RELATIVE EFFICIENCY OF LIGHT ABSORBED BY CAROTENOIDS IN PHOTOSYNTHESIS AND PHOTOTAXIS OF *RHODOSPIRILLUM RUBRUM*

by

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INTRODUCTION

In a preceding paper[1] it was mentioned that, in *Rhodospirillum rubrum*, light mainly absorbed by carotenoids seemed to be more efficient in photosynthesis than in phototaxis. This idea was suggested by the fact that, after correction, in the spectral region of carotenoid absorption, the photosynthesis action spectrum did not coincide with the phototaxis action spectrum established by MANTEN[2,3]. The maxima, owing to carotenoid activity, were much more pronounced in the former spectrum than in the latter one.

However, the interval between the phototaxis and photosynthesis experiments was about two years. Moreover, since some data required for the recalculations of MANTENS' results were not exactly known, it seemed desirable to compare the mentioned carotenoid activities in both processes in experiments with the same bacterial suspension and in light of the same wave-length. The present study covers this programme.

METHODS

Preparation of the bacterial suspensions. Rhodospirillum rubrum strain 4, isolated and described by VAN NIEL[4], was grown anaerobically in a light cabinet at 25–30° C in a liquid medium containing 1% peptone and 0.5% sodium chloride. The cultivation technique was the same as previously described. The cultures were used when 3 or 4 days old.

For experimental use the cultures were centrifuged at about 1200 *g* during 15 minutes. Next the bacteria were resuspended in a solution of 0.015 *M* sodium butyrate in 0.01 *M* phosphate buffer of pH 7.2 up to a concentration of 2 or 3 Tromsdorff units—Tr.u.—per ml. Since phototactic activity was considerably reduced by washing, this procedure was omitted. Finally anaerobic conditions were established by passing a flow of a gas mixture of 95% purified nitrogen and 5% carbon dioxide through the suspension for 15 minutes. This suspension was used in both photosynthesis and phototaxis measurements.

Photosynthesis measurements. Photosynthesis was determined with the aid of a differential manometer described elsewhere[5]. The experimental vessels contained 7 ml suspension. Before closing the manometer systems the above gas mixture was passed through them for 15 minutes.

The bacteria were accommodated in the thermostat at 36° C for 30 minutes in the light, followed by 15 minutes in the dark. Two windows permitted the light to enter into the thermostat. A schematic diagram of the arrangement is represented in Fig. 1. Light emitted by a Philips HP125W high

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pressure mercury lamp—I—, fed by an a.c. stabiliser, was made nearly parallel by means of the lenses L_1 and L_2. A mirror M_1 reflected the light passing L_1 in the same direction as that of the other beam. The filter combination F_1 — copper sulphate 6% 1 cm + the Schott glass filters BG20 4 mm and OG1 2 mm — isolated the 5461 A line, whereas F_2 — copper sulphate 6% 1 cm + Schott OG2 2 mm — transmitted the 5770 and 5791 A lines. A second mirror M_2, placed in the thermostat indicated by the dotted line Th, reflected both beams into the suspensions S.

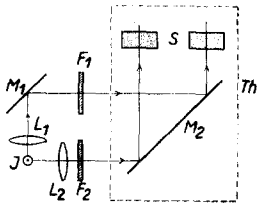

Fig. 1. Schematic diagram of the arrangement used for photosynthesis measurements.

The intensities of the beams could be varied with the aid of Schott NG neutral glass filters. These were chosen in such a way that photosynthesis proceeded at about the same rate in both the green and the yellow light. Care was taken that no light saturation occurred; the intensities of the green and the yellow light amounted to 2.4 and $3.2 \cdot 10^3$ ergs/cm²/sec respectively.

The spectrophotometrically determined "absorption"—scattering included—of the yellow light by the "2" and "3 Tr.u." suspensions in the reaction vessels amounted to 78 and 88%, respectively. The absorption of the green line surpassed that of the yellow ones by about 7%. A correction for the absorption difference of both wave-lengths was applied. Suspensions of different densities were used in order to check that the ratio of the measured photosynthetic rates in both kinds of light was not influenced by alinearity of the relation between light intensity and photosynthesis rate.

In five experiments 25% palladium asbestos—previously heated—was introduced into the vessels in order to absorb possibly developed hydrogen; *cf.* MORITA, SUZUKI AND TAKASHIMA[6]. This measure, however, did not influence the value of the studied ratio. So it was abandoned in further experiments.

Phototaxis measurements. A sample of the bacterial suspensions prepared as described above was suitably diluted with culture medium. Next, a drop of this dilution was mounted on a microthermostat of 36° C as used by THOMAS AND NIJENHUIS[7]. The bacteria were allowed to accommodate under anaerobic conditions in light for about five minutes.

Measurements were done with the aid of a modified bacteriophotometer after MILATZ AND MANTEN[8]. A detailed description of this apparatus will be published in a forthcoming issue[9]. The more simple construction used in the present investigation is represented schematically in Fig. 2. The light of the incandescent lamp I_1 was focussed on the condenser C by means of a lens L_1 in such a way that the diaphragm D was illuminated homogeneously. The filter combination F_1 transmitted a small region of the spectrum with a maximum at 545 mμ. The image of the diaphragm D was projected in the plane of the preparation. By means of a movable mirror M, this green light could be replaced—without a dark interval—by light from the mercury lamp I_2—Philips HP125W—connected to the same a.c. stabiliser as mentioned above. By means of a lens L_2 the image of the mercury arc was projected on slit S. With the aid of L_3 the slit was projected on the condenser C via M. The filter combination F_2 was the same as used in the photosynthesis measurements. A calibrated logarithmic weakener W enabled us to vary the intensity of the beam.

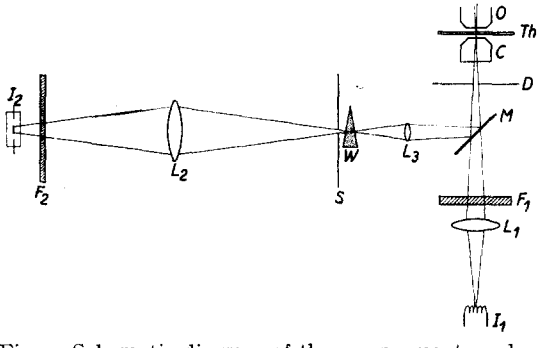

Fig. 2. Schematic diagram of the arrangement used for phototaxis measurements.

The determinations were done as follows. The phototactic action of the mercury lines compared with the light of reference from I_1 was established by adjusting the weakener W in such a way that no shock reactions occurred on changing the beams. Both the upper and lower intensity limits were determined in an analogous way as described by MANTEN[2]. The phototactic action was derived from the formula:

$$W\lambda = \frac{I_{ref}}{\sqrt{I_{max} \cdot I_{min}}}.$$

So, by establishing the phototactic action of both the green and the yellow mercury lines, we were able to compute the ratio $\frac{W_{green}}{W_{yellow}}$.

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RESULTS

The results of experiments with 3- and 4-day old bacteria are represented in Table I and Table II, respectively.

TABLE I

COMPARISON OF THE EFFECTIVENESS W OF GREEN AND YELLOW LIGHT IN PHOTOTAXIS AND PHOTOSYNTHESIS OF BACTERIA FROM 3-DAY OLD CULTURES

Conc. in Tr. u/ml	$\frac{W_{green}}{W_{yellow}}$	
	Phototaxis	*Photosynthesis*
3	85	98
3	81	93
3	80	109
3	90	109
3	87	107*
3	85*	96
3	93	100
2	89*	78
2	86	93
mean	86 ± 3	98 ± 8

* reduced activity

TABLE II

COMPARISON OF THE EFFECTIVENESS W OF GREEN AND YELLOW LIGHT IN PHOTOTAXIS AND PHOTOSYNTHESIS OF BACTERIA FROM 4-DAY OLD CULTURES

Conc. in Tr. u/ml	$\frac{W_{green}}{W_{yellow}}$	
	Phototaxis	*Photosynthesis*
3	78	110
3	76 74	167
3	93 99	176
3	76 80	173
3	81 83	157
2	95*	118*
2	85	108*
2	92* 85*	116*
2	82	222
mean	84 ± 7	150 ± 37

* reduced activity

Though care was taken to maintain favourable culturing conditions (*cf.* THOMAS[1]), activity fluctuations throughout series of experiments could not be avoided. The figures designated by * refer to experiments in which the bacterial activity was reduced to such a degree that the accuracy of the readings decreased. Nevertheless, they were taken into account, too, when computing the mean and the main error. The latter one was calculated according to the formula:

$$\text{m.e.} = \frac{\Sigma (x - \bar{x})}{n - 1}.$$

Table I seems to indicate that, in 3-day old bacteria, the studied ratio in photosynthesis slightly surpasses that in phototaxis. However, as appears from the values of the error it is not permitted to draw a definite conclusion.

To the contrary in 4-day old organisms—Table II—the relative efficiency of light absorbed by carotenoids may be stated to be much more pronounced in photosynthesis than in phototaxis.

As shown in both tables, in phototaxis as well as in photosynthesis readings the variability of the values increases with the age of the bacteria. This may be due to the fact that the temperature of the light cabinet used for growing the cultures was in sufficiently controlled: it varied between about 5° C. So the physiological age of cultures grown at different moments may have differed. The longer the duration of the period of cultivation, the more the influence of these variations will become apparent. This may explain why the results with 4-day old bacteria fluctuate more than those with 3-day old cells.

The difference between the studied ratios with 3- and 4-day old bacteria may be due either to variations in the formation of pigments active in the processes under consideration or in development of—or change in—some enzyme system. Examination of the absorption spectra, of which an arbitrary example is shown in Fig. 3, showed

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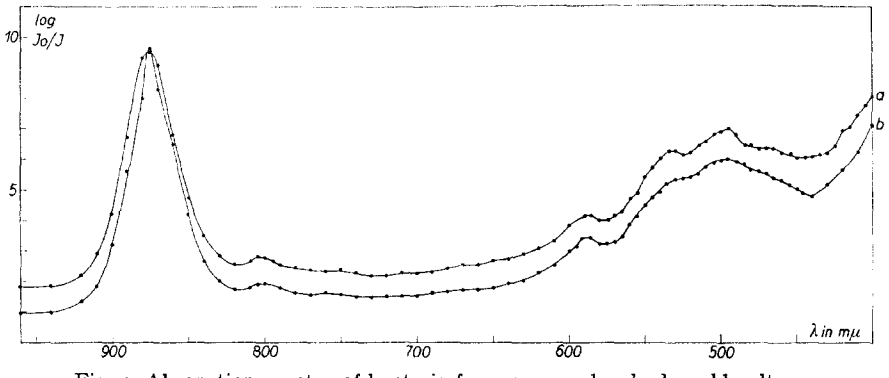

Fig. 3. Absorption spectra of bacteria from 3 -*a*- and 4 -*b*- day old cultures.

us that the deviations herein most probably are too small to account for the difference in question. So, the effect of aging will be due to some change in the enzymic activity. In this respect it may be mentioned here that in bacteria from 14-day old cultures considerable deviations from the normal absorption spectrum were observed (GOEDHEER[9]).

A few additional experiments dealing with the influence of potassium cyanide may be mentioned also. In a previous paper[7] it was stated that this compound affected both phototaxis and photosynthesis of *Rhodospirillum rubrum* in such a way that the light intensity at which the contrast sensitivity starts to decrease coincides with that which just causes photosynthesis saturation. Since, by using sodium light in the mentioned study[7], carotenoid effects were excluded, it seemed interesting to investigate whether potassium cyanide influences the ratio under consideration. The results of these experiments are given in Table III.

Though, with regard to the variability of the results, no decisive conclusion can be drawn, there seems to be some indication that, in phototaxis, the studied ratio tends to increase after addition of potassium cyanide. Some additional photosynthesis experiments were carried out: the results do not—or at least not markedly—deviate from those of experiments in the absence of potassium cyanide.

TABLE III

INFLUENCE OF POTASSIUM CYANIDE ON THE EFFECTIVENESS RATIO OF GREEN AND YELLOW LIGHT IN PHOTOTAXIS AND PHOTOSYNTHESIS. BACTERIAL CONCENTRATION: 2 Tr.u/ml

Conc. KCN in per cent × 10^{-3}	*Age in days*	$\frac{W_{green}}{W_{yellow}}$		
		Phototaxis		*Photosynthesis*
		Control	*KCN*	
0.7	4	77	80	197
1.4	4	77	87	—
1.4	4	—	88	—
1.4	3	77	85	—
3	3	81	86	149
3	4	82	89	110
3	4	79	82	110
6	4	83	82	—
6	4	79	97	—
6	4	81	83	122*
mean	—	80 ± 2	—	—

* reduced activity

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DISCUSSION

Since the present study confirms the previously[1] suspected occurrence of a reduced carotenoid activity in phototaxis as compared to that in photosynthesis we may once more consider the working hypothesis[1] earlier forwarded in order to explain this phenomenon. It was based on the assumption that in *Rhodospirillum rubrum* some as yet unknown energy yielding processes apart from photosynthesis, are stimulated by light absorbed by the carotenoids.

The occurrence of these energy yielding processes was demonstrated in the following way. Bacteria were allowed to accumulate in a small light spot projected on a microscopic preparation under anaerobic conditions. Then the light was turned off. Some minutes afterwards the microscopic field was illuminated homogeneously in order to enable observation. Now the bacteria proved to be distributed homogeneously. So, before becoming motionless, part of the previously accumulated bacteria moved away in the dark. This implies that, after an illumination period, the organisms consume energy. Consequently energy yielding processes must occur. Their nature, however, is still obscure.

EMERSON AND LEWIS[10] demonstrated the occurrence of a comparable phenomenon in *Chlorella*: light absorbed by the carotenoids stimulates oxygen consumption.

According to MANTEN'S hypothesis[2,3], which was supported by subsequent studies[1,7] phototaxis is based on photosynthetic processes. Now, some intermediate product of photosynthesis will be used in various processes, such as the formation of cell constituents—reserve products included—and energy yielding reactions. In some way or another there seems to be a correlation between the concentration of this intermediate and phototaxis. If light absorbed by the carotenoids accelerates the energy yielding processes, it will decrease the concentration in question. Consequently during the stationary state of photosynthesis the concentration of the intermediate will be lower than is suggested by the measured carbon dioxide uptake. A reduction of the intensity of the incident light will lead to a decrease of photosynthesis as well as of carotenoid stimulation of the energy yielding processes. The latter will counteract to some degree the lowering of the concentration of the intermediate due to reduction of the light intensity. For this reason in the case of light absorbed by the carotenoids, the surpassing of the threshold value of the phototactic reaction requires a stronger difference in light intensity than in the cases in which light of other wave-lengths is applied. This means that light absorbed by the carotenoids will be less efficient in phototaxis than in photosynthesis. This working hypothesis is schematically reproduced in Fig. 4.

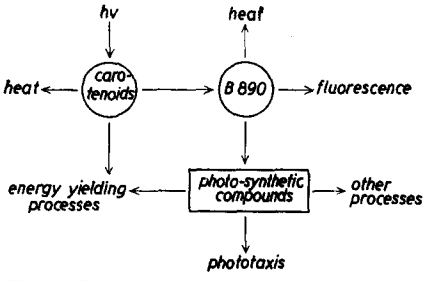

Fig. 4. Hypothetical scheme of the carotenoid action in phototaxis and photosynthesis.

According to DUYSENS[11,12] energy absorbed by carotenoids is transferred to the bacteriochlorophyll type B 890 with an efficiency of about 50%. This value is small in comparison with that of energy transfer from other bacteriochlorophyll types to B 890 and from phycobilins and chlorophyll *b* to chlorophyll *a*, which efficiency is almost 100%. Now it is tempting to suggest that this discrepancy is due to a partial loss of the energy of the excited carotenoid molecules to the said energy-yielding processes.

The energy received by B 890 is partly converted into chemical energy used for

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the synthesis of assimilation compounds. These, in their turn, are used for various processes, such as phototaxis and energy-yielding processes. If now this equilibrium is disturbed by carotenoid-induced increase of the latter processes, the remaining ones, phototaxis included, will be retarded.

In terms of this hypothesis the probable tendency of the relative effectiveness of light absorbed by the carotenoids to increase after addition of potassium cyanide might be interpreted as a blocking effect on the carotenoid-stimulated energy-yielding processes.

It may be remarked that the suggested increase of energy-yielding processes by carotenoid activity is different from the photo-induced interactions in metabolism discussed by KOK[13]. The latter processes are not restricted to light absorption by the carotenoids.

Observations of different chemotactic behaviour towards oxygen of *Rhodospirillum rubrum* grown in light and in the dark made CLAYTON[14] reject "the hypothesis of a direct association between a tactic response and a decrease in the rate of synthetic activity". Instead of this, CLAYTON suggested that "the latent capacity for photosynthesis" may be "the primary associate of the tactic behaviour". A discussion of CLAYTON'S paper is beyond the scope of this study. We only may state that the way in which phototaxis is related to the concentration of the photosynthetic compounds is not indicated in the scheme of Fig. 4.

Finally we should like to emphasize that the above scheme merely represents a working hypothesis, that may offer explanations for some observed phenomena. However, it certainly needs further experimental confirmation.

SUMMARY

The relative efficiency in phototaxis and photosynthesis of light absorbed by carotenoids in *Rhodospirillum rubrum* was determined. Confirming an earlier[1] conclusion, the carotenoid activity proved to be less pronounced in phototaxis than in photosynthesis.

A hypothetical explanation of this phenomenon has been put forward.

RÉSUMÉ

Nous avons étudié l'efficience relative de la lumière absorbée pas les carotenoides chez *Rhodospirillum rubrum*. L'action des carotenoides s'est montrée moins prononcée dans la phototaxe que dans la photosynthèse. Ce résultat affirme la conclusion provisoire.

Nous présentons dans la discussion une hypothèse concernant les phenomènes observées.

ZUSAMMENFASSUNG

Die relative Wirksamkeit des von Carotenoiden von *Rhodospirillum rubrum* absorbirten Lichtes wurde untersucht. Es stellte sich heraus, dass die Aktivität der Carotenoide geringer ist in der Phototaxis als in der Photosynthese. Diese Befunde bestätigen eine ehemahlige Schlussfolgerung.

Eine Hypothese bezüglich der Ursache dieser Erscheinung wird diskutiert.

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