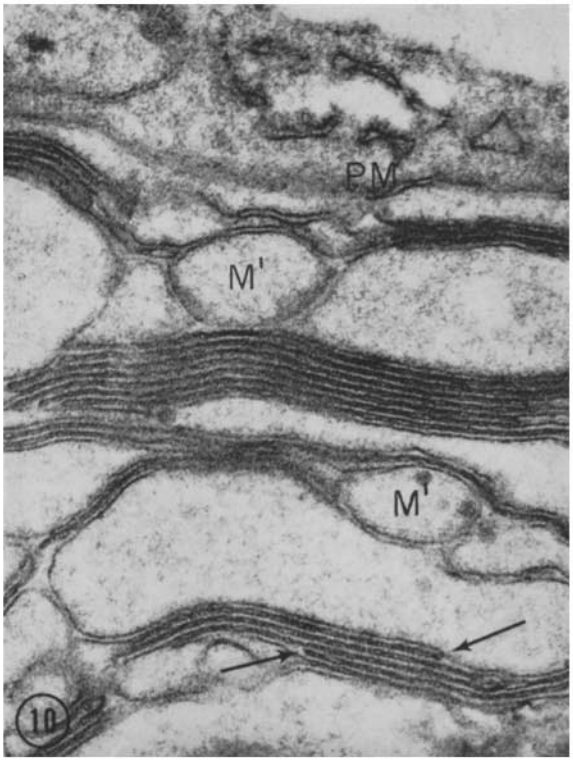
FIGURE 12 *c* Outfoldings of thylakoids have anastomosed (with others of the same or different thylakoids) to partly (or perhaps completely) enclose matrix areas ($M^1$). Such included matrix areas can be seen in Figs. 9 to 11.

FIGURE 12 *d* Invagination of plastid membrane continues for some time (*cf.* Fig. 9). Thylakoids invaginate (*I*) (tips of invaginations seen in Figs. 5 and 10). Regions of matrix become more specialized or more condensed, indicated by denser stippling. Doublets have been produced by pairing and by invagination (*Dp* and *Di*, respectively).

FIGURE 12 *e* Invaginations continue to grow deeper into thylakoids (*cf.* Fig. 10), and starch is formed in the matrix, indicated here next to pyrenoid body (*P*), as seen in Fig. 11.

FIGURE 12 *f* Invagination in thylakoid at the top has almost reached the opposite side of thylakoid; the thylakoid next to this one has been divided into two (*a*, *b*) by fusion of the invagination with the opposite side of the thylakoid (*cf.* Fig. 5 and 6). The vesicle-like matrix regions become flattened ($M^1$).

FIGURE 12 *g* Diagram prepared from a micrograph published by Menke (1961). According to him, area *A* is a *thylakoid*, and the arrows point to outfoldings. According to the present interpretations, area *A* is part of the *matrix*, surrounded by thylakoids with *invaginations* (*i*) and pairing (*p*) indicated by arrows.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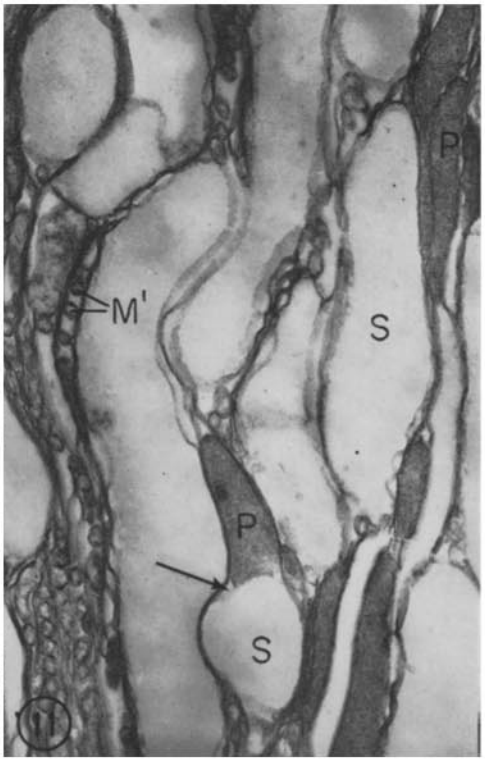
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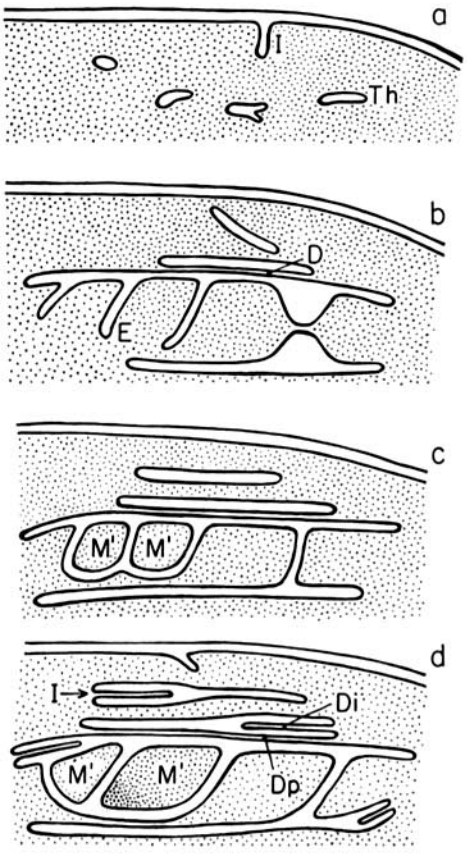
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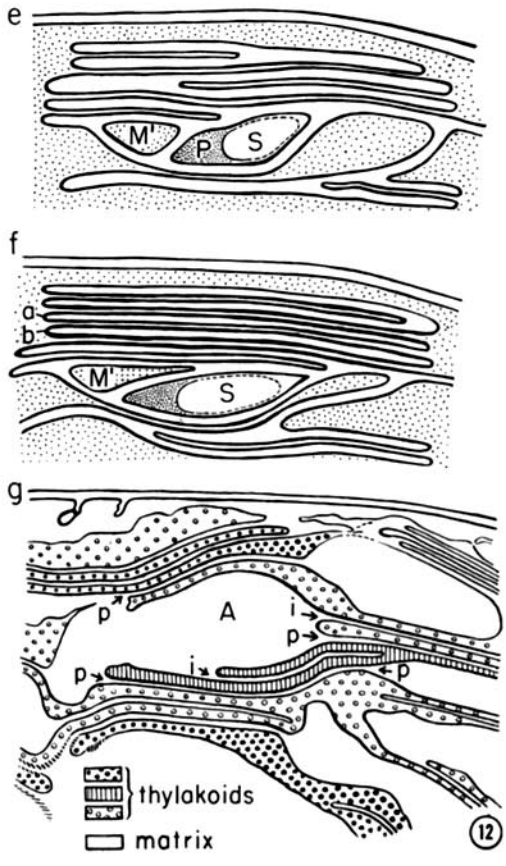
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and look like the pyrenoid bodies reported here, as well as by him, and not like starch (see, *e.g.*, Badenhuizen (1962 *a*) and Manton (1962)).

It can be seen from the present study that starch is formed in the matrix, that it may be present before pyrenoid bodies are present, and that when pyrenoid bodies are present they may show close contact with starch grains. The writer, therefore, considers that the pyrenoid bodies are specialized (or condensed) parts of the matrix which, among others, contain the enzymes that are necessary for starch formation, and that these enzymes are present in the matrix before pyrenoids are formed. This substantiates the idea put forward by Gibbs (1960) that the pyrenoids should be considered as specialized regions of the matrix. It is possible, of course, that thylakoids may cross or enter the pyrenoids. Gibbs (1960) illustrated that this interpretation can be put forward for the pyrenoids of all algae investigated.

Menke (1961) reported that grana were present in *Anthoceros*, whereas Kaja (1959) reported the absence of grana. This does not indicate specific or environmental differences. Grana are present in plastids which are not fully differentiated, but extension of the doublets makes it impossible to distinguish distinct grana in mature plastids.

The structure of the plastids of algae and higher plants can be interpreted adequately when one accepts the interpretation given above, and realizes that the differences in appearance are due to the frequency of invagination, division of thylakoids by invagination, the extent and localization of pairing of thylakoids, and the variation in lateral expansion of the thylakoids in the regions between grana. If an interchange of substances takes place between the thylakoids and the matrix, one can recognize the advantages connected with a localization of the areas where the light reaction of photosynthesis takes place (the grana), and with the extension of some thylakoids into the matrix where the dark reactions most probably take place. It does not seem necessary or advisable to use different terms ("frets," "compartments," "partitions," and "loculi," as used by Weier and Thomson, 1962) to explain the structure of the chloroplasts of higher plants. We have reached the stage at which we can recognize a common structural principle as suggested by Menke (1962), and the one exception according to Menke (1961, 1962) now appears to be a manifestation of the common "Bauprinzip."

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## LITERATURE CITED


1. Badenhuizen, N. P., 1962 *a*, The development of the starch granule in relation to the structure of the plastid, *Proc. Koninkl. Akad.*, **65,** 123.
2. Badenhuizen, N. P., 1962 *b*, Observations on the origin and multiplication of plastids, *Canad. J. Bot.*, **40,** 861.
3. Gibbs, S. P., 1960, The fine structure of *Euglena gracilis* with special reference to the chloroplasts and pyrenoids, *J. Ultrastruct. Research*, **4,** 127.
4. Kaja, H., 1957, Electronenmikroskopische Untersuchungen an den Chromatophoren von *Anthoceros laevis* L., *Ber. Deutsch. Bot. Ges.*, **70,** 343.
5. Manton, I., 1962, Observations on plastid development in the meristem of *Anthoceros*, *J. Exp. Bot.*, **13,** 325.
6. Menke, W., 1960, Weitere Untersuchungen zur Entwickelung der Plastiden von *Oenothera hookeri*. 3. Mitteilung zur Entwickelungsgeschichte der Plastiden, *Z. Naturforsch.*, **15 b,** 479.
7. Menke, W., 1961, Über die Chloroplasten von *Anthoceros punctatus*, *Z. Naturforsch.*, **16 b,** 334.
8. Menke, W., 1962, Structure and Chemistry of plastids, *Ann. Rev. Plant Physiol.*, **13,** 27.
9. Mühlethaler, K., and Frey-Wyssling, A., 1959, Entwickelung und Struktur der Proplastiden, *J. Biophysic. and Biochem. Cytol.*, **6,** 507.
10. Weier, T. E., and Thomson, W. W., 1962, The grana of starch-free chloroplasts of *Nicotiana rustica*, *J. Cell Biol.*, **13,** 89.
11. Wolken, J. J., 1959, The structure of the chloroplast, *Ann. Rev. Plant Physiol.*, **10,** 71.