# ON THE RELATION BETWEEN PHOTOTAXIS AND PHOTOSYNTHESIS IN *RHODOSPIRILLUM RUBRUM*

by

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## INTRODUCTION

It has been suggested by MANTEN[1, 2] that, in *Rhodospirillum rubrum*, phototaxis and photosynthesis are correlated processes. This hypothesis was based on two facts. First the phototaxis action spectrum left no doubt that bacteriochlorophyll is active in this process. Second, the contrast sensitivity in phototaxis remains constant at low light intensities, whereas at high intensities of the field of comparison it is strongly reduced. MANTEN pointed out, that this reduction starts at light intensities at which saturation of photosynthesis is usually to be expected in these bacteria (WASSINK, KATZ AND DORRESTEIN[3]). MANTEN remarked: " . . . it is tempting to suggest that the reduction of the contrast sensitivity is due to the attainment of light saturation in photosynthesis". Shock reactions are supposed to be caused by a sudden decrease in the rate of photosynthesis.

MANTEN's hypothesis was supported by the experiments of THOMAS[4], who established a photosynthesis action spectrum at the visible wave-lengths. The location of the minima and maxima of this spectrum, obtained with the same strain MANTEN used, proved to coincide with that of the phototaxis action spectrum. This means that the same pigments are active both in phototaxis and photosynthesis. Though this fact is strongly in favour of the above-mentioned hypothesis still more evidence is wanted. To this purpose the following experiments were performed:

a. exact determination of the light intensity at which the saturation rate of photosynthesis is reached as well as that at which the contrast sensitivity starts to decrease. Both values were determined with bacteria of the same bacterial suspension.

b. influence of photosynthesis inhibitors on the contrast sensitivity at various light intensities.

## MATERIAL

*Rhodospirillum rubrum* strain 4, originally isolated by VAN NIEL[5], was grown anaerobically in a light cabinet at 25–30° C in a liquid medium containing 1% peptone Poulenc and ½% sodium chloride. During the phototaxis experiments the bacteria

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were suspended either in this medium or in a solution of 0.01 *m* phosphate ($p_H$ = 7.2) and 0.015 *m* sodium butyrate. The latter medium was also used in the photosynthesis experiments. The culture technique was the same as previously described (THOMAS[4]); age of the cultures: 3–4 days.

## METHODS

Fig. 1 shows the equipment used for the determination of the contrast sensitivity. The light emitted by the sodium lamp (I) passed a $CuSO_4$ filter (1 cm, 6%) (A) and was made parallel by a lens ($L_1$). Behind $L_1$ a slit (S) was placed. Near to it a photographic step weakener (W) (cf. also Fig. 2) was adjusted. Table I shows the light transmission of the subsequent fields in percents of that of the most transparant one. The weakener could be moved horizontally by means of a screw. The beam was projected by a mirror (M) on an achromatic Leitz No. 3 objective ($L_2$) (adjusted in the microscope instead of a normal condensor) and it was focussed in the plane of the slide (P) under the objective (O). Thus the image of the step weakener was projected on P.

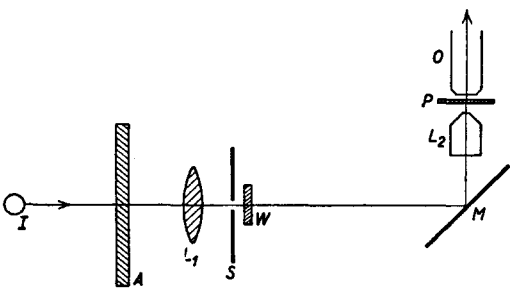


Fig. 1. Diagram of the apparatus

TABLE I

LIGHT TRANSMISSION OF THE SUBSEQUENT FIELDS OF THE PHOTOGRAPHIC STEP WEAKENER

| Field No. | Percentage transmission |
|---|---|
| 0 | 100 |
| 1 | 95 |
| 2 | 87 |
| 3 | 78 |
| 4 | 67 |
| 5 | 61 |
| 6 | 56 |
| 7 | 51 |
| 8 | 46 |
| 9 | 41 |

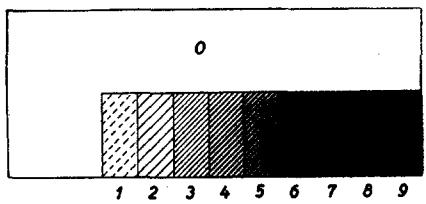


Fig. 2. Step weakener

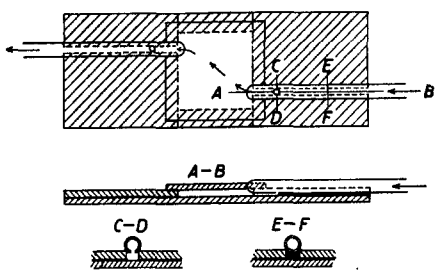


Fig. 3. Microthermostat (all glass)

The two light fields under the microscope were obtained by projecting the image of a part of the step weakener in such a way that the microscopic field was divided into two equal parts by the boundary between field 0 (Fig. 2) and one of the other fields. The intensity of both fields could be varied simultaneously by placing neutral glass filters (Schott NG16) between step weakener and mirror.

The maximal light intensity of field 0 amounted to $2 \cdot 10^4$ ergs/cm$^2$/sec. At this intensity light saturation is reached in photosynthesis. The $CuSO_4$ filter (A), however, could not be used in all experiments since, apart from its infrared absorption, 25% of the sodium light was lost by absorption and reflexion. Only when working with highly sensitive bacteria the filter could be adjusted.

Light intensities were measured with a Cs–$Cs_2O$ barrier layer cell connected to a mirror galvanometer after MOLL. Our thanks are due to Mr L. N. M. DUYSENS for his assistance in building this apparatus.

The temperature of the suspension could be controlled by using a microthermostat (Fig. 3) which enabled us to pass a flow of water of the temperature wanted at a constant rate underneath the slide (a covering glass being used as such).

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The contrast sensitivity was determined by examining at which field of the step weakener the bacteria just show a shock reaction when passing the boundary between this field and the field of comparison (0). So, this procedure is a single cell method.

The computation of the contrast sensitivity occurred as follows. If, *e.g.*, the shock reaction is just observable at the boundary of field 0–3, the contrast sensitivity amounts to 0.78 (Table I). However, if the reaction does not occur at 0–3, whereas it is fully shown at 0–2, it means that the value of the contrast sensitivity lies between 0.78 and 0.87. In the former case the value is plotted in the graph as a single point, in the latter one it is indicated by a vertical line connecting both values, whilst the graph is drawn in between them.

In order to obtain homogeneous and sensitive material the bacterial suspension was centrifuged during some minutes. The spirilla remaining in the supernatant medium were used.

Photosynthesis was determined with the WARBURG technique. The bacteria were centrifuged and taken up to a concentration of 20 Trommsdorff units/cm³ in a medium containing 0.01 *m* phosphate buffer $p_H = 7.2$, and 0.015 *m* sodium butyrate. The gas phase consisted of 5% carbon dioxide and 95% purified ("extra") nitrogen. The last traces of oxygen were removed by passing the gas mixture over an electrically heated copper spiral.

Each vessel was shaken in a beam of sodium light which was made nearly parallel by lenses. Energy measurements were carried out with a thermopile connected to a ZERNIKE mirror galvanometer.

## PRELIMINARY EXPERIMENTS

In order to determine the most suitable temperature to study both phototaxis and photosynthesis some preliminary experiments were done, which may be communicated briefly here. It was stated (GAFFRON[6], THOMAS[4]), that photosynthesis measurements with *Rhodospirillum rubrum* could preferably be made at 35–36° C.

In our phototaxis experiments we had to deal with three complications.

First, the reaction time must be small in order to obtain a complete reversal of the direction of locomotion. Second, the velocity of locomotion must not surpass a certain limit to enable a good observability. Third, it must be possible to obtain light saturation. With regard to these requirements the dependence on temperature of reaction time, as well as of velocity of locomotion, was investigated with spirilla treated in the above mentioned way. During these experiments both contrast and light intensity (about $5 \cdot 10^3$ ergs/cm²/sec) were kept constant. In total 10 experiments were performed. Fig. 4 shows the relation between reaction time and temperature at bacteria of two different cultures. The temperature dependency of locomotion is presented in Fig. 5. The velocity of locomotion is expressed in the time required for covering a distance of 70 $\mu$. In both graphs the results obtained from two different cultures are plotted. Except for the readings at high temperatures in Fig. 3 the values are means of 20 determinations.

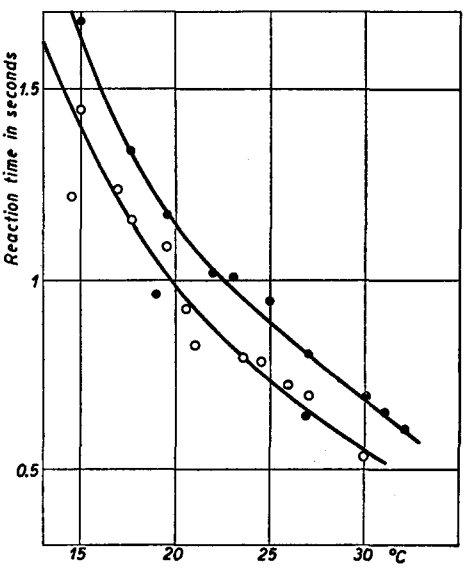


Fig. 4. Influence of temperature on reaction time. Results of two experiments

Next, the relation between temperature and contrast sensitivity was studied. This was done with the same suspension for two light intensities ($6.5 \cdot 10^3$ and $2 \cdot 10^4$ ergs/

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Fig. 5. Influence of temperature on the velocity of locomotion. Results of two experiments

cm²/sec) of the field of comparison. Four experiments were performed. One of them is presented in Fig. 6. It is shown that (reading the graph from right to left) at the light intensity of $6.5 \cdot 10^3$ ergs/cm²/sec the contrast sensitivity starts to decrease at lower temperature than it does at $2 \cdot 10^4$ ergs/cm²/sec. This phenomenon fits well in MANTEN's hypothesis, for, if phototaxis be based on photosynthesis, light saturation in the variable field will be reached sooner at lower temperature. In this way the difference between the photosynthetic rates corresponding to both fields will diminish. So a decrease of the contrast sensitivity will be found at lower temperatures when lower intensities are used.

## RESULTS

### *I. Relation between the minimal light intensities required for saturation of photosynthesis and for decrease of the contrast sensitivity in phototaxis*

With regard to the above-mentioned data as well as to measurements of photosynthesis (THOMAS[4]) the combined experiments were run at 30–31° C, using bacteria of the same culture, suspended in sodium butyrate solution. The suspensions used for the phototaxis experiments were saturated with the same gas mixture as used in the Warburg vessels. Fig. 7 shows one of these experiments. Since the transition ranges between the horizontal and the declining parts of the graphs are rather extended

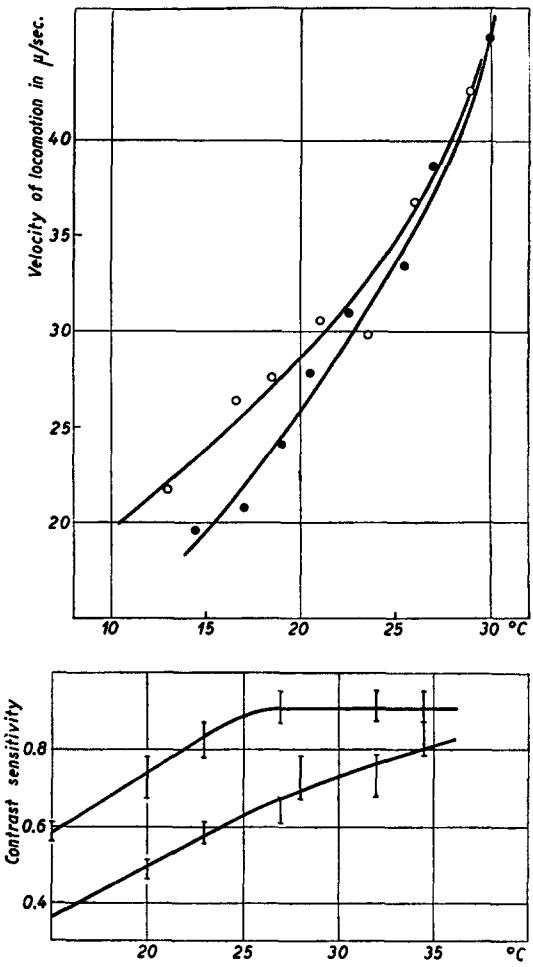


Fig. 6. Influence of temperature on contrast sensitivity at two light intensities

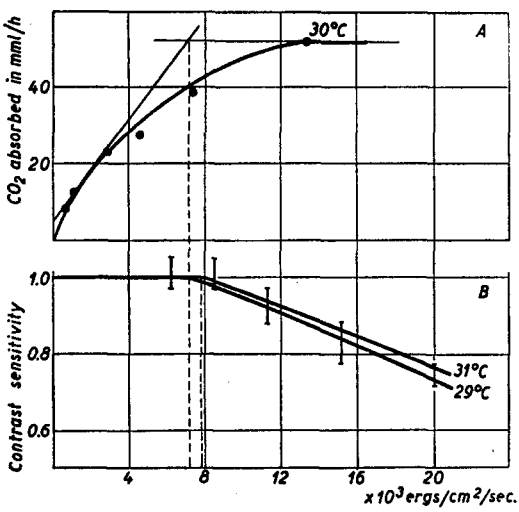


Fig. 7. Photosynthesis (A) and contrast sensitivity (B) in relation to light intensity

we determined the theoretical transition point by drawing the tangents. In order to do this as objectively as possible one tangent was represented by a straight line drawn through three "low intensity points". The other tangent was made by drawing a line parallel to the abscissa through the "light saturation point". As can be read from the graph in this way, the light intensity at which the saturation rate of photosynthesis is reached corresponds rather well with that at which the contrast sensitivity starts to decrease.

Fig. 8, in which both critical light intensities mentioned above are plotted versus each other, shows that this holds in the other experiments too. The point indicated "KCN" is derived from an experiment in which KCN (0.03%) had been added. The position of the other points is determined by the natural variability of the saturation rates of the studied processes. So we may conclude that a direct proportionality exists between both phototaxis and photosynthesis with regard to the illumination at which the dependency on light intensities of both processes is changing.

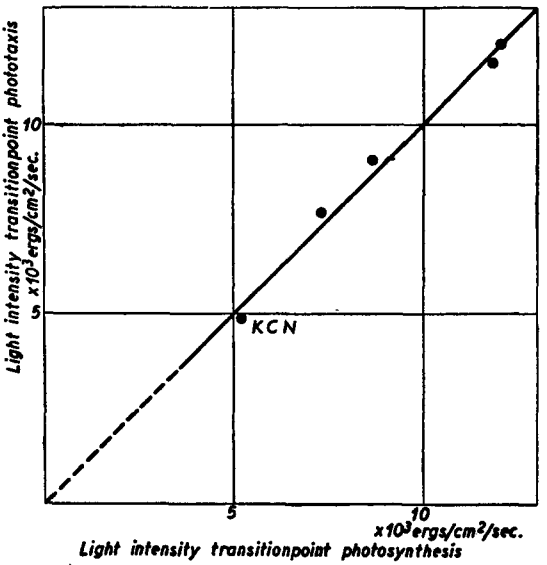


Fig. 8. Relation between the light intensity values at the transition points in the rate of photosynthesis and the value of contrast sensitivity in dependency on light intensity

*II. Influence of potassium cyanide*

a. *Effect on photosynthesis.* According to WARBURG[7], using *Chlorella*, sodium cyanide preferably inhibits the BLACKMAN reaction. GAFFRON[8] demonstrated that photosynthesis in a *Thiorhodacea, Thiocystis*, is very sensitive to this compound. A quantitative determination of its influence proved to be difficult, since the sodium cyanide soon disappears by reaction with the sulphur compounds. WASSINK, KATZ AND DORRESTEIN[3] succeeded in obtaining reproducible results in an other *Thiorhodacea Chromatium* strain D. Here, in the same way as in green algae, (*Chlorella* (WARBURG[7], EMERSON[9], WASSINK, VERMEULEN, REMAN AND KATZ[10]), *Scenedesmus* (GAFFRON[11])), and diatoms (WASSINK AND KERSTEN[12]) the BLACKMAN reaction is preferably inhibited.

This also holds for the *Athiorhodacea Rhodospirillum rubrum*. Fig. 9 shows a marked impedance of the BLACKMAN reaction by 0.0015% cyanide.

b. *Effect on phototaxis.* In series of four experiments potassium cyanide was added in concentrations ranging from 0 to 0.002%. One of these is presented in Fig. 10. It is shown that KCN, dependent on its concentration, shifts the decrease of the contrast sensitivity to lower light intensities. This relation is demonstrated furthermore by Fig. 11, in which the KCN concentrations are plotted versus the light intensities at which the contrast sensitivity starts to decrease. In this figure the data of all experiments are summarized.

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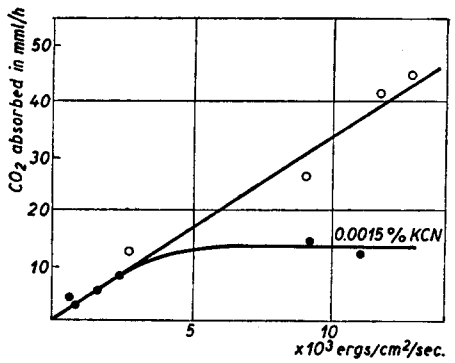


Fig. 9. Influence of KCN (0.0015%) on photosynthesis (o: control)

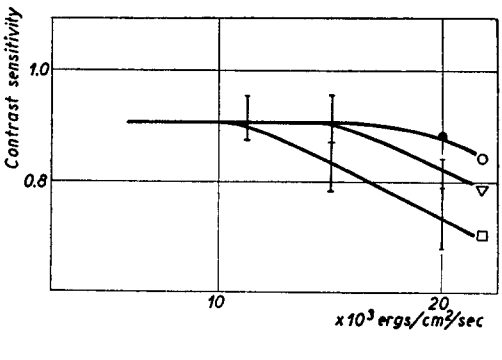


Fig. 10. Influence of KCN (o: control, ▽: 0.001%, □ : 0.0015%) on contrast sensitivity

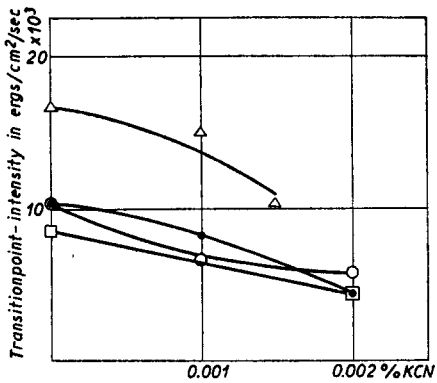


Fig. 11. Relation between the concentration of KCN and the light intensity at which the contrast sensitivity starts to decrease (four experiments)

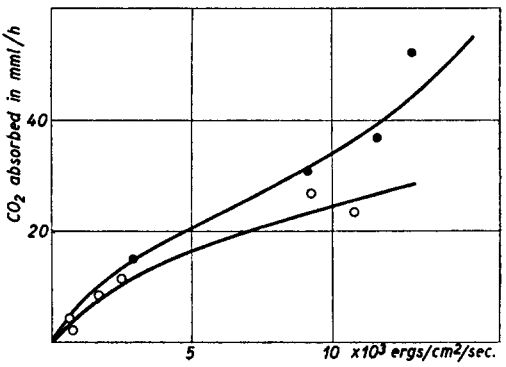


Fig. 12. Influence of ethyl urethan (0.03%) on photosynthesis (•: control)

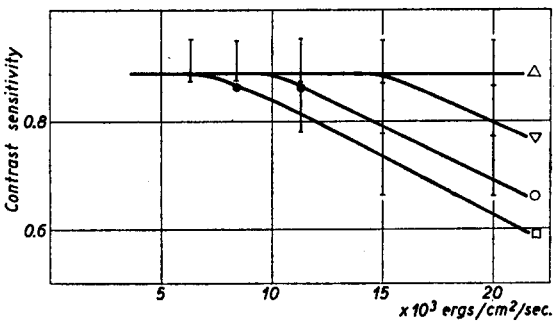


Fig. 13. Influence of ethyl urethan (□ : control, o: 0.01%, ▽: 0.02%, △ 0.03%) on contrast sensitivity

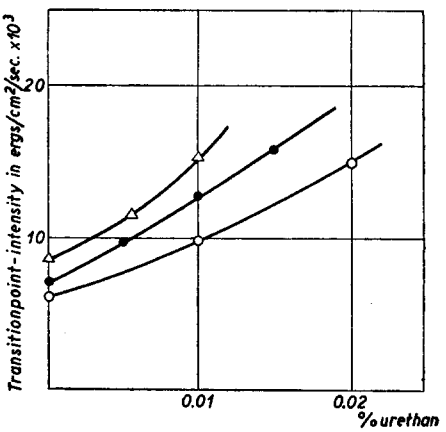


Fig. 14. Relation between the concentration of ethyl urethan and the light intensity at which the contrast sensitivity starts to decrease (three experiments)

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*III. Influence of ethyl urethan*

a. *Effect on photosynthesis.* Unlike to cyanide urethan acts on both the photochemical and the dark reactions as it was found to do in green algae, diatoms and *Thiorhodaceae* by WARBURG[7], WASSINK *et al.*[3, 12, 10]. One of our experiments is shown in Fig. 12, indicating that *Rhodospirillum rubrum* is sensitive to urethan in the same way as the above-mentioned organisms.

b. *Effect on phototaxis.* Three experiments were done in which ethyl urethan was added. For an example see Fig. 13.

Ethyl urethan appears to shift the decrease of the contrast sensitivity to higher light intensities. The data on the relation between ethyl urethan concentration and light intensity at which the contrast sensitivity starts to decrease are summarized in Fig. 14. In these experiments the temperature was kept at about 25° C. In this way the contrast sensitivity starts to decrease at a lower light intensity, enabling us to extend the range of concentrations used.

## DISCUSSION

As was shown above (Fig. 8), the decline of contrast sensitivity starts at the light intensity at which light saturation is reached in photosynthesis. This turned out to be true for spirilla of different "states of activity". The "activity" of different suspensions varies somewhat in both processes, even if the culture conditions are kept constant. By careful growing of the bacteria the differences can be reduced. We did not succeed, however, in eliminating them totally (THOMAS[4]). In this study, however, we used this variability as a means to check the above relation at different light intensities. In one experiment the activity state was depressed artificially by means of potassium cyanide. Also in this case the critical light intensities for changing the rate of both phototaxis and photosynthesis are the same.

These results prove that MANTEN[1, 2] was right in assuming the existence of such a relation, in this way giving support to his theory that phototaxis is based on photosynthesis.

Further evidence for this theory is furnished by our observations on the influence of photosynthesis inhibitors on phototaxis. It was shown that cyanide shifts the transition point intensity in the phototaxis curve towards lower values, whereas by urethan it is shifted towards higher light intensities.

As mentioned above cyanide preferably inhibits the BLACKMAN reaction in photosynthesis. That means that the light intensity at which this reaction becomes saturated will be lowered by cyanide. This implies that the contrast sensitivity in phototaxis also starts to decrease at lower light intensities, if this phenomenon is based on a sudden drop in the rate of photosynthesis. For light saturation will be obtained in the variable field at reduced light intensity. In this way the photosynthesis rates in both the variable and the constant field will equal each other sooner, and so the contrast sensitivity will be decreased as mentioned above.

Urethan, on the other hand, inhibits both the light and dark processes in photosynthesis, as shown by WASSINK *et al.* From fluorescence measurements these authors concluded that urethan primarily attacks the energy transfer system. In a theoretical paper (DORRESTEIN, WASSINK AND KATZ[13]) it is suggested that urethan competes in accepting the energy of an excited (bacterio-)chlorophyll molecule. So in the presence of

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this substance the excitation energy of the chlorophyll is released in four ways: as heat, as fluorescence, as chemical energy for the dark photosynthesis processes, and as energy transferred to urethan.

In terms of this theory the influence of urethan on phototaxis can be readily explained. Since the excitation energy is partly transferred to this substances, higher light intensities are required to obtain saturation in photosynthesis. Hence, the difference in photosynthetic rates in the bacteria in both light fields in the phototaxis experiments, and, with them, the contrast sensitivity will only be decreased at higher light intensities.

The above data strongly support MANTEN's point of view that phototaxis may be based on photosynthetic processes.


## SUMMARY

The relation between phototaxis and photosynthesis in *Rhodospirillum rubrum* has been studied. The light intensity at which saturation is reached in photosynthesis proved to coincide with that at which the contrast sensitivity starts to decrease.

Potassium cyanide, which preferably inhibits the BLACKMAN reaction in photosynthesis, decreases the light intensity at which the contrast sensitivity starts to decrease.

Ethyl urethan, which preferably affects the energy transfer in photosynthesis, increases this intensity.

These data strongly support MANTEN's hypothesis, according to which phototaxis is based on a sudden decrease of the photosynthetic rate.

## RÉSUMÉ

Nous avons étudié le rapport entre la phototaxie et la photosynthèse chez *Rhodospirillum rubrum*.

La saturation de la photosynthèse est obtenue par la même intensité de la lumière incidente, qui produit une diminution de la sensibilité de contraste.

La cyanure de potassium, qui inhibe surtout la réaction de BLACKMAN, diminue l'intensité à laquelle la sensibilité de contraste est réduite.

L'éthyluréthane, qui influence principalement le transfert d'énergie, augmente cette intensité.

Ces résultats soutiennent l'hypothèse de MANTEN, qui suppose que la phototaxie est basée sur une diminution instantanée de la photosynthèse.

## ZUSAMMENFASSUNG

Die Beziehung zwischen Phototaxis und Photosynthese wurde beim *Rhodospirillum rubrum* untersucht. Die Lichtintensität, bei welcher die Sättigung der Dunkelprozesse der Photosynthese erreicht wird, erniedrigt ebenfalls die Kontrastsensibilität.

Blausäure, welche die Dunkelprozesse hemmt, erniedrigt die obengenannte Intensität, während Äthylurethan, welche die Energie-übertragung angreift, diese Intensität erhöht.

Diese Ergebnisse bestätigen die Hypothese von MANTEN, der annimmt, dass die Phototaxis sich auf einer schnellen Erniedrigung der Photosynthese basiert.


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