Botany. — *Influence of Magnesium on the Relation between Chlorophyll Content and Rate of Photosynthesis.* By J. C. VAN HILLE. (Communicated by Prof. L. G. M. BAAS BECKING).

(Communicated at the meeting of October 30, 1937.)

Introduction.

In the Journal of General Physiology vol. **18**, p. 573, W. E. FLEISCHER (1) published the data of his experiments on the relation between chlorophyll content and rate of photosynthesis in *Chlorella.*

This alga was grown in nutrient solutions with graded quantities magnesium, nitrogen and iron. In the cultures with low concentrations of one of these elements the cells of *Chlorella* were chlorotic.

3 to 6 days after inoculation the photosynthesis and chlorophyll content were determined. The results of the experiments are:

a. that the rate of photosynthesis is proportional to the chlorophyll content, when the latter is varied by iron supply or nitrogen supply.

b. the behaviour of the *Chlorella* cells in nutrient solutions with graded quantities of magnesium is quite different.

FLEISCHER writes: "At low concentrations of magnesium the rate of "photosynthesis is relatively independent of the chlorophyll content. As "the magnesium concentration is increased, the rate of photosynthesis "rises rapidly and during the rise is relatively independent of the chloro-"phyll content. Eventually the rate of photosynthesis reaches the value "indicated by full nutrient determinations and at that point the relation "between the rate of photosynthesis and chlorophyll content is comparable "to the relation existing in the iron and nitrogen graphs for similar values."

Methods.

In my work I followed the methods of FLEISCHER. The work was done with a pure strain of *Chlorella pyrenoidosa,* while FLEISCHER worked with a pure strain of *Chlorella* of undetermined species.

I also used *Chlorella vulgaris* var. *viridis* which yielded the same results.

Both strains have been obtained from E. PRINGSHEIM, Praha.

The same nutrient solution was used as in FLEISCHER experiments.

$FeSO_4$	0,03 g.
Na_2SO_4	1,42 g.
Na citrate	1,— g.
KNO_3	1,26 g.
KH_2PO_4	1,22 g.
Glucose	15,— g.
Aq. dest.	1 L.

The standard solution of Mg was a solution of $MgSO_4$, which contained 1 mg of magnesium per cm^3. FLEISCHER used $MgCl_2$ for his standard solution.

Chlorella was cultivated in 100 cm^3 Erlenmeyer flasks containing 50 cm^3 nutrient solution.

The cells were cultivated in the constant light at 0,9 m distance from a 500 Watt bulb. The temperature in the incubating room varied from 24° to 27°. To secure a regular growth the cultures were shaken continuously.

The photosynthesis and the respiration were determined after the method of WARBURG in the single manometer. The cells were centrifuged and suspended in WARBURG's mixture Nr. 9. Photosynthesis and respiration were determined during at least half an hour each.

Readings were made every ten minutes. If no regular values were obtained during half an hour the readings were continued for some ten minutes longer. The temperature of the thermostat was 25,8° C.

The chlorophyll content was determined by measuring the light absorption by λ 6600 of the methylalcoholic chlorophyll extract of the cells, used for the photosynthesis determination, in the spectralpyrometer (3).

As no absolute chlorophyll determinations were carried out, the concentration of the chlorophyll was indicated by the logarithm of the absorption, after it had been ascertained, that the laws of BEER and LAMBERT are followed by the chlorophyll solutions of the occurring concentrations.

Cell-volumes were determined by centrifuging the cell suspensions in THROMSDORF tubes during 1,5 minute at a definite rate.

Results.

Though the methods followed for determining photosynthesis and chlorophyll content did not differ in any essential point from the methods of FLEISCHER, I could not confirm his results of the most interesting part of his investigations: the influence of magnesium.

As FLEISCHER gives no data of the Mg concentrations which give rise to the abnormally low photosynthesis values, I tried a wide range of magnesium concentrations, beginning with no magnesium at all up to the normal magnesium rate, corresponding with an addition of 2,5 cm^3 of the standard solution.

It appeared that *Chlorella* needs very little magnesium. Even if no magnesium at all was added to the nutrient solution, there was some development of the alga, though distilled water and pure salts were used.

The growth of the alga must have been a little slower than in FLEISCHER's case, since I never could carry out the experiments three days after the inoculation, the growth then being insufficient.

Only once I could make the determinations after four days of growth, but for the greater part the first determinations of a series were made five or six days after inoculation.

In fourday old cultures the photosynthesis and the chlorophyll content were quite proportional, as appears from table I, column 2.

It also appears (table I, column 3) that there is no question of chlorosis as the quantity of chlorophyll contained in 1 mm^3 of cells is also rather constant.

TABLE I.

Data of Mg series 4 days (and 6) after inoculating.

cm^3 of Mg standard sol. added to the nutrient sol.	photosynthesis / chlorophyll	chlorophyll / mm^3 cells	chlorophyll / cm^3 nutrient sol. after inoculation		mm^3 cells / cm^3 nutrient sol. after inoculation	
			4 days	**6 days**	4 days	**6 days**
0	1.31	38	32	**35**	0.85	**1.17**
0.001	1.44	35	26	**44**	0.75	**1.14**
0.003	1.54	37	32	**68**	0.88	**2.31**
0.007	1.12	33	20	**106**	0.62	**2.8**
0.02	1.33	24	12	**63**	0.52	**1.9**
0.03	1.23	34	18	**85**	0.52	**2.4**
0.04	1.21	28	18	**103**	0.63	**3.0**
0.05	1.59	31	35	**256**	1.1	**7.7**

As can be seen from table I, column 4 and 5, there is not yet a clear difference in the development of the cultures as the chlorophyll content and cell amount per cm^3 nutrient solution do not widely differ.

It may be concluded that after four days no influence of the different quantities of Mg added to the nutrient solution can be stated.

However, after six days the differences are evident. The growth of the cultures with a small quantity of magnesium is less than the growth of the cultures with more magnesium. (Table I, column **4** and **5**).

When the experiments with the same cultures are continued it is seen that, in spite of the growth, the photosynthesis, calculated per chlorophyll-unit or per cell-volume becomes lower. There is an indication, that the less magnesium in the nutrient solution, the more rapid is the decrease of photosynthesis, but in this regard the cultures of different series behaved differently and did not give uniform results.

The quantity of chlorophyll decreases after some time. The photosynthesis, however, also decreases independently from the chlorophyll content.

It may happen that the photosynthesis has reached the compensation point (assimilation = respiration), before much chlorophyll has disappeared.

In certain cases, however, the chlorophyll decomposition proceeds more

TABLE II.

cm^3 of Mg standard sol. added to the nutrient sol.	Number of days after inoculation	$\frac{\text{photosynthesis}}{\text{chlorophyll}}$	$\frac{\text{photosynthesis}}{mm^3 \text{ cells}}$	$\frac{\text{clorophyll}}{cm^3}$ nutrient sol.	$\frac{mm^3 \text{ cells}}{cm^3}$ nutrient sol.
0.01	7	2.63	114	66	1.5
	9	2.06	76	240	7
	14	1.51	55	547	15
	38	0.50	8	281	17.4
0.03	6	2.46	90	35	1
	9	2.21	64	375	13
	14	1.47	58	547	14
	38	0.27	4.2	383	25

quickly than the decrease of the photosynthesis and then the values of the $\frac{\text{photosynthesis}}{\text{chlorophyll}}$ quotient become higher again, however, without reaching the value of the first days of the growth.

The quotient $\frac{\text{photosynthesis}}{\text{cell volume}}$ is always decreasing. That the death of a great number of the cells was not the cause of this phenomenon appeared from several examinations of 50 and more days old cultures. No more than 4 % of the cells could be coloured with an eosin-solution.

As no regular relation could be demonstrated between photosynthesis and chlorophyll content, it seems probable that the controlling factor of the photosynthesis of an "aging" culture of *Chlorella*, is in the BLACKMAN reaction.

The dark-process, that in the opinion of WARBURG, WILLSTÄTTER a.o. could not be anything but a decomposition of a peroxide was also studied by me, using the method of WARBURG and YABUSOE, by the development of oxygen in the dark after the addition of H_2O_2 to a *Chlorella* suspension.

It appeared (table III) that two quantities of cells from different cultures

TABLE III.

BLACKMAN reaction and peroxide decomposition of two cultures, 20 days after inoculation with the same chlorophyll content.

Mg content	0.02	0.06
BLACKMAN reaction	0.7	1.6
Peroxide decomposition	5.5	1.2

of the same series and age and of the same chlorophyll content did not agree in their rate of photosynthesis and peroxide decomposition.

The culture with less magnesium showed a lower photosynthesis but a greater ability of H_2O_2 decomposition than the other one.

EMERSON and GREEN (2) stated that the BLACKMAN reaction could be decreased without decreasing the rate of peroxide decomposition, and that there existed no significant similarity between the temperature curves of the BLACKMAN reaction and the peroxide decomposition.

I can add to their results, that the rate of the BLACKMAN reaction and the rate of peroxide decomposition are influenced in entirely different ways in cultures with different nutrient solutions, in which the photosynthesis has been decreased by age.

It must be emphasized, that the quotient $\frac{\text{photosynthesis}}{\text{chlorophyll}}$ shows often differences in the cultures from the same nutrient solution but which do not belong to the same series.

Small fluctuations in the temperature of the incubating room may be an influencing factor in this case.

The quotient $\frac{\text{photosynthesis}}{\text{mm}^3\ \text{cells}}$ is subject to still greater fluctuations. Here the addition of glucose to the nutrient solution is one of the most important factors.

LITERATURE.

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Geology. — *The age of the Elsloo Beds.* By WILHELMINA A. E. VAN DE GEYN. (Communicated by Prof. L. G. M. BAAS BECKING).

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In the Scharberg at Elsloo on the steep banks of the river Meuse, various Tertiary beds crop out from under a diluvial covering about 10 m thick.

Between some glauconiferous sandy beds is a conglomerate which contains many phosphatic nodules and fossils — chiefly sharks' teeth and