Reduction at Higher Pressure.—A solution of 72.0 Gm. (0.5 mole) of I in 250 ml. of 95% ethyl alcohol was hydrogenated at room temperature and 130 Atm. in the presence of 30 Gm. of 5% rhodium on carbon. Reaction was slightly exothermic, the temperature rising to 46°. Uptake was complete in about 1 hour. The catalyst was removed, the solution concentrated, and the residue distilled. It boiled at 237–240° [lit. 246° at 745 mm. (5)] at atmospheric pressure and solidified quickly. The product weighed 74.5 Gm. and melted at 89–91°.

To determine the purity of the product,[4] 2.5 Gm. was dissolved in a mixture of 20 ml. of 99% benzene and 1% ether, put over a silica gel column, and eluted with a 95% benzene and 5% ether mixture. Fractions were collected in 30-ml. portions. These were followed by means of thin-layer chromatography. In one fraction a minor component was seen. It was isolated and found by infrared examination to have a peak at 5.78 μ, indicative of an unconjugated carbonyl group. Since there was no evidence of aromaticity, it was assumed to be 1-decalone. One more fraction produced a mixture in which the major component predominated (85%). Thereafter only *cis,cis* 1-decalol was obtained, 1.5 Gm., m.p. 91–92°.

In a larger run (1.0 mole) at 200 Atm. pressure, the temperature rose to 54–55°. In this instance the yield of distilled III was lower (76.5%).

[4] Performed by D. E. Dickson, Regis Chemical Co., Chicago, Ill.

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Lipid Content of *Chlorella* "Aerated" with a CO_2–Nitrogen *versus* a CO_2–Air Mixture

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Contrary to some earlier reports, substitution of nitrogen for air in the CO_2–air mixture conventionally used to "aerate" cultures of *Chlorella* had little effect on dry weight or on protein and lipid content of *C. pyrenoidosa* harvested after 8 weeks, whether the cells were provided with a good nutrient solution or with one deficient in nitrogen. In a manganese-deficient medium, the yield of dry weight and the protein and lipid content of the cells produced was approximately the same whether a CO_2–air or a CO_2–nitrogen gas stream was used, but the dry weight and lipid content were markedly lower and the protein content was higher than in cultures developed in the standard complete medium.

IT HAS BEEN REPORTED (1, 2) and confirmed (3) that cells of *Chlorella pyrenoidosa* have a higher lipid content when cultured in a nitrogen-deficient mineral nutrient solution than when cultured in one with an adequate concentration of nitrogen. However, the highest level of lipid found by the later authors was markedly lower than the 65% in 4 weeks and approximately 75% in 10 weeks, reported by the earlier workers (1, 2). Seeking sources of the discrepancy is difficult because of the lack of specific details with respect to environmental conditions in the earlier papers. Since composition of the "aeration" stream might have been an important variable, this has now been investigated and is the principal subject of this report.

The "aeration" mixture customarily used in *Chlorella* experiments in this laboratory and elsewhere for many years consists of 5% carbon dioxide and 95% air. However, a personal communication from one of the earlier authors states that they "found it essential to use 5% CO_2 in *nitrogen* in order to maintain cultures in good shape for the long time needed to obtain the highest lipide content" and that "we too observed clumping of cells in old cultures in SM (nitrogen-deficient) medium grown with CO_2 in air . . . But even in the 70–80 day old, very high lipide cultures grown with CO_2 in N_2, the cells remained freely suspendible and showed negligible sticking together" (4).

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RESULTS

In three experiments (each run in duplicate) averaged in Table IA, substitution of CO_2–nitrogen for the CO_2–air stream had only minimal effect on the dry weight of cells produced and on their protein and lipid content, whether the alga was cultured in the standard medium or in the nitrogen-deficient one. Regardless of which gas mixture was provided, the lipid content (as per cent of dry weight) was about 19% higher, and the protein level was about 10% lower in the medium deficient in mineral nitrogen than in the standard. Under the experimental conditions employed, we found no evidence that composition of the culture solution affected the response of the cells to substitution of nitrogen for air in the gas stream or to support the cautiously worded suggestion that *Chlorella* may "under the special conditions producing high *R*-value (namely, mineral nitrogen starvation and supplied with 5% CO_2 in nitrogen), fix atmospheric nitrogen" (1).

It has been stressed that high lipid values were never attained when the "residual fixed nitrogen concentration was greater than 0.001 molar" in the medium (1). Although the conditions provided in the present experiments are believed to have

TABLE I.—YIELDS FROM *C. pyrenoidosa* IN STANDARD (STD.)[a] AND IN "HIGH-LIPID-INDUCING" (SM)[a] MEDIA AND IN THE STANDARD MEDIUM LACKING MANGANESE (−Mn)[a] PROVIDED WITH CO_2–AIR AND CO_2–NITROGEN "AERATING" MIXTURES[b]

	A				B	
	Std.		SM		Mn	
	1 Air	*2* N	*3* Air	*4* N	Air	N
Dry wt. (mg./ml. of culture)	8.393	8.725	1.115	1.198	3.463	3.583
Protein (% dry wt.)	19.8	19.8	10.1	9.5	29.3	30.6
Lipid (% dry wt.)	33.2	36.7	52.6	55.8	17.6	16.6

[a] Culture media.

	Std.	SM	Std. − Mn
KNO_3	0.025 *M*	...	0.025 *M*
$MgSO_4 \cdot 7H_2O$	0.020 *M*	0.01 *M*	0.020 *M*
KH_2PO_4	0.018 *M*	0.01 *M*	0.018 *M*
$(NH_4)H_2PO_4$	...	0.000825 *M*	...
$(NH_4)_2HPO_4$	...	0.000714 *M*	...
KCl	...	0.03 *M*	...
$FeSO_4 \cdot 7H_2O$	5×10^{-5} *M*	5×10^{-5} *M*	5×10^{-5} *M*
K citrate	5×10^{-5} *M*	5×10^{-5} *M*	5×10^{-5} *M*
Zn (as $ZnSO_4 \cdot 7H_2O$)	0.400 p.p.m.	0.400 p.p.m.	0.400 p.p.m.
Cu (as $CuSO_4 \cdot 5H_2O$)	0.004 p.p.m.	0.004 p.p.m.	0.004 p.p.m.
Mn (as $MnSO_4 \cdot 4H_2O$)	0.400 p.p.m.	0.400 p.p.m.	...
B (as H_3BO_3)	0.020 p.p.m.	0.020 p.p.m.	0.020 p.p.m.

[b] Culture conditions: Light = 1250 f.-c. (Mazda source) at the level of the flasks. Temperature: 22.5 ± 2° C. Culture period: 8 weeks. Aeration mixtures: air = 5% CO_2 + 95% air; N = 5% CO_2 + 95% nitrogen (both humidified to prevent evaporation of culture solutions).

simulated closely those used by the earlier workers, the cultures did not reduce the "residual" nitrogen level substantially below 0.001 *M*. The "residual" nitrogen level we found in the initially nitrogen-deficient cultures supplied with the CO_2–nitrogen gas stream was 0.00087 *M versus* 0.00093 *M* in similar cultures provided with CO_2–air.

In our experiments, substitution of nitrogen for air in the gas stream had no effect on the appearance or the morphology of the cells in SM medium. After 10 days to 2 weeks, cells in SM medium, irrespective of the composition of the "aeration" mixture, adhered tenaciously in clumps which could not be separated sufficiently to permit cell counts and which would not remain suspended in the medium. All SM cultures displayed the physical characteristics described previously (3).

In a preliminary study of trace elements, it was observed that omission of manganese from the standard medium resulted in cultures that, upon gross inspection, appeared very similar to those produced in the nitrogen-deficient medium. In view of that observation and of the relation of manganese to processes, *e.g.*, photosynthesis and enzyme reactions, fundamental to nitrogen metabolism, cultures in a manganese-deficient solution (−Mn) were included in the present experiments. It should be emphasized that the cultures were manganese-deficient but not manganese-free. Solutions were prepared with twice-distilled water, the last distillation being in an all Pyrex still, and cells for inoculation of the manganese-deficient cultures were rinsed twice in such water. However, undetermined amounts of intracellular manganese undoubtedly were carried into the new cultures with the inocula, and no attempt was made to remove possible trace impurities of the element from the salts used to prepare the culture medium. Estimates based on reports in the literature (5–8) suggest that in the present experiments the concentration of manganese in the −Mn medium was above 5×10^{-5} and below 5×10^{-3} p.p.m. A reasonable approximation appears to be about 5×10^{-4} p.p.m.—a reduction of roughly 800-fold below the initial concentration in the standard and in the SM media.

The experiments revealed marked differences between cells developed in the SM and in the −Mn media (Table IA, columns *3* and *4 versus* Table IB), despite the apparent similarity of the cultures upon gross visual inspection. The experiments showed also that results in the manganese-deficient medium were essentially similar whether the CO_2–air or the CO_2–nitrogen mixture was employed (Table IB).

The most striking feature of the data from cells developed in manganese-deficient cultures in comparison with data from standard cultures is the relatively high protein and low lipid content of cells from the −Mn medium (Table IB *versus* IA, columns *1* and *2*). In 8 weeks, −Mn cultures supplied with the CO_2–air mixture produced about 40% as much dry weight as cultures in the standard medium, but, relative to their dry weight, had approximately 48% more protein and about half as much lipid. In cultures receiving the CO_2–nitrogen gas stream, protein content, relative to dry weight, was 54% greater in cells from manganese-deficient cultures than in those produced in the standard medium and the lipid content was reduced to less than half. These effects are not interpreted as stemming from a direct effect of manganese on nitrogen metabolism, but rather as being indirect consequences of manganese deficiency.

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