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The Effect of Manganese on Chloroplast Structure and Photosynthetic Ability of Chlamydomonas reinhardi[1]

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Abstract. Manganese deficiency was induced in mixotrophically-grown cultures of the green alga *Chlamydomonas reinhardi* and the effects of this deficiency on photosynthetic activity and cell structure were investigated. Manganese-deficient cells are unable to carry out, at normal rates, photosynthetic reactions involving system II of the photosynthetic electron transport chain. Reactions requiring only system I are not inhibited. Normal system II activity returns within 2 hr in the light following addition of manganous ions to deficient cells.

Significant structural alterations for deficient cells were observed in the chloroplast, in the arrangement of discs into stacks. Stacking distributions for normal, deficient, and recovered cells were quantitatively characterized and compared by the introduction of an experimentally-defined distribution function and its attendant parameters. The results provide evidence for a role for manganese in maintaining chloroplast structure.

Green plants exhibit a higher manganese requirement for phototrophic growth than for heterotrophic growth (4, 16). Measurements of various parameters of photosynthesis indicate a manganese involvement in the oxygen-evolving system of photosynthesis, system II (6, 9, 15, 17), but it is presently impossible to further specify the reaction site and the manner in which manganese exerts its effect. However, Possingham and co-workers (18) found that manganese deficiency in spinach leaves was accompanied by a structural breakdown confined to the chloroplasts. They briefly considered the possibility that the progressive disorganization of lamellar structure could be related to the reduction in photosynthetic ability.

The possible structural involvement of manganese in photosynthesis suggested the value of an investigation into the effect of manganese on chloroplast structure in a system in which photosynthetic function could be readily determined. *Chlamydomonas reinhardi*, a unicellular green alga, is well suited for such a study: Manganese deficiency can be induced at levels sufficient to inhibit photosynthesis but not severe enough to interfere with growth on an externally-supplied carbon source (22). Photosynthetic capability can be surveyed without difficulty. Manganese ions can be easily added to bring about recovery of normal photosynthetic activity in manganese-deficient cells.

In this paper we present the results of a study of manganese deficiency and chloroplast structure in *C. reinhardi*. Employing electron microscopy we have examined chloroplast structure in cells under conditions of manganese starvation and immediately after the restoration of photosynthetic ability following the addition of manganese. Our resultant characterization of chloroplast structure of normal, manganese-deficient, and recovered cells provides evidence for a role for manganese in maintaining chloroplast structure.

Materials and Methods

Culture Preparation. Wild-type *Chlamydomonas reinhardi* (strain 137c) was grown on normally prepared liquid minimal medium (21) supplemented with 0.2% sodium acetate. These cells will be referred to as *normal* cells. *Manganese-deficient* wild-type cells were obtained through growth and sequential transfer in manganese-deficient minimal medium also supplemented with 0.2% sodium acetate. This medium was prepared by excluding manganese chloride from the trace metal supplement to the medium and by reducing manganese contamination from other sources. Glassware was thoroughly cleaned by soaking in concentrated nitric and sulfuric acids. Plasticware was cleaned in 3 N hydrochloric acid. Water used in the medium was obtained by passage of tap water through a Barnstead Model BD-1 Bantam demineralizer equipped with a mixed-bed resin cartridge and was used when it gave a specific conductance of less than 0.05 μmho. Several procedures were followed to reduce the amount of

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manganese introduced along with the nutrient salts used in the medium. These methods included recrystallization, and co-precipitation with magnesium sulfate and potassium hydroxide as described by Munns and Johnson (14). Matthey Specpure salts, commercially prepared purified salts, were used when available. *Recovered* cells were obtained by adding a concentrated solution of manganese chloride to restore manganese-deficient cell cultures to normal manganese levels.

All 3 types of cultures were grown at 25°, illuminated continuously at an intensity of 4000 lux from daylight fluorescent lamps, and agitated on a Gyrotory shaker.

Photosynthesis Measurements. Determination of the *p*-benzoquinone (PBQ) Hill reaction was carried out manometrically at 25° and 30,000 lux with whole cells washed and resuspended in 0.067 M phosphate buffer. Prior to illumination the flasks were gassed with nitrogen for 10 min.

The method of measurement of photosynthetic carbon dioxide fixation has been described (19). Carbon dioxide fixation by photoreduction was measured manometrically. Cells were washed and resuspended in 0.1 M bicarbonate buffer, pH 8. The flasks were gassed with 5 % carbon dioxide in hydrogen in the dark for 30 min prior to illumination. The temperature was maintained at 25° and the light intensity at 10,000 lux.

Chloroplast fragments were prepared by a sonication method described elsewhere (19) except for the use of 5 mM phosphate buffer, pH 7.4, as the suspending medium. Hill reactions with 2,6-dichlorophenol-indophenol (DPIP) as oxidant, and NADP photoreduction from water or from reduced DPIP and ascorbate (24) were measured following the procedures of Levine and Smillie (11).

The Hill reaction rate, measured with DPIP as the oxidant, was used as an indicator of system II activity in determining recovery. Fragments were prepared from aliquots of cells from a manganese-deficient culture prior to and following the addition of sufficient manganese to restore the concentration of the deficient culture medium to 2.5×10^{-5} M. When comparing recovery in the light with that in the dark, one-half of the deficient culture grown in the light was removed to a light-tight sterile flask. Equal quantities of manganese ions were then added to both flasks.

Chlorophyll concentration and pigment distribution were determined on 80 % acetone extracts. Chlorophyll concentration was calculated by Arnon's modification (1) of the method of MacKinney (13).

Electron Microscopy. Cells grown in manganese-deficient medium were prepared for electron microscopic examination when chloroplast fragments of these cells carried out the DPIP-Hill reaction at levels 30 % or less than that of similarly treated normal cell fragments. For recovered cells the DPIP-Hill reaction rate became equivalent to that of normal cells within 2 hr after the addition of manganese to deficient cells. The cells were prepared for microscopy as soon as normal Hill reaction rates were obtained.

Normal, deficient, and recovered cells were all prepared for observation in the same manner. The cells were fixed for 1 hr at 0° in 2 % osmium tetroxide in 0.05 M cacodylate buffer, pH 7.4, with 0.002 M calcium chloride. The cells were dehydrated in cold ethanol, transferred to propylene oxide, and then embedded in Epon resin (12). Capsules containing the fixed cells were allowed to harden in a 60° oven. Sections were cut with glass knives on a Porter-Blum MT-2 ultramicrotome. The sections were mounted on uncoated nickel grids and stained for 5 min each in uranyl acetate and lead citrate. Electron micrographs of the sections were obtained with an RCA EMU-3D electron microscope.

Results

Photosynthetic Capabilities. Photosynthetic reactions, including the Hill reaction with PBQ, carbon dioxide fixation, and carbon dioxide fixation by photoreduction, were measured on whole cells. NADP photoreduction and DPIP-Hill reaction were measured on cell fragments. The reaction rates observed are given in table I.

The absence of manganese led to reduced Hill reaction rates (table I a,b). Sequential transfer of cultures in deficient medium resulted in the reduction of Hill reaction ability measured with DPIP to as little as 5 % of normal. The rate of NADP photoreduction by deficient cells with water as the source of electrons was reduced to about 20 % of that by normal cells (table Ic). However, when the electrons for this reaction were derived from reduced DPIP and ascorbate, the rate of NADP photoreduction of the deficient cells was greater than the normal rate (table Id). The rate of carbon dioxide fixation for deficient cells was 30 % of the rate of normal cells (table Ie). After adaptation to a hydrogen atmosphere, manganese-deficient cells were capable of carrying out carbon dioxide fixation by photoreduction at rates comparable to that of normal cells in which oxygen evolution was blocked with 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) (table If). Unpoisoned normal cells deadapted at low light intensities.

Total recovery of oxygen-evolving ability, as indicated by return to normal Hill reaction rates measured with DPIP as oxidant, took place within 2 hr after the addition of manganese to manganese-deficient cultures (Fig. 1). This recovery time was verified on several preparations of deficient cells including those with initial Hill reaction rates that were 5 % of the normal cells. Return to control levels in the light proceeded more rapidly and more completely than in the dark. Chlorophyll concen-

tration remained practically unchanged during the recovery period. The ratio of chlorophyll *a* to chlorophyll *b* was 2.3 in both kinds of cells, indicating that no alteration in the pigment distribution resulted from the deficiency.

Table I. *Photosynthetic Rates of Normal and Manganese-Deficient* Chlamydomonas reinhardi

PBQ (*p*-benzoquinone) Hill reaction was measured on whole cells in a nitrogen atmosphere in a Warburg vessel. The reaction mixture contained whole cells equivalent to 0.2 mg chlorophyll in 0.067 M phosphate buffer, pH 6.8. PBQ, 15 μmoles, was added from the side-arm prior to illumination.

DPIP (2,6-dichlorophenol-indophenol) Hill reaction was measured at 600 nm in a Cary model 14 spectrophotometer. The reaction mixture (0.8 ml) contained chloroplast fragments equivalent to 3 to 4 μg of chlorophyll and the following in μmoles: potassium phosphate, pH 7, 10; KCl, 5; DPIP, 0.05. DPIP was omitted from the control sample.

NADP Hill reaction was measured at 340 nm in a Cary model 14 spectrophotometer. The reaction mixture (0.8 ml) contained chloroplast fragments equivalent to 12 μg chlorophyll and the following in μmoles: $MgCl_2$, 0.015; NADP, 0.2. Ferredoxin purified from *C. reinhardi* was added in excess. The reaction mixture was brought to volume with 5 mM phosphate buffer, pH 7.5. Ferredoxin and NADP were not added to the control sample.

NADP photoreduction with electrons derived from DPIP and ascorbate was measured at 340 nm in a Cary model 14 spectrophotometer. The reaction mixture (0.8 ml) contained chloroplast fragments equivalent to 12 μg chlorophyll and the following in μmoles: $MgCl_2$, 0.015; DCMU, 0.02; DPIP, 0.05; ascorbate, 5; NADP, 0.2. Ferredoxin was added in excess. Volume was brought to 0.8 ml by addition of 5 mM phosphate buffer, pH 7.5. Ferredoxin and NADP were omitted from the control sample.

Carbon dioxide fixation was measured in a reaction mixture containing whole cells equivalent to 0.25 mg of chlorophyll, 310 μmoles of bicarbonate buffer, pH 8, and $NaH^{14}CO_3$, 10 μcuries.

Carbon dioxide fixation by photoreduction was measured on whole cells in an atmosphere of 5 % CO_2 in H_2. The reaction mixture contained whole cells equivalent to 0.15 mg chlorophyll in 0.1 M bicarbonate buffer, pH 8. DCMU, 0.03 μmole in methanol, was added to flasks containing normal cells. An equivalent volume of methanol was added to flasks containing manganese-deficient cells.

Reaction	Rate of normal (μequivalents of electrons per hr per mg chlorophyll)	Rate of deficient	Percent of normal
a. PBQ-Hill reaction	120	36	30
b. DPIP-Hill reaction	160-400	15-50	5-30
c. NADP-Hill reaction	120	25	21
d. NADP photoreduction from DPIP and ascorbate	28	40	140
e. CO_2 fixation	280	85	30
f. CO_2 fixation by photoreduction	42	57	133

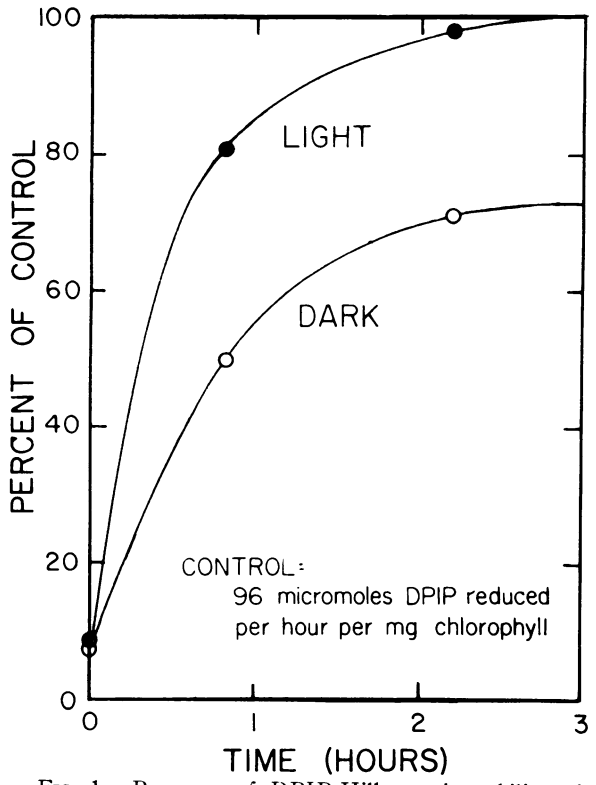

FIG. 1. Recovery of DPIP-Hill reaction ability of manganese-deficient cells incubated in the light and in the dark in the presence of manganese. Manganous ions were added at time 0. (See table I for composition of the reaction mixture.)

Cell Structure. Normal Cells. The cellular organization of normal wild-type cells of *C. reinhardi* has been described (20), and is largely typical of many other green algae. Each cell contains a single cup-shaped chloroplast which occupies about 50 % of the cell volume. The chloroplast is surrounded by 2 membranes that are separated by a variable distance and which together comprise the chloroplast envelope. The fundamental structural unit within the chloroplast is the disc or thylakoid which appears in electron micrographs as 2 parallel membranes joined at the ends to form a flattened vesicle. Each of the parallel membranes of the disc is called a lamella. Within the chloroplast, the discs are arranged parallel to the chloroplast envelope. Typically, each disc is organized with 1 or more other discs into stacks characterized by a close association of adjacent disc surfaces. No space can be resolved between discs so that the internal lamellae appear twice as thick as the 2 outermost lamellae of a stack (Fig. 2a). An individual disc is quite extensive and usually is associated with different stacks in different regions of the chloroplast.

Each chloroplast also contains a pyrenoid, lipoidal bodies, starch grains, chloroplast ribosomes, and

several rows of granules, termed the eyespot, which are just under the envelope in the anterior portion of the chloroplast.

Manganese-Deficient Cells. Deficient cells in many respects are identical to normal cells. Deficient cells, however, are smaller than normal cells and tend to remain clumped within the original cell wall for some time after cell division. The only other prominent difference between normal and deficient cells is in the organization of discs into stacks within the chloroplast. There is a reduced tendency of the discs to form stacks, as evidenced by the appearance of many isolated, or singlet, discs, and of discs already within stacks to dissociate from each other (Fig. 2b). The chloroplast matrix and inclusions are otherwise comparable.

Recovered Cells. The recovered cells, prepared for examination as soon as the Hill reaction rate returned to normal, resemble deficient cells in that daughter cells remain surrounded by the mother cell wall. However, as in normal cells, the chloroplast discs are associated into stacks (Fig. 2c) and very few regions of disorganization are apparent.

Statistics of the Stacking Distributions

In this section we develop quantitative measures of chloroplast structure, as revealed by electron microscopy, with the aim of determining the degree of correlation, if any, between structural changes and system II capability. The quantitative measure employed is the distribution function $g(n)$ which specifies the fraction of discs in stacks of size n. The distribution function is included in a footnote[3] in the list of definitions of quantities discussed in this section.

Electron micrographs of 7 different samples of cells—2 normal, 3 deficient, and 2 recovered—were taken, and the prints of best photographic quality were used in the analysis. Two perpendicular lines were drawn through the center of each print parallel to the edges, and the number and size of stacks of discs intersecting these sampling lines were counted. This simple and arbitrary procedure was strictly adhered to in order to ensure random sampling.

A schematic illustration of the method is shown in Fig. 3, which shows a counting line intersecting 15 discs arranged into 7 stacks. The distribution function corresponding to this miniature stacking sample is as follows: $g(1)=3/15=20\ \%$; $g(2)=4/15=27\ \%$; $g(3)=3/15=20\ \%$; $g(4)=0$; $g(5)=5/15=33\ \%$; $g(6)=g(7)=\ .\ .\ .=0$. The function $g(n)$ is normalized, $\sum_n g(n)=1=100\ \%$, and specifies the manner in which the discs arrange themselves into stacks.

A total of 263 electron micrographs, containing 7124 discs arranged into 2390 stacks, were studied. The total number of micrographs, stacks, and discs for the 7 samples are given in table II, as well as the average stack size, $\overline{n}$, and the fraction of singlet discs, $g(1)$, in each case. The numbers in parenthesis indicate the range spanned by 2 standard deviations ($\pm 2\sigma$), representing a level of confidence of 95 %.

The standard deviation for $\overline{n}$ was estimated as follows: A separate $\overline{n}_i$ was computed for the data obtained from each micrograph i, and then

$$[\sum_i |\overline{n}_i - \overline{n}|^2/m]^{1/2}$$

was calculated, where m is the number of micro-

[3] List of Symbols and Definitions. n = stack size, number of discs in stack; $N(n)$ = number of stacks of size n; $n \cdot N(n)$ = number of discs in stacks of size n; $\sum_n N(n)$ = total number of stacks; $\sum_n nN(n)$ = total number of discs; $\overline{n} = \sum_n nN(n)/\sum_n N(n)$, average stack size; $g(n) = nN(n)/\sum_n nN(n)$, fraction of discs in stacks of size n; $n(g_{max})$ = peak position of $g(n)$; $<n> = \sum_n ng(n)$, centroid (center of gravity) of $g(n)$; $<n^2> = \sum_n n^2 g(n)$; $s = [<n^2> - <n>^2]^{1/2}$, root-mean-square spread of the distribution.

Table II. *Stacking Data for the Individual and the Composite Samples*

Sample	No. of micrographs	No. of stacks ($\sum N$)	No. of discs ($\sum nN$)	Avg stack size ($\overline{n}$)	Fraction of singlet discs [$g(1)$ in %]
N1	37	260	788	3.03 (± 0.28)	5.8 (± 1.7)
N2	20	198	698	3.52 (± 0.59)	3.9 (± 1.5)
R1	59	487	1604	3.29 (± 0.30)	5.9 (± 1.2)
R2	21	160	584	3.65 (± 0.55)	5.3 (± 1.9)
D1	46	515	1360	2.64 (± 0.27)	15.2 (± 1.9)
D2	43	402	1089	2.71 (± 0.54)	21.2 (± 2.4)
D3	37	368	1001	2.72 (± 0.38)	15.7 (± 2.3)
N	57	458	1486	3.24 (± 0.26)	4.9 (± 1.1)
R	80	647	2188	3.38 (± 0.27)	5.7 (± 1.0)
D	126	1285	3450	2.68 (± 0.23)	17.3 (± 1.3)

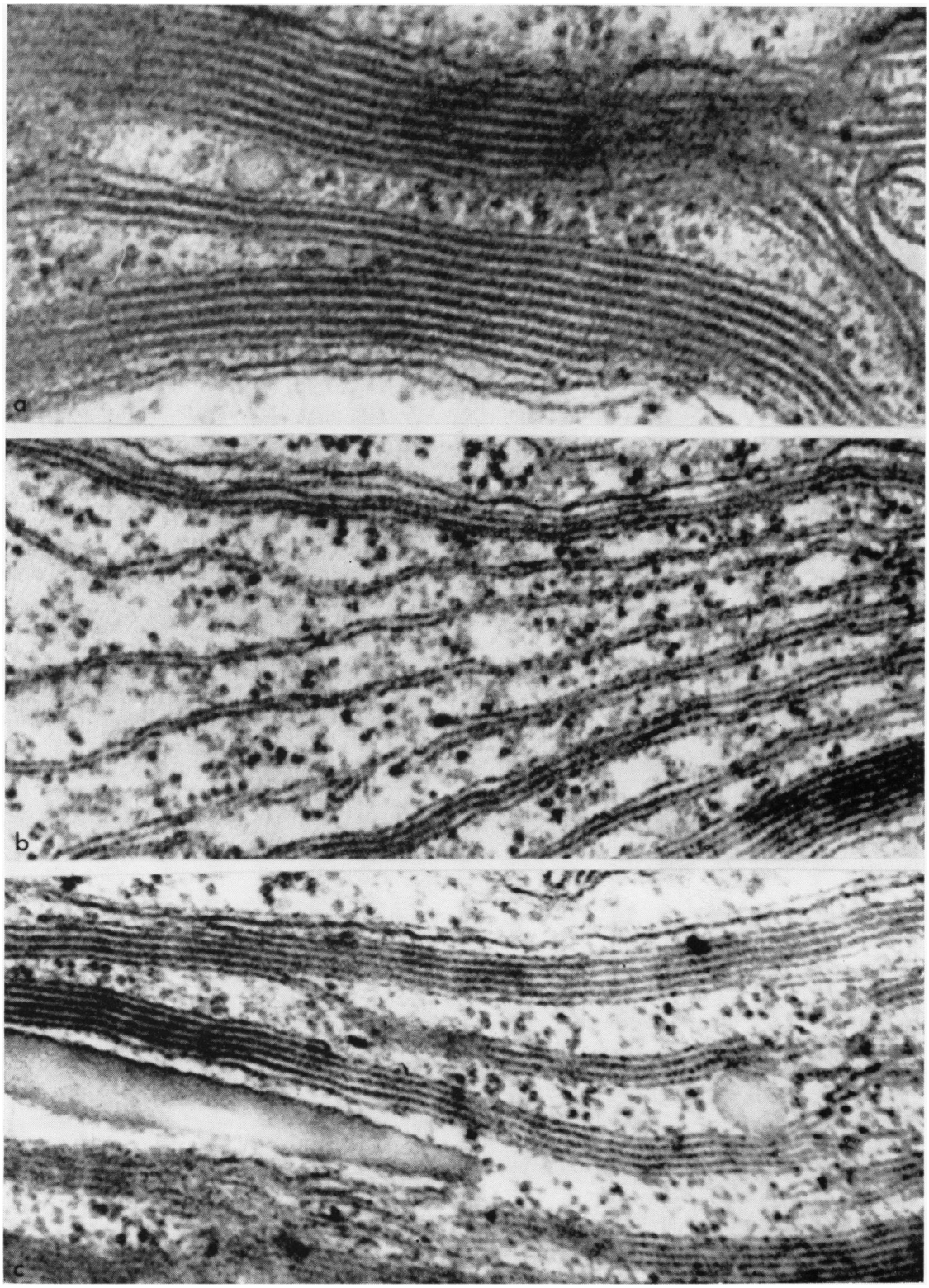

FIG. 1. Section of *Chlamydomonas reinhardi* chloroplast from (a) normal cell (magnification 121,000×), (b) a deficient cell (magnification 88,500×), (c) a recovered cell (magnification 88,500×).

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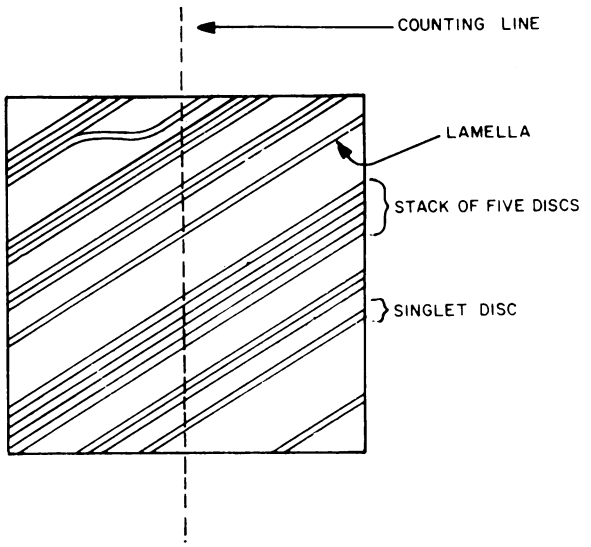

FIG. 3. Illustration of the method of analysis of a chloroplast region containing discs arranged into stacks of various sizes.

graphs in the sample. This result is divided by $\sqrt{m}$ to obtain δ, the standard error of the mean $\bar{n}$. For $g(1)$, the frequency of occurrence of discs as singlets, δ was estimated by applying the relation for a binomial distribution (3), obtaining:

$$\delta = [g(1)\{1 - g(1)\}/\Sigma nN]^{1/2}.$$

An examination of the last 2 columns of table II reveals that with respect to $\bar{n}$ and $g(1)$, the 7 samples fall into 2 distinct sets: the normal (N) and the recovered (R) samples belonging to 1 set; the deficient (D) samples belonging to the other.

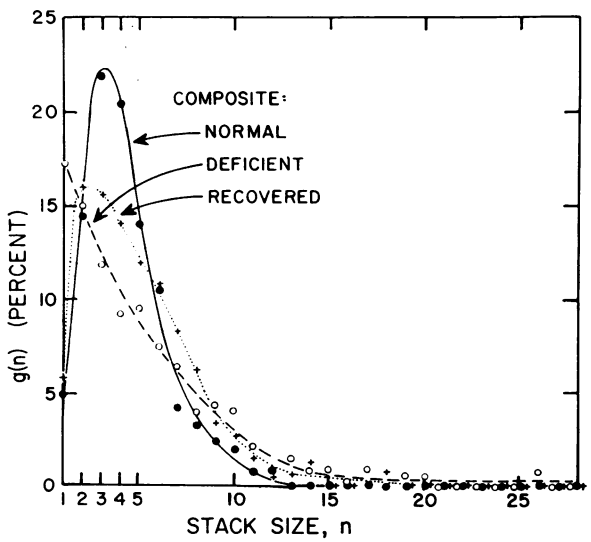

FIG. 4. Distribution functions of the composite samples. Normal cells, ●; deficient cells, ○; recovered cells, +.

Within each set of samples the ranges of $\bar{n}$ overlap, while comparing samples of different sets we find that D_1 fails to overlap N_2, R_1, and R_2; and that D_2 just barely overlaps R_2. For $g(1)$, the ranges of the 4 normal and recovered samples fall within range of each other but well outside the ranges of the deficient samples.

The 2 normal, 2 recovered, and 3 deficient samples were separately merged to form 3 composite samples, the data for which are given in the last 3 rows of table II. The observations of the preceding paragraph suggest that this is a very reasonable simplification to make. For the composite samples, the values of $\bar{n}$ and $g(1)$ are statistically indistinguishable for N and R, whereas for N and D (and, of course, R and D) the differences are statistically significant. The total number of discs ($\Sigma nN/m$) is conserved in all 3 types of cells (see the last row, table III).

The distribution functions $g(n)$ for the composite samples are shown in Fig. 4. A smooth curve has been drawn through each set of points to clarify the behavior. For normal samples $g(n)$ is sharply peaked at $n = 3$–4, $[g(3) + g(4) = 43\,\%]$, whereas for deficient cells $g(n)$ falls off continuously and gradually from $n = 1$. For recovered cells $g(n)$ again reveals a peak at $n = 2$–3 but it is shallower, $[g(2) + g(3) = 32\,\%]$, and broader than that for normal cells.

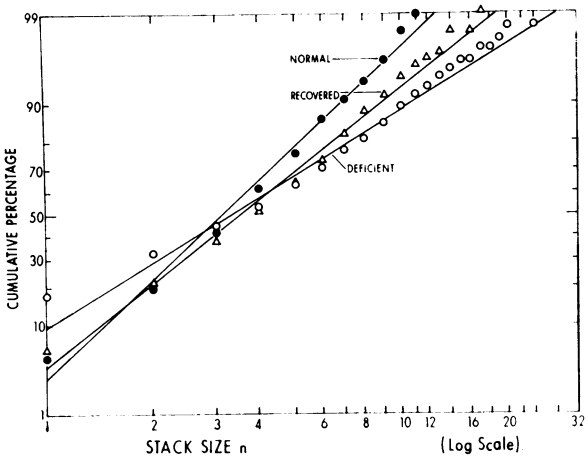

FIG. 5. Log-normal representation of the cumulative disc distribution of the composite samples. Normal cells, ●; deficient cells, ○; recovered cells, △.

Quantitative characteristics of $g(n)$ for normal, deficient, and recovered cells are presented in table III. The first 2 quantities listed, $g(1)$ and $\bar{n}$, exhibit virtually complete recovery. The variation in the fraction of singlet discs is particularly striking, tripling in value from N to D and closely returning to its initial value from D to R. The average stack size, however, is a relatively insensitive measure of

the changes in distribution. The quantities $n(g_{max})$, $<n>$, and s exhibit only partial recovery, the value for R in each instance lying intermediate between the values for N and D. The parameter s which is a measure of the spread of the distribution, reveals clearly how much broader $g(n)$ is for deficient cells than for normal cells.

The curves of Fig. 4 indicate that the deficient distribution contains more very large stacks than the normal distribution, as well as more singlet stacks. The recovered distribution, while approximating the normal distribution at $n = 1$, does not recover completely at large n and retains more than the normal proportion of large stacks. This characteristic is demonstrated by the quantity s, in table III. The opposite behavior of peak position and centroid in table III is a consequence of the fact that manganese deficiency results in an increase in the number of both small ($n = 1$) and large ($n > 10$) stacks. Similarly, the insensitivity of $\overline{n}$ results from the near compensation of these 2 effects.

In Fig. 5, the distribution functions are displayed in another form. The cumulative distribution $\sum_{i}^{n} g(n)$ is plotted against log n in a manner for which a normal distribution would yield a straight line. The reason for presenting the data in this log-normal representation (3, 7) is simply that the plots are roughly linear. The $n = 1$ intercept of each plot is, of course, $g(1)$ whereas the slope is inversely related to the spread of the distribution ($\sim s^{-1}$). This figure reveals somewhat more clearly than Fig. 4 the differences in the spreads of the distributions (slope for D<slope for R<slope for N) as well as the incomplete recovery at large stack sizes.

The results of this statistical analysis can be summarized as follows: For normal cells $g(n)$ peaks sharply, nearly half of the discs occurring in stacks of 3 and 4. The effect of manganese deficiency is to spread out the distribution function, increasing the number of singlet discs as well as of large stacks, at the expense of stacks of intermediate size. For recovered cells the fraction of singlets returns to the value for normal cells, while the recovery at large stack sizes is less complete. The most pertinent parameters for characterizing these changes, among those listed in table III, are $g(1)$ and s.

Discussion

When cells of *C. reinhardi* are grown in the absence of manganese they exhibit the characteristics that are typical of manganese deficiency observed in other photosynthetic organisms: There is a decrease in ability to evolve oxygen and to fix carbon dioxide by photosynthesis, with no accompanying decrease in ability to carry on carbon dioxide fixation by photoreduction. The inability of deficient cells to perform the Hill reaction while displaying an unimpaired ability to reduce NADP from DPIP and ascorbate supports the contention that manganese is involved, directly or indirectly, in system II of photosynthesis. Furthermore, it establishes the integrity of the electron transport chain associated with system I in manganese-deficient cells.

Rapid restoration of system II activity occurs upon the addition of manganese salts to manganese-deficient *C. reinhardi*. This 2 hr recovery time is comparable to that determined for other algae (2, 5, 6, 8, 16). The more complete return to normal photosynthesis in the light than in the dark upon addition of manganese may be simply accounted for by an enhanced uptake of manganese across the cell membrane in the light. Supporting this view is evidence from electron spin resonance studies that manganese uptake is stimulated by light in *C. reinhardi* even when photosynthetic electron flow from the manganese-requiring system II is blocked by chemical treatment with DCMU or by mutation (23).

In *C. reinhardi* the absence of manganese results in an inhibition of system II activity and a concomitant disruption of chloroplast organization as we have described. When manganese is restored Hill reaction ability returns to normal and there is a return of several parameters of the stack distribution, especially $g(1)$, the fraction of singlet discs, to normal levels. From the exact correlation between the appearance and disappearance of singlet discs and the inhibition and recovery of Hill reaction activity, we conclude that the appearance of singlet

Table III. *Parameters of the Composite Distribution Functions*

Characteristic of the distribution	Symbol	Normal	Recovered	Deficient
Fraction of singlets	$g(1)$	5 %	6 %	17 %
Avg stack size	$\overline{n}$	3.2	3.4	2.7
Peak position	$n(g_{max})$	3.3	2.3	1.0
Centroid of $g(n)$	$<n>$	4.3	5.1	5.5
rms spread of $g(n)$	s	2.2	3.2	4.9
Discs in large stacks	$\sum_{n>10} g(n)$	2 %	6 %	11 %
Discs per cell		26.1	27.4	27.4

discs is a primary effect of manganese deficiency. The proportion of very large stacks ($n > 10$) also observed in manganese-deficient cells does not recover completely in the sequence N→D→R and may represent a secondary effect of the manganese deficiency.

On the basis of our observations and analysis of the electron micrographs, 3 interpretations are possible. The unstacking of chloroplast discs can be (a) the *result* of the inhibition of system II activity, (b) the *cause* of the loss of system II activity, (c) related to manganese deficiency but *independent* of any role of manganese in system II. We reject (a) since cells growing or regreening in the presence of DCMU, a system II inhibitor, do not show unstacking of discs (10). If (b) is correct, manganese is performing a structural role in permitting system II activity by promoting the proper association of discs into stacks. It is possible to construct some very simple models for such a structural role and to compare these with the experimental results given here (tables I and III, Fig. 4). For example, if lamellae must be in contact to facilitate oxygen evolution, then we can show that the fraction of effective lamellae is: $1 - 1/\bar{n}$. This quantity is 10 % smaller for manganese-deficient than for normal cells (table III), thus predicting an effect which, while in the right direction, is too small by a factor of 7 to 9 (table Ib). A slight refinement to include a dropoff in efficiency for deeply-buried lamellae in the interior of large stacks (due to inaccessibility of needed substrates or limited opportunity for removal of products of photosynthesis) results in a larger effect, though one still too small by a factor of 3. Although quantitative agreement with experiment is not obtained, the qualitative features of these simple models for (b) are suggestive. Nevertheless, it is not possible at present to rule out (c) as an alternative.

The influence of manganese on chloroplast structure does help to explain some other observations. If the manganese functioning in photosynthesis is localized on a membrane surface, the observed rapid recovery of photosynthetic ability would be possible through an easy binding of manganese with its acceptor site on the exposed lamellar surfaces of manganese-deficient cells. After the re-association of discs into stacks following the addition of manganese most of the manganese should be difficult to remove and such removal should, as observed, require relatively harsh treatment (17).

Acknowledgments

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