STUDIES IN THE PHOTOTAXIS OF RHODOSPIRILLUM RUBRUM

Thesis by

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ABSTRACT

The bacterium Rhodospirillum rubrum is capable of a photosynthetic reduction of CO_2 similar to plant photosynthesis. A decrease in the illumination of R. rubrum induces it to reverse its direction of swimming; an investigation of the probability of this phototactic response as a function of the parameters of the stimulus, such as the intensity of illumination and its time pattern, should enhance our understanding of biological irritability.

The present investigation is preliminary to such an undertaking. It was attempted to learn more about the responding system through studies of the interaction between the pigments, phototaxis, and metabolism of R. rubrum. A determination was made of the relative spectral effectiveness of light in promoting a phototactic response; this measurement provided evidence that in addition to bacteriochlorophyll the predominant carotenoid pigment of rubrum absorbs phototactically active light. It has been suggested that the tactic response of R. rubrum is associated directly with a decrease in its metabolic rate; studies of the tactic response to oxygen yielded evidence that this hypothesis must be abandoned.

The remarkable adherence to the Weber Law reportedly displayed in the phototaxis of R. rubrum was reinvestigated; an adherence over a much smaller range of intensities was observed than had been reported earlier. The masking, under certain conditions, of a potentially close Weber Law adherence was discussed.

CONTENTS

I. INTRODUCTION

Because of their striking pigmentation, their singular phototactic movements, and their unique photosynthetic metabolism, the purple bacteria (Thiorhodaceae and Athiorhodaceae) have been the subject of numerous studies in the past seventy years. These studies have fallen into three broad classes: observations of the response to changes of illumination, examinations of the pigments involved, and investigations of the metabolism.

a. Phototactic response: In 1883 Engelmann (1) published his observations of the behavior under illumination of Bacterium photometricum, a polarly flagellated representative of the Thiorhodaceae. Engelmann observed that if a B. photometricum, swimming in an illuminated region, encounters a darker region it will usually recoil, pause briefly, and then resume swimming in whatever direction it points after the "shock reaction". In 1907 Molisch (2) reported similar observations, principally on Rhodospirilla. These bacteria, swimming with equal facility forward and backward (the presence of one flagellated end makes this distinction possible), reverse their direction of swimming if subjected to a sufficiently great and abrupt decrease of illumination. An increase of illumination, on the other hand, evokes no response. The phototactic responses of purple bacteria result from decreases in the intensity of light impinging upon them. In a uniform field of illumi-

nation they show no tendency to orient themselves so as to swim toward the source of light, as do Euglena and Peridinium. These latter organisms rotate about their axis of progression when swimming; an "eye-spot" of local pigmentation thus receives changes of illumination unless the swimming direction is parallel to the direction of propagation of a uniform beam of light. These changes of illumination induce tactic responses which tend to align the organism so as to swim toward the source of light. Thus in a convergent beam Euglena will tend to swim toward the light source even though this tendency carries it in the direction of decreasing intensity; in contrast the purple bacteria will tend to accumulate in the more intense end of the beam as a result of the phototactic response to the entry into a region of lesser intensity.

Engelmann made the observation, confirmed by Molisch, that purple bacteria come to rest after a few minutes in darkness, regaining their motility after a few seconds' exposure to light. Also the speed of swimming appeared to increase with increasing illumination. Both workers noticed that oxygen inhibits the photo-response and that the purple bacteria avoid high concentrations of oxygen, presumably by a chemotactic response similar to the phototactic one. Finally, Molisch observed that a sustained decrease of illumination evokes in Rhodospirillum a succession of swimming reversals; when the first reversal fails to restore a

bacterium to its former high illumination it appears to try again and again.

Following Molisch, Buder (3) undertook an exhaustive study of the mechanics of locomotion of purple bacteria, observing the changes in flagellar movement which accompanied the phototactic response. Buder also made a number of semi-quantitative observations relating the response of Thiospirillum to the magnitude and duration of the light stimulus. First, he observed that the sensitivity to light is concentrated at the flagellated end of the organism. Second, he observed a response to increases of intensity rather than decreases when the original intensity was 1000 meter-candles and higher. At lower intensities he examined cursorily the possibility that the phototactic response might follow the Weber Law. This law, better described as an approximate empirical rule, requires that the least perceptible change in an environmental factor is a constant fraction of the original intensity of the factor. For example, if the threshold for discrimination of pitch by the human ear is one percent, then a tone of 300 cps can just be distinguished from one of 297 cps or of 303 cps, while a tone of 3000 cps must be altered by 30 cps before the change can be perceived. In the case of bacterial phototaxis, adherence to the Weber Law would require that the least perceptible (as evidenced by a phototactic response)

decrease in light intensity $(\Delta I)_m$ be a constant fraction of the original intensity I_o; $(\Delta I)_m/I_o = C$. Buder observed that the Weber Law was not adhered to over the range of intensities studies by him; he found that at 20 meter-candles the threshold stimulus is a 10% decrease, while at 200 m. c. the liminal decrease is 25%. Further, Buder recognized, as had Molisch, the existence of accommodation; the effectiveness of a decrease of illumination becomes greater as the decrease is made more abrupt. Finally, he observed that a pulse of decreased illumination might be ineffective because of its short duration, and that two such subliminal pulses can be summed, if closely spaced, to evoke a response.

The next important study of the kinetics of bacterial phototaxis was made by Schrammeck in 1935 (4). Schrammeck, working with Rhodospirillum rubrum, found that the sensitivity to decreases of intensity does indeed follow the Weber Law closely over a great intensity range. He observed that from about .05 m.c. to about 300 m.c. a decrease of 5% of the initial intensity was sufficient to elicit a phototactic response in the majority of an active population of the bacteria. Below .05 m.c. the liminal fractional decrease was found to rise, attaining a limiting value of 100% at about .005 m.c., which was the threshold intensity for discrimination between light and darkness. Above 300 m.c. the liminal fractional

decrease also rose as the intensity of illumination became saturating for the bacteria. It is possible that Buder might have observed adherence to the Weber Law had he worked with the low intensities employed by Schrammeck.

A few more facts pertaining to the kinetics of phototaxis have emerged recently in the course of researches by Manten (5) and by Thomas and Nijenhuis (6): The latent period between application of stimulus and initiation of response, of the order of one second, becomes less as the magnitude of the stimulus is increased, and falls from 1.5 sec to .5 sec as the temperature is raised from 15°C to 30°C (for a given stimulus). The speed of swimming, which Thomas and Nijenhuis observed to be about 20 μ/sec at 15°C, is approximately doubled by the same increase in temperature. Finally, the intensity at which the phototactic sensitivity begins to fall (the saturating intensity) increases with increasing temperature, suggesting that the saturation is due to a rate-limiting dark reaction, as the case of plant photosynthesis.

From the foregoing account it appears that our knowledge of the kinetics of the responses of purple bacteria to light stimuli is quite fragmentary; as an example of cell irritability the phototaxis of bacteria would seem to deserve the same thorough kinetic study that has been made of the excitation, by electric current, of nerves.

b. Pigments: While Engelmann and earlier observers

had surmised that in the purple bacteria they were dealing with a complex of more than one pigment, it remained for Ewart to show in 1897 (7) that a red and a green component could be separated by differential extraction with solvents. Molisch (2) obtained absorption spectra of these components which accounted, in the visible region, for the maxima in the absorption spectra of the living bacteria obtained by Engelmann (1,8,9) and by himself. He speculated cogently that the red and green components were analogous to the carotenes and chlorophyll of algae.

More recent studies have brought our knowledge of these pigments to the following state: As was shown by French (10,11), both the green and the red pigments are conjugated to proteins. Infra-red maxima in the absorption spectra of intact purple bacteria are due to the green pigment. They occur in various positions between 800 mμ and 900 mμ (at about 875 and 800 mμ in the case of Rhodospirillum rubrum); the diverse locations of these maxima in different species are probably due to conjugation of the pigment to different proteins. In all cases an alcoholic extract of the green pigment, no longer conjugated to a protein, shows only one infrared maximum at 774 mμ , as was demonstrated by Wassink et al. (12,13). Additional absorption maxima of the green pigment, not shifted by

extraction with alcohol, occur at 590 mμ and in the violet. The work of Schneider (14), of Fischer at al. (15, 16), and of Stern and Pruckner (17) has shown the green pigment to be a tetrapyrrole similar in structure to chlorophyll a; at present it is referred to as bacteriochlorophyll.

The red component, responsible for absorption maxima in the blue and green (at about 480, 510, and 550 mμ in the case of R. rubrum), yields numerous fractions which have been characterized as carotenoids of various kinds by Karrer et al (18,19,20,21). More recent work by van Niel and Smith (22) and by Polgar, van Niel, and Zechmeister (23) has shown that in R. rubrum there is one predominant carotenoid, spirilloxanthin, which comprises at least 95% of the red pigment complex. The readiness with which spirilloxanthin undergoes trans-cis isomeric changes upon extraction throws doubt upon the existence in vivo of the welter of different carotenoids separated by various workers. Spirilloxanthin was found by Polgar, van Niel and Zechmeister to be a carotenoid having thirteen double bonds and the empirical formula $C_{40}H_{54}(OCH_3)_2$; in benzene it shows absorption maxima at 545, 508, and 479 mμ .

A discussion of the role played by these pigments in the activities of the purple bacteria will follow a brief characterization of their metabolism.

c. Metabolism: The chronological development of our understanding of the metabolism of the purple bacteria has been presented clearly and thoroughly in a well-annotated review by van Niel in 1944 (24). The subject will be reviewed in some detail in Sec. V; we shall limit ourselves here to a short sketch based on the above-mentioned paper and on a recent lecture by van Niel (25).

The principal metabolic activity is the reduction of CO_2 with the concomitant oxidation of any of a number of organic substances or other suitable hydrogen donors; simple organic acids and alcohols, H_2, and H_2S serve well as substrates. The role of the substrate as H donor is shown strikingly by the quantitative conversion of secondary alcohols to the corresponding ketones by the Athiorhodaceae (26). The oxidation of some substrates can yield CO_2 or higher intermediates which can in turn be metabolized to cell materials, making an external supply of carbonate unnecessary for growth. Light, when it is available to the purple bacteria, serves as a source of energy for the reduction of CO_2. The Athiorhodaceae are not limited, however, to a photosynthetic metabolism; under aerobic conditions in darkness they are capable of reducing CO_2 chemosynthetically, obtaining the necessary energy from the reduction of O_2. With a newly isolated strain this capability must usually be developed by a period of adaptation to oxygen.

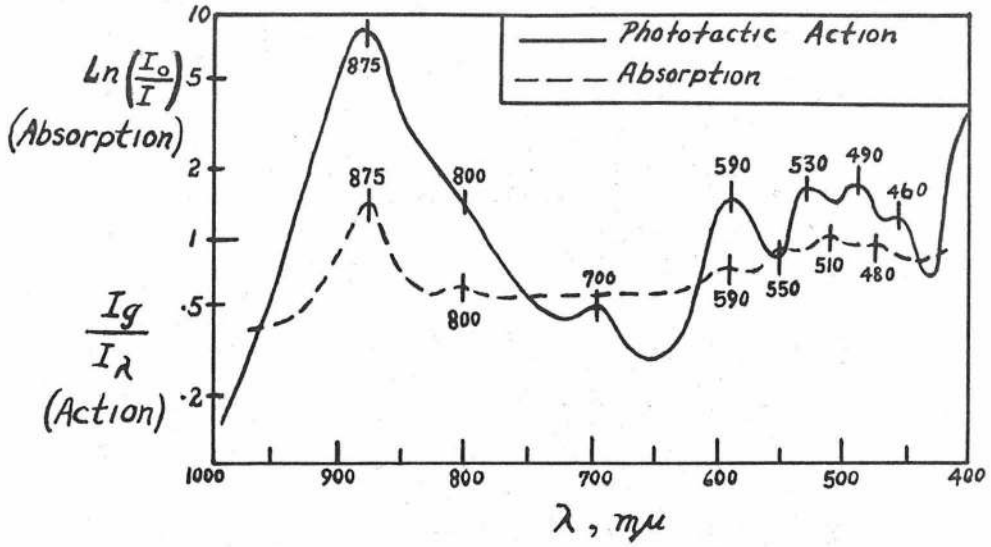
d. Relations between tactic behavior, metabolism, and pigments: It has been assumed, in keeping with our knowledge of algal and higher plant photosynthesis, that the primary absorber of photosynthetically active light is the bacteriochlorophyll. If the carotenoids absorb photosynthetically active light the energy is presumed to be transferred to the chlorophyll molecule, possibly by a mechanism analogous to internal conversion as suggested by Arnold and Oppenheimer (27).

The first action spectrum of photosynthesis (rate of CO_2 uptake vs. wave length at constant light intensity) was obtained for Rhodospirillum rubrum by French in 1937 (28). The spectrum, showing peaks in the infra-red and yellow and no structure in the blue, indicated that the bacteriochlorophyll alone absorbs photosynthetically active light. Furthermore, numerous experiments on growth of purple bacteria in a spectrum (mentioned in 24) have shown that growth does not occur in the blue portion of the spectrum. It should be remembered, however, that the intensity in a spectrum is usually much lower in the blue than in the red, both because of the greater dispersion of a prism in the blue and because of the smaller emissivity of the common light sources in that region. In an equal-intensity spectrum growth might have been observed in the regions of carotene absorption as well as in the regions of chlorophyll absorption.

An action spectrum of phototaxis of R. rubrum was obtained in 1948 by Manten (5). In Manten's measurements a divided field of illumination, each half at a different wave length, was projected onto a slide bearing a layer of bacterial culture. In general the bacteria responded phototactically when crossing the boundary dividing the field; one side, of one color, appeared darker to the bacteria than the other side, of another color. Manten found, however, that the two halves of the field could be so adjusted in intensity that they appeared equally bright to the bacteria; i.e., so that no phototactic response would occur at the boundary. Using filtered green light of a certain intensity I_g in one half of the field, Manten determined the intensity I_λ of light of wave length λ which, when applied in the other half of the field, matched the green half (i.e., abolished the tactic response at the boundary). By repeating this experiment at many wave lengths and plotting I_g/I_λ vs. λ, Manten obtained an action spectrum which expresses the brightness, as perceived by R. rubrum, of light as a function of its wave length. The word "perceived" in the foregoing sentence is used, of course, in an operational sense based on the act of phototaxis. Manten's action spectrum is shown in Fig. 1; for comparison a plot of an absorption spectrum of a suspension of the living bacteria, also obtained by Manten, is shown (the

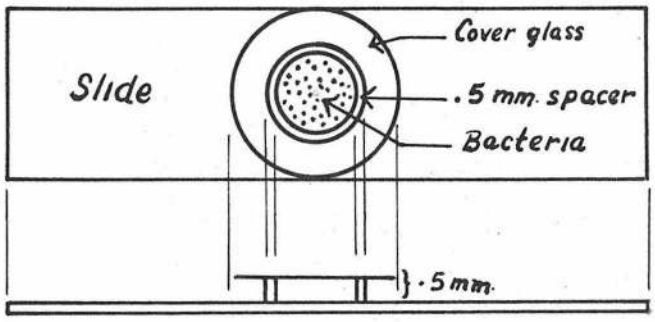
absence of sharp structure in the absorption spectrum is due to strong scattering). The absorption maxima at 875, 800, and 590 mμ are due to the bacteriochlorophyll; those at 550, 510, and 480 mμ are due to spirilloxanthin. It is apparent that although the phototactic action spectrum shows structure in the blue, the structure does not reflect absorption by the spirilloxanthin. Manten concluded that light absorbed by the spirilloxanthin is not perceived by the bacteria (as evidenced by their phototaxis) and that the blue structure in his action spectrum corresponds to absorption by other carotenoids. The great perponderance of spirilloxanthin over all other red and yellow pigments weighs against such a possibility; this question will be considered in detail later.

Manten hypothesized that the phototactic response of R. rubrum is mediated by an abrupt change in the rate of photosynthesis; if this is the case the action spectra for phototaxis and for photosynthesis should have the same form. Investigating this, Thomas (29) obtained an action spectrum of CO_2 uptake agreeing substantially with Manten's phototactic action spectrum and contradicting French's findings (28). Subsequently Thomas and Nijenhuis (6) found that the saturating light intensity for photosynthesis coincides with that for phototaxis in R. rubrum, and that the two are reduced equally by KCN and raised by urethane.



PHOTOTACTIC ACTION AND ABSORPTION SPECTRA FOR RHODOSPIRILLUM RUBRUM (A. MANTEN)

Fig. 1



OBSERVATION CHAMBER

Fig. 2

The foregoing description of two conflicting photosynthetic action spectra and of a phototactic action spectrum showing unexpected behavior in the blue indicates that the question of the relationships between the pigments and the vital activities of the purple bacteria is not well resolved at present.

e. Use of Rhodospirillum rubrum in the study of irritability: While much work has been done on the metabolism and the pigments of R. rubrum, its phototaxis as a simple example of biological irritability has been subjected to little quantitative study. In contrast, the responses of nerve fibers to stimuli have been studied most exhaustively in an effort to characterize the manner in which a living cell responds to a change in its environment.

In its resting state the membrane of a nerve fiber is electrically polarized. If an electric current is passed through a portion of the fiber the membrane is depolarized to some extent, so that the surface of the stimulated portion of the fiber becomes electrically negative compared with the rest of the surface. The surface then returns to its resting state if the strength and duration of the stimulating current were such that the stimulus was below a threshold value. With a stimulus above this value the locally depolarized condition develops into a fully excited state, signalled by an overshoot of

polarization in the opposite direction from the original one. The excited condition then spreads over the remainder of the nerve fiber membrane, propagated by small circulating currents at the boundary of the excited region. In the meantime the region initially excited returns to its resting state, followed by the region adjacent to it, so that a wave of repolarization follows the wave of excitation. An above-threshold stimulus results, then, in an excitation of the fiber which is signalled by a band of electrical negativity progressing down the length of the fiber. The degree of negativity is independent of the magnitude of the initial stimulus as long as the latter is above the threshold for excitation (the response is all-or-none).

Immediately after excitation the fiber is abnormally resistant to another stimulation; this refractory condition, induced by the foregoing stimulation and excitation, wears off as the fiber remains in a resting state. The rise of refractoriness during stimulation is manifested also in the phenomenon of accommodation: if the stimulating current is increased gradually it may fail to excite the nerve, even though it rises to a strength which would have been well above threshold had it been turned on abruptly.

With a stimulus consisting of a square pulse of current of strength I and duration t, the excitation of

the nerve fiber requires a minimum (threshold) value of the product It; this reciprocity of intensity and duration fails for very long durations because of the rise of refractoriness during stimulation. Beyond a critical duration (the utilization time) no further prolongation of the pulse is effective; the current strength must attain a threshold value (the rheobase) for excitation.

If a subliminal pulse of stimulating current is followed closely by another the two may add, by virtue of the current-duration reciprocity, to give an above-threshold stimulus. This summation breaks down if the pulses are not so closely spaced; the refractoriness induced by a subliminal pulse can even prevent the excitation of the fiber by a subsequent pulse which ordinarily would be well above threshold.

Finally, the maintenance of a constant current of the proper strength can cause rhythmically repeated excitations of a nerve fiber, the frequency of impulses being determined by the current strength and by the speed of recovery of the fiber after each excitation.

Attempts to account for the effects we have just considered have involved the use of two variables in defining the state of the nerve fiber. One, the degree of excitation, symbolizes the extent to which the process culminating in excitation has progressed. The other,

the degree of refractoriness, denotes the amount of stimulation which is required for excitation. By making appropriate assumptions regarding the dependence of these variables upon each other and upon time one can account for all of the phenomena mentioned in the last few paragraphs. Such a treatment has been carried out successfully by Bonhoeffer (30) for the activation of iron wire in nitric acid; this intriguing model of nerve excitation displays all of the phenomena just described. In this case the degree of excitation was identified with the electrochemical potential across the surface of the wire and the degree of refractoriness was identified with the threshold for activation of the wire (a threshold pulse of stimulating current is required for activation). These variables could be measured at various stages of the excitatory process; a logical extrapolation of these observations predicted an interplay between the variables which accounted for the effects of utilization time, rheobase, accommodation, summation, and rhythmicity.

It would be of interest to determine what features of the response of a nerve fiber are characteristic of the responses of living cells in general, and whether the quantitative aspects of these features are similar in different cases of irritability. The phototactic response of R. rubrum should be highly suitable for such an investigation, being a definite all-or-none response of a single

cell to a stimulus which is easily controlled and measured. A temporary decrease of illumination might be used analogously to the current pulse employed in nerve studies; the responses of the bacteria to various types and sequences of such light-reduction pulses should yield information on the presence or absence of such phenomenaas refractoriness, accommodation, reciprocity of strength and duration of stimulus, rheobase, utilization time, summation, and rhythmicity. If such effects are found to exist, and to resemble the effects found in nerves in their quantitative aspects, they may then be interpreted provisionally as resulting from the interplay of a degree of excitation and a degree of refractoriness. The physico-chemical processes mediating the stimulation to the response can be expected to differ widely in the two cases of phototaxis and nerve excitation; in the former case the mediation appears to involve the metabolic activities of the cell. Accordingly the physical meanings of the terms "degree of excitation" and "degree of refractoriness" might be quite different in the two cases. Nevertheless, it would be of interest to determine what features of the overall relation between stimulus and response are held in common, quantitatively as well as qualitatively, by bacterial phototaxis and by nerve excitation, and by other responses of living systems.

f. Experiments undertaken: Progress in our understanding of cell irritability must not depend solely on superficial observations of the relations between stimulus and response. Without a detailed investigation of the nature of the responding system one would obtain a collection of relationships which would admit of many plausible interpretations. For this reason it was decided to repeat some basic experiments with R. rubrum which have led to contradictory results, employing more refined methods where possible.

Perhaps the most unsatisfactory state of affairs in our knowledge of the purple bacteria is the contradictory evidence concerning the action spectra of phototaxis and photosynthesis and the uncertainty surrounding the role of the carotenoid pigments in absorbing photosynthetically and phototactically active light. Accordingly a repetition of the phototactic action spectrum measurement was undertaken, using a technique similar to Manten's but with a higher degree of spectral purity. An investigation of factors which could invalidate such action spectra was also undertaken.

The significance in biology of the Weber Law (that the liminally perceived change in a stimulus is a constant fraction of the original strength of the stimulus) has been debated at great length, and the remarkable Weber Law adherence exhibited in bacterial phototaxis, as

reported by Schrammeck (4), has been a key case in point. Since a mechanism causing at least an approximation to Weber Law adherence might be a fundamental property of irritable cells it was decided that the striking results reported by Schrammeck would bear confirmation, again with whatever refinements were feasible.

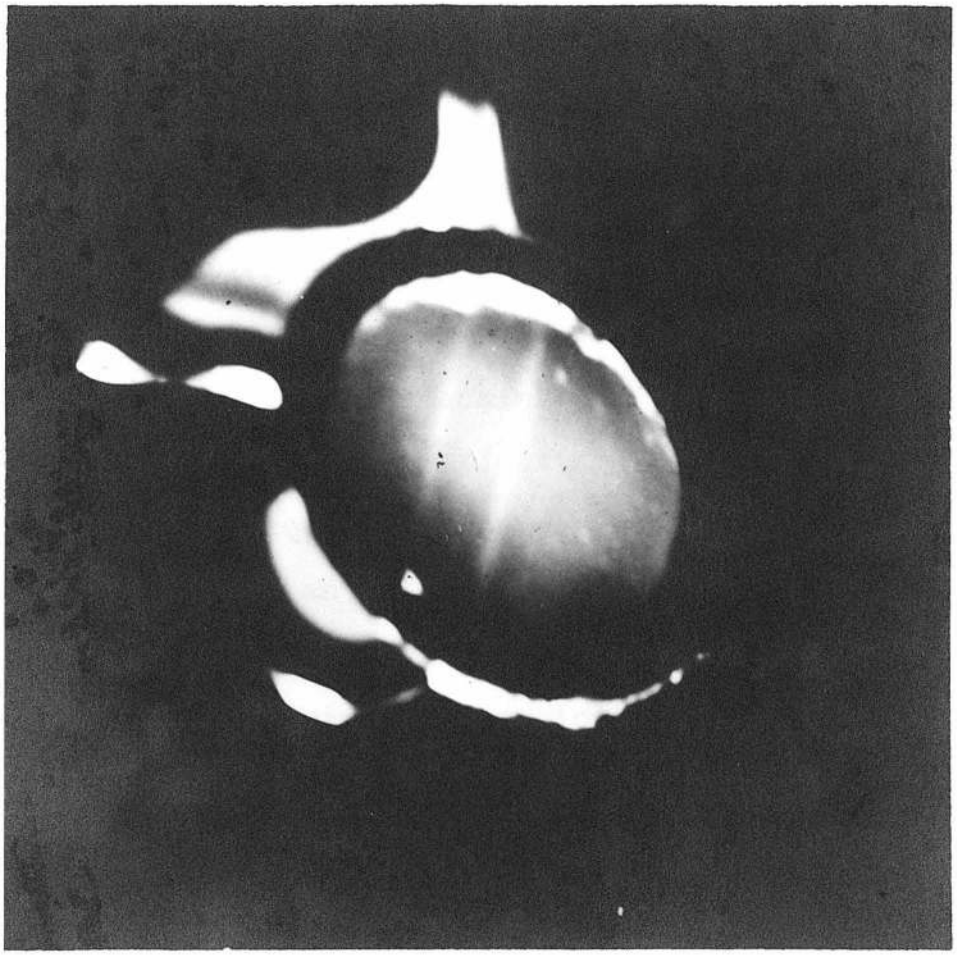
The hypothesis of Manten, that the phototactic response of R. rubrum is mediated by an abrupt decrease in the rate of photosynthesis, suggests the following extension: the tactic response (to any agent, including light, which evokes a response) is mediated by an abrupt decrease in the rate of synthesis, whether it be photosynthesis or chemosynthesis. This idea was investigated through studies of the chemotaxis to oxygen under various conditions.

Finally, a superficial survey of the kinetics of the phototactic response was conducted, preliminary to an anticipated quantitative study analogous to the studies of nerve excitation kinetics.

II. ACTION SPECTRUM OF PHOTOTAXIS

a. Method: The principal reason for repeating a determination of the phototactic action spectrum was to gather more precise information on the activity of the carotenoid pigments; the obvious technical refinement to be exploited was the use of greater spectral purity. For this reason a monochromator was constructed which yielded sufficient intensity for measurements with a spectral purity of 10 to 15 mμ throughout the region investigated.

The following observational procedure was adopted: A layer of dense bacterial culture .5 mm in thickness was mounted on a microscope slide, a circular glass spacer supporting the cover glass and the whole sealed together with vaseline (Fig. 2). Two strips of light were imaged in the plane of the culture. One, a control strip of white light, was kept at a fixed intensity of about four times the absolute threshold for phototaxis (the least intensity which causes a perceptible accumulation of bacteria in the light strip). The other strip, of monochromatic light, was applied with varying intensities. The two strips, surrounded by darkness, were imaged in the layer of bacterial culture for four minutes. Then the entire preparation was illuminated uniformly and viewed obliquely without magnification against a dark background; under these conditions the accumulation of



Phototactic Accumulation

x6

Fig. 3

bacteria where the light strips had been was easily seen (Fig. 3): This procedure was repeated with different intensities in the monochromatic strip until the degrees of accumulation of bacteria in the two strips were observed to be equal; the intensity I_λ in the monochromatic strip which matched the effect of the white strip was noted. Then the entire procedure was repeated at another wave length λ' of monochromatic light; the ratio $I_\lambda / I_{\lambda'}$ expresses the relative effectiveness of the two colors of light in evoking phototaxis. By plotting $1/I_\lambda$ vs. λ, expressed in quanta per square micron per millisecond and normalized to $I_{880\ m\mu} = 1$, an action spectrum of phototaxis analogous to that of Manten was obtained.

b. Preliminary culture investigations: Before action spectrum measurements could be made it was necessary to find the culture conditions and observational procedures which would yield greatest visibility of the patterns of phototactic accumulation. The following conditions were found empirically to be desirable:

1. Maximum density of actively motile bacteria attainable without centrifuging and resuspending.
2. Thickness of layer of bacterial culture of about .5 mm in observation cell.
3. Use of a long strip of light about .2 mm to 1 mm wide in producing the bacterial accumulation.
4. Freedom from interference by chemotactic effects (this was achieved by one hour's dark adaptation of the preparation before use; during this time the patterns of bacterial accumulation caused by local gradients of oxygen concentration became dissipated).

5. Storage of the preparation in the dark between measurements (the reduced metabolic activity in the dark appeared to favor the continued vitality of the dense culture of bacteria).

The strain of R. rubrum employed in these investigations (strain S-1, kindly supplied by Prof. C. B. van Niel of the Hopkins Marine Station, Pacific Grove, California) proved to be remarkably tolerant of oxygen; it was readily cultivated aerobically in the dark, and no special precautions were needed in the way of oxygen exclusion to bring out the full phototactic sensitivity of the bacteria.

After considerable testing the following medium was adopted for the growth of dense, active cultures of the bacteria:

Sodium pyruvate	1%
Yeast extract	.3%
Potassium phosphate buffer, pH 7	.2%
$(NH_4)_2SO_4$	.1%
$MgCl_2$	.05%
$CaCl_2$	.01%
$NaHCO_3$	.01%
Tap water	98.3%

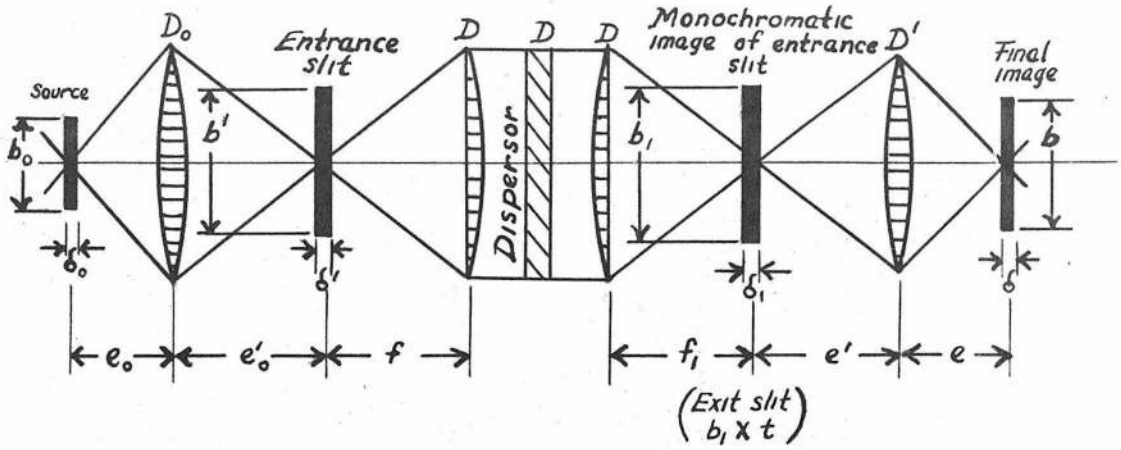
The phosphate buffer and the calcium and magnesium chlorides were autoclaved separately in distilled water to avoid turbidity and the bicarbonate was sterilized by filtration. Fully grown cultures were obtained in this medium from moderate inocula in about four days at 35°C. The cultures were grown under continuous illumination anaerobically (in completely filled glass-stoppered bottles).

c. Monochromator design: By far the most laborious part of the program was the construction of a monochromator

yielding sufficient intensity to permit measurements involving a reasonably high degree of spectral purity (about 10-15 mμ). While the design of the monochromator evolved largely as a result of trial and error and of the equipment available, a few principles became apparent which seem to be of sufficient interest to warrant a digression into the theory of monochromator design.

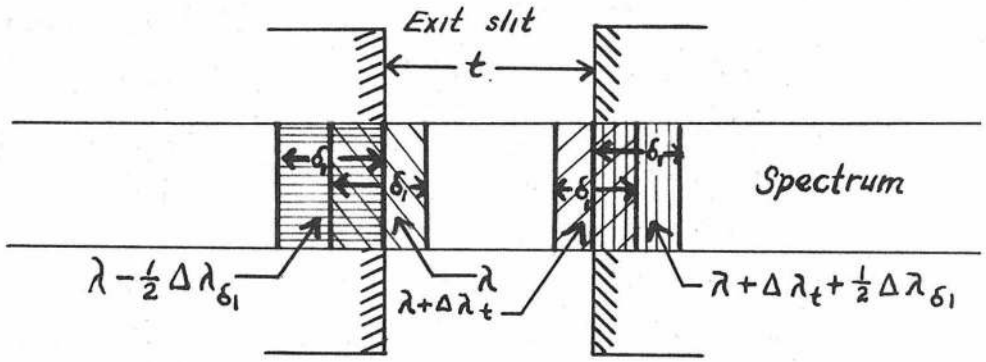
In the present case it was desired to illuminate a rectangular strip of specified size with the greatest possible intensity and with a specified spectral purity. Practical considerations require that a light source be imaged at an entrance slit, that the light admitted through the entrance slit be collimated and sent through a dispersor such as a grating or prism, that the resulting spectrum be focussed in the plane of an exit slit, and that an image of the exit slit form the final illuminated rectangle. Such an arrangement is shown schematically in Fig. 4 for the case of lens optics; the essential features of our treatment would be the same if the use of mirrors were considered.

Being generally the most difficult item to obtain in large size, the dispersor is the primary determinant of the aperture of the system . In Fig. 4 the dispersor is shown to have an aperture D; it is preceded by a collimating lense of diameter D or greater and of focal length f. At the focus of the collimator is an entrance



GENERALIZED MONOCHROMATOR

Fig. 4



COMPOSITION OF SPECTRUM

Fig. 5

slit of dimensions $b' \times \delta'$; framed in it is the image of a light source of minimum dimensions $b_o \times \delta_o$. The lens which images the source at the entrance slit is of diameter D_o with object and image distances e_o and e_o' such that $D_o/e_o' \geq D/f$; the minimum source dimensions $b_o \times \delta_o$ are chosen to make $b_o/b' = \delta_o/\delta' = e_o/e_o'$. Beyond the dispersor is a lens of diameter D or greater and of focal length f_1; with a monochromatic light source the entrance slit would be imaged at the focus of this lens. Such an image would have the dimensions $b_1 \times \delta_1$ where $b_1/b' = \delta_1/\delta' = f_1/f$. With a spectrally continuous light source the single image of the entrance slit is replaced by a continuous sequence of overlapping images comprising the spectrum; an exit slit of dimensions $b_1 \times t$ transmits a portion of this spectrum. Finally a lens of diameter D' forms an image, of dimensions $b \times \delta$, of the exit slit. It is this rectangle, with sides b and δ, which is to be illuminated as strongly as possible with light of wave length between λ and $\lambda + \Delta\lambda$. Our task now is to see how the intensity obtained is related to the required dimensions b and δ, the required spectral purity $\Delta\lambda$, the characteristics of the dispersor, and the geometry of the system. For simplification we shall assume the source, slits, and final image to be sufficiently small that the off-axis field of view does not become a limiting factor. The approximations of first-order optical theory ($D/2f \approx \sin^{-1} D/2f$, etc.) will be used

throughout our treatment.

If L is the total radiant energy passing through the entrance slit and i_o is the brightness of the source we can write the proportionality

$$L \sim (D_o/e_o)^2 i_o b_o \delta_o .$$

Since $e_o/e'_o = b_o/b' = \delta_o/\delta'$ we can write

$$L \sim (D_o/e'_o)^2 i_o b' \delta' ;$$

since $D/f = D_o/e'_o$ we can write

$$L \sim (D/f)^2 i_o b' \delta' ;$$

and since $f/f_1 = b'/b_1 = \delta'/\delta_1$ (b_1 and δ_1 are the dimensions of a monochromatic image of the entrance slit) we can write

$$L \sim (D/f_1)^2 i_o b_1 \delta_1 .$$

Of the total energy L passing the entrance slit a fraction L_λ will be admitted through the exit slit of width t. This fraction will be determined by the ratio of t to the length of the entire spectrum and by the spectral characteristics of the dispersor and the light source; only the first of these factors need be considered at any given wave length. The length of the spectrum will be proportional to the product of f_1 and the angular dispersion of the dispersor. The angular dispersion will of course be a function of wave length in the case of a prism; however, it can be resolved into a factor $g(\lambda)$, expressing the wave length dependence, and a factor Q which is common to all wave lengths. It is this latter factor which is

essential to our considerations at any given wave length; abstracting it we say that the length of the spectrum varies as $f_1 Q$, so that $L_\lambda / L \sim t/f_1 Q$, or

$$L_\lambda \sim (t/f_1 Q)\ (D/f_1)^2 i_o b_1\ \delta_1 .$$

We must now consider the relationship between t, δ_1, and the spectral purity $\Delta\lambda$. If $\Delta\lambda_a$ is the change in wave length associated with a displacement "a" along the spectrum we have $a = f_1 [Qg(\lambda)] \Delta\lambda_a$. Assuming that $g(\lambda)$ is essentially constant over the range $\Delta\lambda_a$ we shall normalize it to unity by adjusting the numerical value of Q and write $a = f_1 Q \Delta\lambda_a$. Let us now examine in detail the composition of the spectrum in the neighborhood of the exit slit. This is shown in Fig. 5, with monochromatic images of the entrance slit sketched in at some critical wave lengths. The exit slit passes no light of wave length less than $\lambda - (1/2)(\Delta\lambda_{\delta_1})$ or greater than $\lambda + \Delta\lambda_t + (1/2)(\Delta\lambda_{\delta_1})$; accordingly the spectral purity of the emergent light is $(1/2)\Delta\lambda_{\delta_1} + \Delta\lambda_t + (1/2)\Delta\lambda_{\delta_1}$. Thus we can write $\Delta\lambda = \Delta\lambda_t + \Delta\lambda_{\delta_1} = (\delta_1 + t)/f_1 Q$, or $\delta_1 = f_1 Q \Delta\lambda - t$. Substituting into the expression for L_λ we have

$$L_\lambda \sim (D/f_1)^2 (i_o b_1 / f_1 Q) t (f_1 Q \Delta\lambda - t).$$

Differentiating with respect to t we find that L_λ is maximized when $t = \delta_1 = (1/2) f_1 Q \Delta\lambda$; i.e., the exit slit should be of the same width as a monochromatic image of the entrance slit. Substituting for t and dropping the factors of 1/2 we now have

$$L_\lambda \sim (D/f_1)^2 (i_o b_1 / f_1 Q)(f_1 Q \Delta\lambda)^2$$

or

$$L_\lambda \sim i_o D^2 Q(\Delta\lambda)^2 b_1/f_1.$$

The illumination of the final image will be $i_\lambda = L_\lambda/b\delta$, giving

$$i_\lambda \sim i_o D^2 Q(\Delta\lambda)^2 (1/f_1\delta)(b_1/b).$$

Since $b_1/b = \delta_1/\delta$ and $\delta_1 = t = (1/2)f_1 Q\Delta\lambda$, the factor $(1/f_1\delta)(b_1/b)$ becomes $\delta_1/f_1\delta^2$ or $Q\Delta\lambda/2\delta^2$, yielding the final result

$$i_\lambda \sim i_o Q^2(\Delta\lambda)^3 D^2/\delta^2.$$

At first sight this result seems strange in two ways. First, the length b of the required final image does not appear explicitly. This is due, of course, to the fact that a longer image can always be obtained with no gain or loss in intensity by making the source and the slits correspondingly longer, provided that the off-axis field of view does not become a limiting factor. More striking is the fact that neither f nor f_1 appears as an essential variable in the final result; one generally expects the F-ratio, D/f, to be of fundamental importance in determining optical intensities. That this is not the case here is a consequence of our stipulation that a specified area be illuminated, as can be seen by the following consideration: suppose that, having designed a monochromator along the lines of Fig. 4, one attempts to obtain greater intensity by choosing new lenses in which all focal lengths are one half the former focal lengths. This would leave

the sizes of all images unaltered, and a fourfold gain in intensity might be anticipated. However, the linear dispersion of the spectrum would be halved, and to restore the required spectral purity it would be necessary to halve the widths of entrance and exit slits. The final image would then be one half its former width, and to restore it to the required width it would be necessary to alter the last lens of the system, doubling the magnification of the exit slit in forming the final image. Attending this doubled magnification would be a fourfold decrease in intensity which would just nullify the gain achieved by halving the focal lengths of the first three lenses in the system.

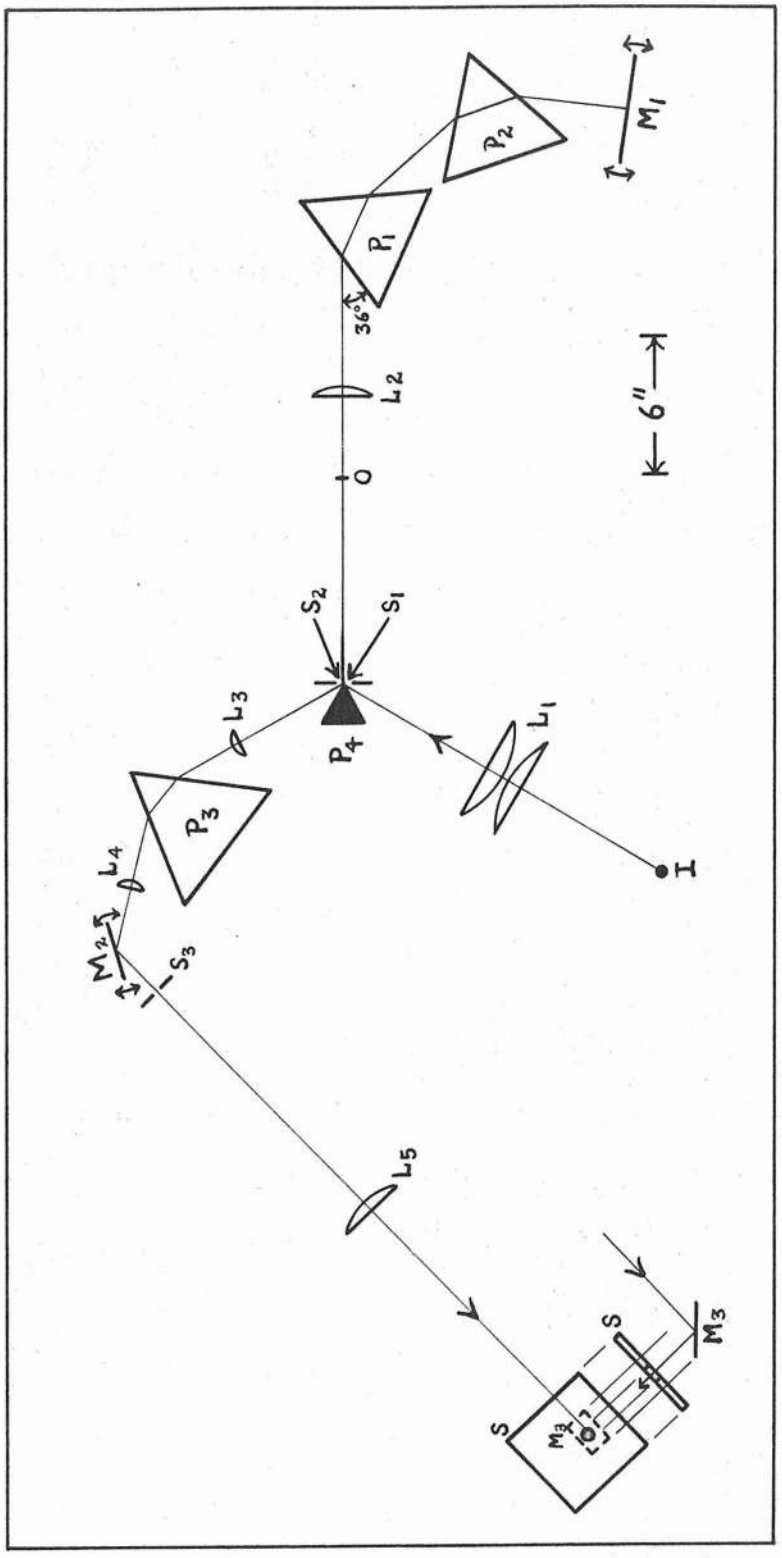
Had we desired simply the greatest light flux having a specified spectral purity, without regard to the area illuminated, we would have found the quantities f and f_1 to be significant; we would also have had to consider the limitations imposed by the field of view when source and slits are lengthened indefinitely.

d. Instrumentation:

1. The monochromator: The monochromator actually constructed for the measurement of the action spectrum of phototaxis of R. rubrum failed in many ways to conform to the principles just outlined, nevertheless it fulfilled its purpose satisfactorily. Its basic features are shown schematically in Fig. 6. The light source I was imaged by the condensing lenses L_1 at the

entrance slit S_1; P_4 was an opaque reflecting wedge the apex of which formed one boundary of both entrance and exit slit. Having been reflected through S_1 the light was collimated by the lens L_2 and sent through the prisms P_1 and P_2. The mirror M_1 returned the light through these prisms to L_2; this lens then focussed the chromatically dispersed beam in the form of a spectrum at the exit slit S_2. The exit slit transmitted a portion of the spectrum; O was a black velvet obstacle which prevented a direct reflection of light from S_1 to S_2 via the surfaces of L_2. Despite this precaution the monochromatic light emerging from S_2 was contaminated with enough stray light to require further purification; this was accomplished by the intervention of an extra dispersing stage consisting of the lenses L_3 and L_4 and the prism P_3. The monochromatic light from S_2 was brought to a focus at a third slit S_3 via the mirror M_2; most of the stray light, having been dispersed into a spectrum, failed to pass the third slit. Finally the light passed through the lens L_5 and was reflected vertically by the mirror M_3 to the microscope stage S; L_5 formed an image of the slit S_3 in a layer of bacterial culture which rested on the stage.

The light source employed was a G. E. 500 watt projection lamp; it should be noted that the biplanar filament of a G. E. 750 watt projection lamp would have



BASIC MONOCHROMATOR

Fig. 6

yielded an appreciably enhanced flux through the entrance slit. The use of a carbon arc, which would have trebled the output of the monochromator, was avoided because of the greater instability and difficulty of operation.

The prisms P_1, P_2, and P_3 were six inch Jena dense flint prisms loaned through the courtesy of the Astrophysics Department of the California Institute of Technology; P_4 was a polished steel wedge upon which a layer of aluminum, followed by a protective layer of quartz, had been evaporated. L_2, L_3, L_4, and L_5 were lenses of photographic quality; nevertheless they showed significant chromatic aberration in the near infra-red. To ensure precise imaging of the third slit at the microscope stage at all wave lengths the chromatic aberration of the lens L_5 was determined photographically with the aid of infra-red sensitive plates.

2. Accessories: A number of devices accessory to the calibration and operation of the monochromator will be mentioned briefly. First, the selection of wave length was accomplished by rotating the mirror M_1, causing a lateral shift of the spectrum across the exit slit. An optical lever connected the position of M_1 with the position of a hairline on an illuminated scale calibrated in wave length. The calibration was performed by replacing the light source by the output of a Beckman Spectrophotometer, the mirror M_1 being

rotated until incoming light of a specified wave length emerged from the exit slit. The spectral lines of a mercury arc were also used as a check against the spectrophotometer. At each wave length the mirror M_2 had to be set appropriately to frame the image of the exit slit in the third slit; the position of this mirror was controlled by an arm fixed to the mirror and terminating in a pointer which moved over a calibrated scale.

The three slits, of adjustable widths, could be set accurately by observing their magnified images; the output of the monochromator was varied by varying the voltage applied to the lamp I.

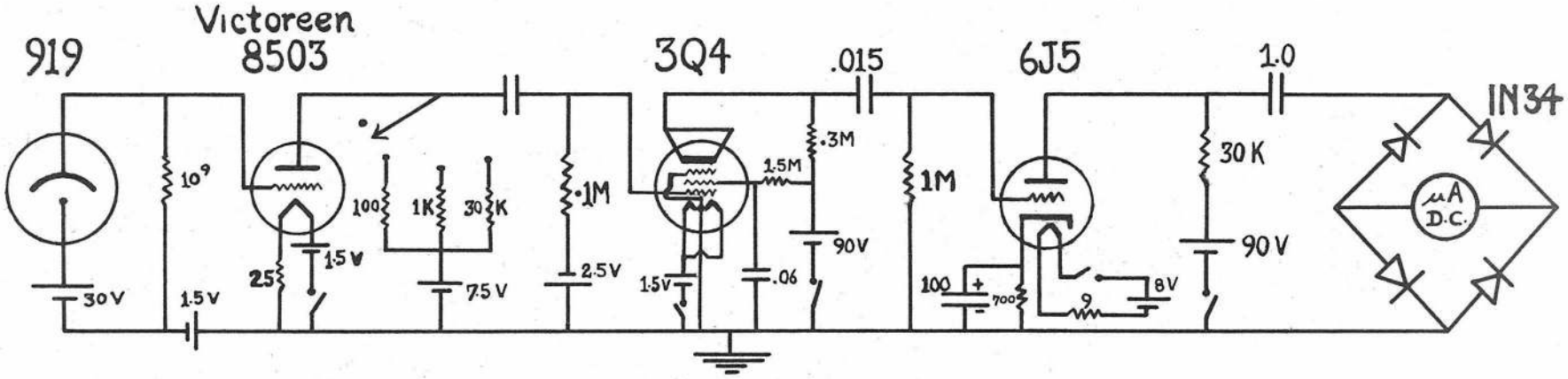
To provide a strip of white light adjacent to the monochromatic strip in the bacterial culture, the image of an illuminated slit was projected onto the microscope stage via a small mirror located just behind the lens L_5 (between L_5 and S_3). A provision was also made for superimposing a background of white light upon the strip of monochromatic light in order to raise the intensity level of the strip; this procedure was found useful when working at wave lengths of 500 mμ and less. The superposition was accomplished by introducing, between S_3 and M_2, an unsilvered glass plate which reflected white light into the optical path.

Finally, the mirror M_3 could be rotated through 90°

about a vertical axis. When this was done the bacterial culture became illuminated uniformly by a lamp under the microscope stage and any previously induced phototactic accumulations could be observed.

3. Phototube and amplifier: A phototube and AC amplifier was used to measure the intensity of the monochromatic strip to which the bacteria were exposed. A removable mirror deflected the light emerging from L_5 to the cathode of a phototube, where the image of S_3 was formed. A ground glass plate in front of the phototube diffused the light over the cathode; without this precaution the response of the phototube was very sensitive to small movements of the image.

The intervention of a fan modulated the light into 60 cps pulses, permitting AC amplification. The pulsating photocurrent, of the order of 10^{-10} to 10^{-13} amperes, was passed through a high resistance in the grid circuit of a Victoreen electrometer triode; following a voltage amplification stage and a current amplification stage the output was rectified by germanium crystal diodes and measured by a DC microammeter. The circuit is shown in Fig. 7; the choice of three resistance values in the plate circuit of the first stage provided approximately a hundredfold range in amplification. The range of the microammeter was also extended by the use



Capacitances in μf. *Resistances in ohms.*

PHOTOTUBE — AMPLIFIER CIRCUIT

Fig. 7

of shunting resistors, so that the instrument could be used for measuring photocurrents ranging from 10^{-13} to 10^{-9} amperes.

The entire circuit excepting the 6J5 heater supply and the meter was shielded with soft iron, light being admitted to the phototube through a window in the soft iron box. The phototube, 10^{9} ohm resistor, and electrometer triode were mounted in a sealed dehydrated container to reduce current leakage caused by moisture. Microphonic noise, arising principally in the 3Q4, was eliminated effectively by packing this tube and the 6J5 in non-absorbent cotton (the absorbent variety is more inflammable).

While there is much room for improvement in the circuit just described, the sensitivity and stability proved to be quite adequate for the task at hand.

The calibration of the phototube-amplifier involved three tasks: calibration of linearity (meter reading vs. incident light flux), of relative spectral sensitivity, and of absolute sensitivity. The linearity was calibrated by observing the change in microammeter reading when the light incident on the phototube was reduced by one half; successive reductions were carried out over the entire useful range of the instrument. Accurate halvings of the incident flux were achieved by interposing a divided shutter in the beam impinging on the phototube;

the shutter was so oriented that opening either half of it yielded the same meter reading. When both halves were opened the reading was the same as with the shutter absent; with both halves closed the reading was zero.

The spectral variation of sensitivity was calibrated as follows: By the use of filters in conjunction with mercury and sodium arcs, monochromatic light of the following wave lengths was obtained: 405, 435, 546, 589, 691, 819, and 1014 mμ (the light provided by the monochromator itself was too feeble for calibration purposes). The light could be directed either at the phototube window or, via an identical optical path, at a vacuum thermocouple connected to a galvanometer. The linearity of the thermocouple-galvanometer combination was checked and found satisfactory within the precision of measurement; its spectral sensitivity was assumed to be uniform. A comparison of the responses of phototube-amplifier and thermocouple-galvanometer then yielded the relative sensitivity of the phototube to light of the wave lengths employed. It was found that if all points of the published spectral sensitivity curve of the 919 phototube (normalized to unity at the maximum sensitivity) were raised to the .65 power the resulting curve fit the experimental points within 5% in all cases; the curve thus derived was accordingly taken as representing the spectral sensitivity of the phototube.

An accurate knowledge of the absolute sensitivity of the phototube was not required; the responsiveness of the bacteria varies so widely with varying culture conditions and other environmental factors that a knowledge of absolute intensity having precision greater than fifty percent would have been superfluous. The absolute sensitivity of the thermocouple-galvanometer was estimated by observing the response to the radiation from a 100 watt lamp at 2.3 meters and referring to the published efficiency of the lamp. The sensitivity of the phototube-amplifier at a given wave length was then estimated by comparing its response with the response of the thermocouple-galvanometer to monochromatic light. Since the sensitivity of the thermocouple was known in terms of radiant intensity (ergs/cm^2 sec), while the sensitivity of the phototube was desired in terms of radiant energy flux (ergs/sec), the light admitted to the phototube was passed through a small aperture of known area situated over the phototube window.

When the phototube-amplifier was used in conjunction with the monochromator, the intensity in the light strip applied to the bacteria was derived from a measurement of the radiant flux through a knowledge of the area of the strip; flux/area = intensity. In addition to a correction for the spectral sensitivity of the phototube

a small correction was applied for the absorption of light by the culture medium, which was pale amber in color.

4. Performance of monochromator and phototube-amplifier: The amplifier was free of detectable noise except when operated at maximum gain; under this condition a meter reading of .4 to .6 microamperes appeared as a result of electrostatic hum. This noise level was subtracted from all readings obtained at maximum amplification; the readings themselves were generally above 50 μa and never below 5 μa. Readings above 50 μa were reproducible within 2% over the course of several hours; the day-to-day sensitivity of the phototube-amplifier remained constant within 5%. As a check on the stability of the spectral sensitivity, the output of the monochromator under specified operating conditions was measured every month; here again variations greater than about 5% were not observed. In performing this check the monochromator slits were set at 1.0 mm and a 500 watt projection lamp, used only for this purpose, was operated at 115 $\pm$ .5 volts in place of the lamp used every day.

Since the calibration of the spectral sensitivity of the phototube was performed with a precision of better than 5% we can compound this with the 5% error due to drift in spectral calibration and the 2% irreproducibility of readings and predict that the

accuracy of comparative intensity measurements at two wave lengths was better than $(.05^2 + .05^2 + .02^2)^{1/2}$ or about 7%. In comparing two wave lengths within 50 mμ of each other the accuracy was probably well within 2% since errors due to unprecise knowledge of spectral sensitivity are smaller over a narrow range of wave length than over a great range (this is a consequence of the fact that the spectral response curve of a phototube is a smooth curve lacking abrupt irregularities).

The output of the monochromator was found to be free of significant stray light contamination by the following method: The strip of light illuminating the bacteria (the image of slit S_3) ordinarily consisted of monochromatic light plus stray light. Monochromatic light of wave length 580 mμ could be removed with a didymium filter, leaving the stray light (slightly attenuated) alone in the strip. Finally a background of white light could be introduced into the strip independently of the stray light. By observing the phototactic response to the white light with and without the stray light it was found that no influence of the stray light could be detected; also the stray light alone produced no evidence of phototaxis.

The spectral purity at a given slit setting and wave length was observed directly by replacing the source I by a mercury arc. For example, if the mercury green

line could be seen through the exit slit when the wave length setting of the monochromator was anywhere between 542 and 552 mμ , the spectral purity was taken to be 10 mμ .

The chief shortcoming of the monochromator was its markedly decreased output in the blue and violet, caused primarily by absorption attending five passages through prisms (in the absence of absorption the reduced emissivity of a tungstenlamp in the blue is largely compensated by the increased dispersion of a glass prism). Had the extent of the absorption been anticipated fully the monochromator would have been constructed with fewer prisms in a non-Littrow arrangement; as it was there was abundant intensity above 600 mμ , but measurements of the phototaxis could not be pursued accurately below 480 mμ . Fortunately most of the significant absorption maxima of the carotenoid pigments fall at 480 mμ and higher.

e. Observations: The method of determining the action spectrum of phototaxis of R. rubrum has already been outlined; the data from a typical experiment are presented in Table I. The first two columns are self-explanatory. In the next two columns the lamp voltage determines the intensity I_λ , expressed in quanta per square micron per millisecond, of the monochromatic strip the effect of which is compared with the control strip of white light. After a four minute exposure to the two strips of light the preparation was viewed under uniform illumination; the fifth column in Table I

expresses the relative degrees of accumulation of bacteria where the two strips had been. The meanings of the numbers ranging from 2 to -2 in the fifth column can be tabulated as follows:

2, accumulation in monochromatic strip much stronger than in white strip.

1, accumulation in monochromatic strip definitely stronger than in white strip.

$\frac{1}{2}$, accumulation in monochromatic strip just perceptibly stronger than in white strip.

0, accumulation in monochromatic strip equal to that in white strip.

$-\frac{1}{2}$, accumulation in monochromatic strip just perceptibly weaker than that in white strip.

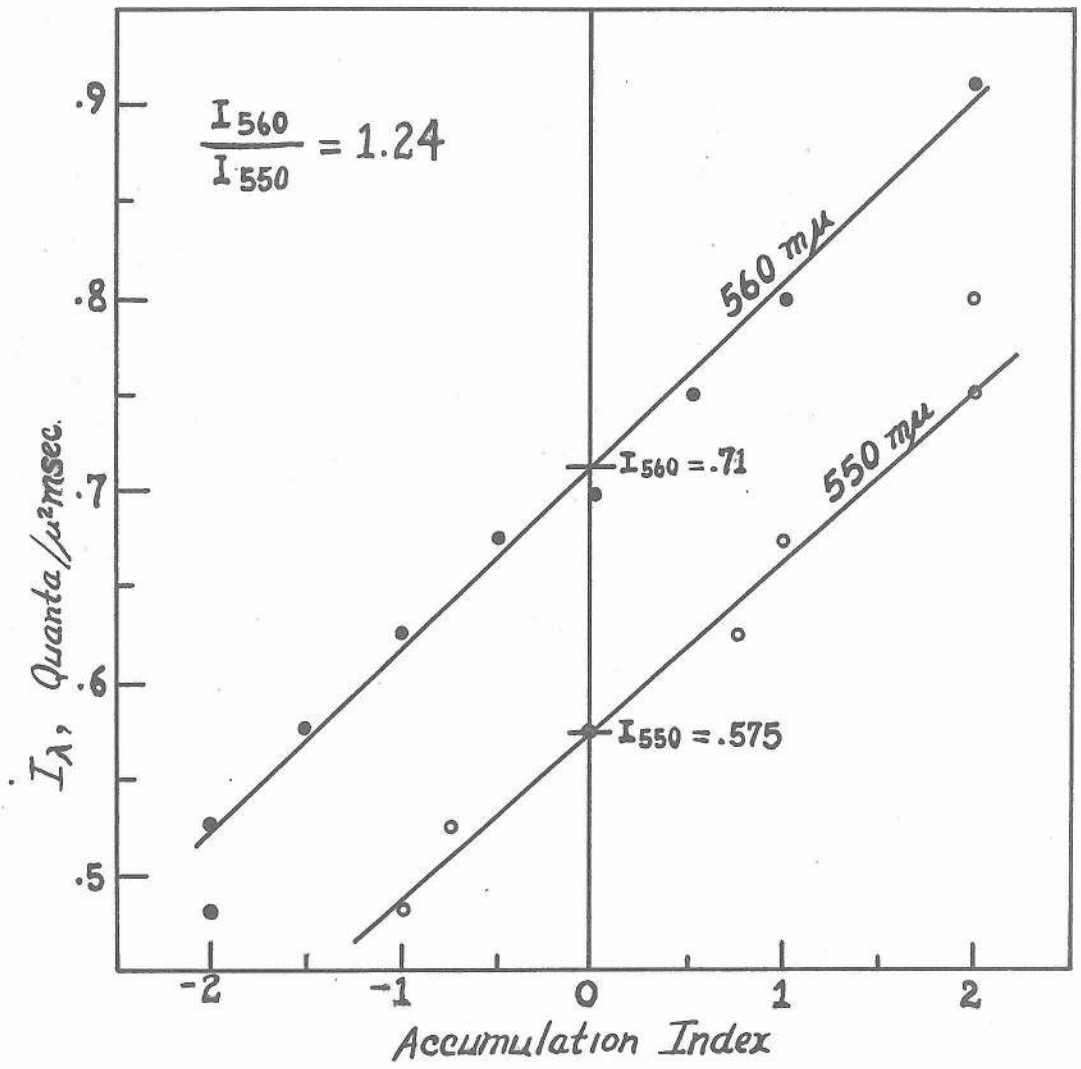
-1, accumulation in monochromatic strip definitely weaker than that in white strip.

-2, accumulation in monochromatic strip much weaker than that in white strip.

These indices of relative accumulation are not defined quantitatively, but in a null method of observation such as this the absolute values of deviations from the null need not be well known for precise judgment of the null itself. It was found helpful in locating the null and in judging the quality of the experiment to plot the accumulation index versus the intensity of the monochromatic strip; this has been done in Fig. 8 for the data of Table I. In this experiment the monochromatic intensities which matched the effectof the white strip were found to be .575 (at 550 mμ) and .71 (at 560 mμ); these intensities at their corresponding wave lengths were accordingly taken as equivalent in their ability to evoke phototaxis. The

Time	λ, mμ	Lamp volts	I_λ quanta/μ^2sec	Relative taxis
1254	550	100	.675	1
1301	560	92.5	.675	$-\frac{1}{2}$
1308	560	97	.80	1
1315	550	105	.80	2
1321	550	95	.575	0
1328	560	89	.575	$-1\frac{1}{2}$
1335	560	92.5	.675	$-\frac{1}{2}$
1342	550	90	.48	-1
1349	560	84.5	.48	-2
1355	560	101	.91	2
1402	560	95	.75	$\frac{1}{2}$
1408	550	103	.75	2
1415	550	97.5	.625	$\frac{1}{2}$ to 1
1422	560	91	.625	-1
1429	560	86.5	.525	-2
1435	550	92.5	.525	$-\frac{1}{2}$ to -1
1442	550	95	.575	0
1448	560	93.5	.70	0

Table I.



(EXPLANATION IN TEXT)

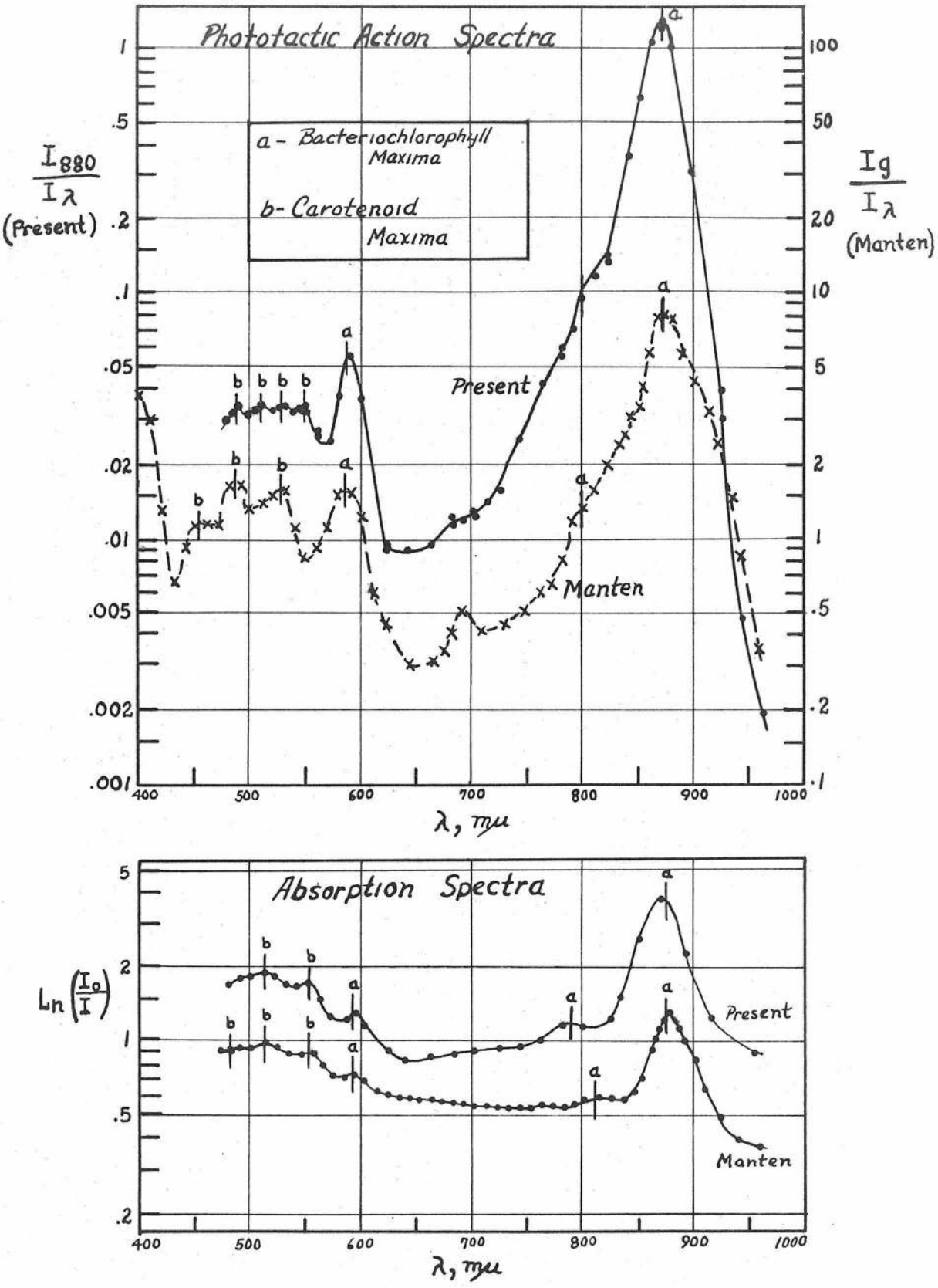
Fig. 8

"equivalent intensity" ratio I_{560}/I_{550} was then .71/.575 or 1.24, and since it was known from earlier experiments that I_{880}/I_{560} = .027, the value I_{880}/I_{550} = .027x1.24 = .033 could be entered in the action spectrum.

The complete action spectrum, a plot of I_{880}/I_{λ} vs. λ, is shown in Fig. 9 together with an absorption spectrum of a suspension of living R. rubrum. The absorption spectrum, obtained with the same equipment as was used in the measurement of the action spectrum, was probably distorted seriously by spectrally nonuniform scattering. Since many workers, notably French (11), Wassink et al (12,13), and van Niel and Smith (22), have made careful measurements of absorption spectra both of the living bacteria and of extracts of their pigments, it was desired in this study merely to observe the locations of absorption maxima. In this respect the absorption spectrum was in substantial agreement with one obtained by Manten (5); for comparison Manten's absorption spectrum and his phototactic action spectrum are included in Fig. 9.

f. Discussion: Above 570 mμ the present action spectrum is in good agreement with that obtained by Manten, showing the characteristic bacteriochlorophyll maxima at 590 and 870 mμ and smaller shoulders at 685 and perhaps at 800 mμ .

Below 570 mμ the two action spectra bear almost no resemblance to each other. Manten's spectrum, showing pronounced maxima at 530, 490, and 460 mμ (the maximum at



ABSORPTION SPECTRA AND PHOTOTACTIC ACTION SPECTRA OF RHODOSPIRILLUM RUBRUM

Fig. 9

400 mμ is due to bacteriochlorophyll) and minima at 550, 500, and 470 mμ , suggests that spirilloxanthin does not absorb phototactically active light, but that one or more of the less abundant carotenoids does. The relative activities of these less abundant carotenoid(s) and the bacteriochlorophyll are reflected in the relative heights of the various maxima; the maxima at 490 and 530 mμ (carotenoid) and at 590 mμ (bacteriochlorophyll) are seen to be about equal in height.

In contrast with Manten's action spectrum, the present action spectrum does show structure in the blue and green which suggests activity on the part of the spirilloxanthin. The maxima at 550 and 510 mμ coincide with spirilloxanthin absorption maxima; the agreement between the phototactic action maximum at 490 mμ and the spirilloxanthin absorption maximum at 480 mμ is more questionable. The maximum observed by Manten at 530 mμ appears in the present action spectrum, again suggesting activity on the part of the less abundant carotenoids. Indeed, the maximum in the action spectrum at 490 mμ , observed also by Manten, may be associated with one of these scarcer carotenoids and may have masked a spirilloxanthin maximum at 480 mμ .

The conflicting locations of maxima in the two action spectra are no more striking than the differences in relative heights of carotenoid and bacteriochlorophyll

maxima. It will be recalled that in Manten's action spectrum the peaks at 490 and 530 mμ , ascribed to activity on the part of scarcer carotenoids, are fully as high as the bacteriochlorophyll peak at 590 mμ . In the present action spectrum the peak at 590 mμ rises much higher than any of the carotenoid maxima, even though some of these are associated with the abundant spirilloxanthin.

What can be said of the role of carotenoids in phototaxis, on the basis of these contradictory action spectra? Manten's spectrum suggests that spirilloxanthin is phototactically inert but that one or more scarcer carotenoids are quite active. The present spectrum, showing maxima at 490 and 530 mμ comparable in height to other maxima at 510 and 550 mμ , suggests that spirilloxanthin is phototactically active but that one or more of the scarcer carotenoids are much more active in proportion to their concentration in the bacteria. If both spectra are to be taken seriously they must be reconciled by the assumption that the two strains of Rhodospirillum rubrum employed differed widely in their pigment content. Both strains (S-1 and S-4) were isolated by van Niel at Pacific Grove, California; their absorption spectra as shown in Fig. 9 indicate the presence of spirilloxanthin and bacteriochlorophyll in roughly the same relative amounts. The scarcer components of the carotenoid complex do not appear in these relatively

crude absorption spectra, and their concentrations in the two strains of bacteria could conceivably differ by a large factor. Such a difference could cause exactly the variation in action spectra with which we are confronted. Had the strain S-4 used by Manten been more deficient in the scarcer carotenoids, the maxima in his action spectrum at 490 and 530 mμ might have been suppressed to a level far below the chlorophyll peak at 590 mμ ; at the same time the less pronounced maxima due to spirilloxanthin might have emerged from the masking influence of the other peaks. Conversely an increase in the concentration of the scarcer carotenoids in strain S-1 might have caused the present action spectrum to resemble that obtained by Manten.

The assumption, required in the foregoing interpretation, of low spirilloxanthin activity coupled with high activity on the part of much less preponderant carotenoids is not very satisfying. In addition to violating one's teleological notions, it is a difficult assumption to interpret convincingly on theoretical grounds. If one accepts the idea that the phototactic response is mediated via the photosynthetic metabolic pathways, it might be held provisionally that the transfer of energy to the bacteriochlorophyll molecule from some of the lesser carotenoids proceeds with much greater efficiency

than does the transfer from spirilloxanthin. The possibility remains, of course, that either or both action spectra were vitiated by uncontrolled environmental influences; some of these were investigated in detail and will be discussedin the next section.

III. STABILITY OF PIGMENTS IN THEIR PHOTOTACTIC ACTIVITY

a. Factors Invalidating Action Spectra: To explore possible sources of error in the phototactic action spectrum, and to gain further information about the pigments of R. rubrum, it was decided to investigate the influence of various environmental factors on the phototactic sensitivity to light absorbed by the carotenoids and by the bacteriochlorophyll.

Before describing these investigations it would be well to discuss some of the influences which tend to invalidate action spectra and to rule out those to which the phototactic action spectrum was not susceptible.

1. Screening: The light incident on the system under investigation may be attenuated to an unknown degree by inert pigments before it reaches an active pigment which contributes to the action spectrum. In the present case the attenuation by the thin layer of bacterial culture was too slight to introduce a significant screening error; moreover the small attenuation by the culture medium was taken into account.

2. Strong absorption: A valid action spectrum should follow a weighted superposition of the absorption curves of the pigments concerned (the weighting occurs

because different pigments show different degrees of activity). The appearance of an absorption curve depends markedly on the amount of absorbing pigment, and if a pigment is present in excessive amounts its absorption curve may be featureless, showing total absorption at all wave lengths. The corresponding action spectrum would then be equally devoid of structure (this effect can be seen in photosynthetic action spectra of opaque leaves). In the present case this difficulty did not appear for the same reason that screening was not troublesome; the degree of absorption by the system was slight at all wave lengths. The absorption spectra shown in Fig. 9 were, to be sure, obtained from thick, dense suspensions of purple bacteria. However, their presentation in the form $\ln(I_o/I)$ vs λ rendered them comparable, except for scattering effects, to action spectra obtained from thin suspensions. Referring to the relation $I/I_o = e^{-acx}$, where I/I_o is the attenuation by a pigment whose absorption coefficient is a, and where c is the concentration of the pigment in a medium presenting a thickness x to the light beam, we find that a plot of $\ln(I_o/I)$ vs. λ is equivalent to a plot of acx vs. λ. In the phototactic action spectrum the quantity plotted against wave length is the reciprocal of the intensity required for a given phototactic effect. It is assumed that this required intensity varies inversely as the fraction of it which is absorbed by an active

pigment; an intensity of 100, 2% absorbed, is equivalent to an intensity of 50, 4% absorbed. Plotting the reciprocal of the required intensity is then equivalent to plotting the fractional absorption $(I_o-I)/I_o$, which is equal to $1-e^{-acx}$. For the small values of acx prevailing in the action spectrum measurements, $1-e^{-acx}$ is approximately equal to acx, so that the quantity plotted in the action spectrum is comparable in its essential form to the quantity plotted in the absorption spectrum.

3. Variability of material investigated: Variations in the composition of the system under investigation can lead to completely anomalous action spectra; this possibility has already been considered in connection with the disagreement between the present action spectrum and that obtained by Manten. It is unlikely, however, that the strain of bacteria used in the present action spectrum determination varied significantly during the course of the measurements. Different parts of the spectrum were returned to a number of times during the investigation, and the results were always reproducible within the expected precision of the method.

4. Variability of environment: Long-term variations in environmental factors were ruled out as a source of error for the same reason that variability of the strain was excluded in the preceding paragraph. More rapid

fluctuations, occurring during the course of an experiment, did not introduce a perceptible irregularity in the data; at any rate they would have been averaged by the procedure of switching back and forth between two wave lengths during an experiment. It is believed that the measurements were sufficiently precise that the structure appearing in the action spectrum was in no case an artifact of scattered data; the difference between successive maxima and minima was always greater than the difference between duplicate measurements in the same region of the spectrum.

5. Inactivation or sensitization of one or more pigments: The possibility remains that throughout the action spectrum measurement a condition prevailed which tended to suppress or enhance the activity of one or more pigments. If such an influence had been lacking or present to a different degree in Manten's action spectrum the disagreement between the two spectra could well be accounted for without invoking a difference in the composition of the two strains. It was decided therefore that the influence of certain obvious environmental factors on the relative activities of the bacteriochlorophyll and the carotenoids should be explored. The factors investigated included temperature, oxygen concentration, culture age, and illumination in various aspects. The influence of changes in the composition of the culture medium should have

been investigated; this has not been done at the present writing.

b. Method of Investigation: The relative activities of the bacteriochlorophyll and the carotenoid systems was measured by a method similar to that employed by Manten in his action spectrum measurements. A divided field of illumination was projected on the bacterial preparation; one half consisted of light absorbed only by the bacteriochlorophyll and the other half, separated from the first by a sharp boundary, consisted of light absorbed only by the carotenoids. The relative intensities of the two halves were then adjusted so that no phototactic accumulations of bacteria were induced. This adjustment admitted of considerable precision; a variation of intensity as little as 3% from the matching intensity would lead to a noticeable phototactic accumulation pattern along the boundary. The match of intensities having been established, its stability under the influence of certain environmental changes was examined; in this way the relative inactivation or sensitization of one pigment system or the other could be detected.

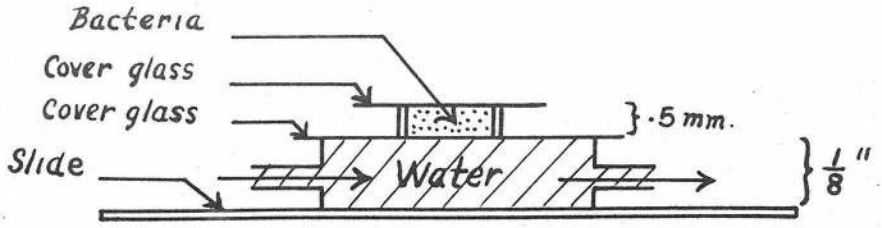
The instrument providing a sharply divided field of illumination will be described in the next section in connection with investigations of the Weber Law adherence. To isolate light in one half of the field which was

absorbed solely by the bacteriochlorophyll, a Wratten No. 23A filter was used in conjunction with 1/4 inch of 10% $CuCl_2$ in water; this combination transmitted light from about 570 to 700 mμ . To isolate light in the other half which was absorbed only by the carotenoids a Wratten No. 61 filter was used with the $CuCl_2$ filter just described; this combination passed light from about 500 to 570 mμ .

The influence of culture age was investigated by comparing the matching intensities when applied to cultures of varying physiological age and to preparations which had been resting in the observation cell, in the dark, for lengths of time varying from a few minutes to ten hours.

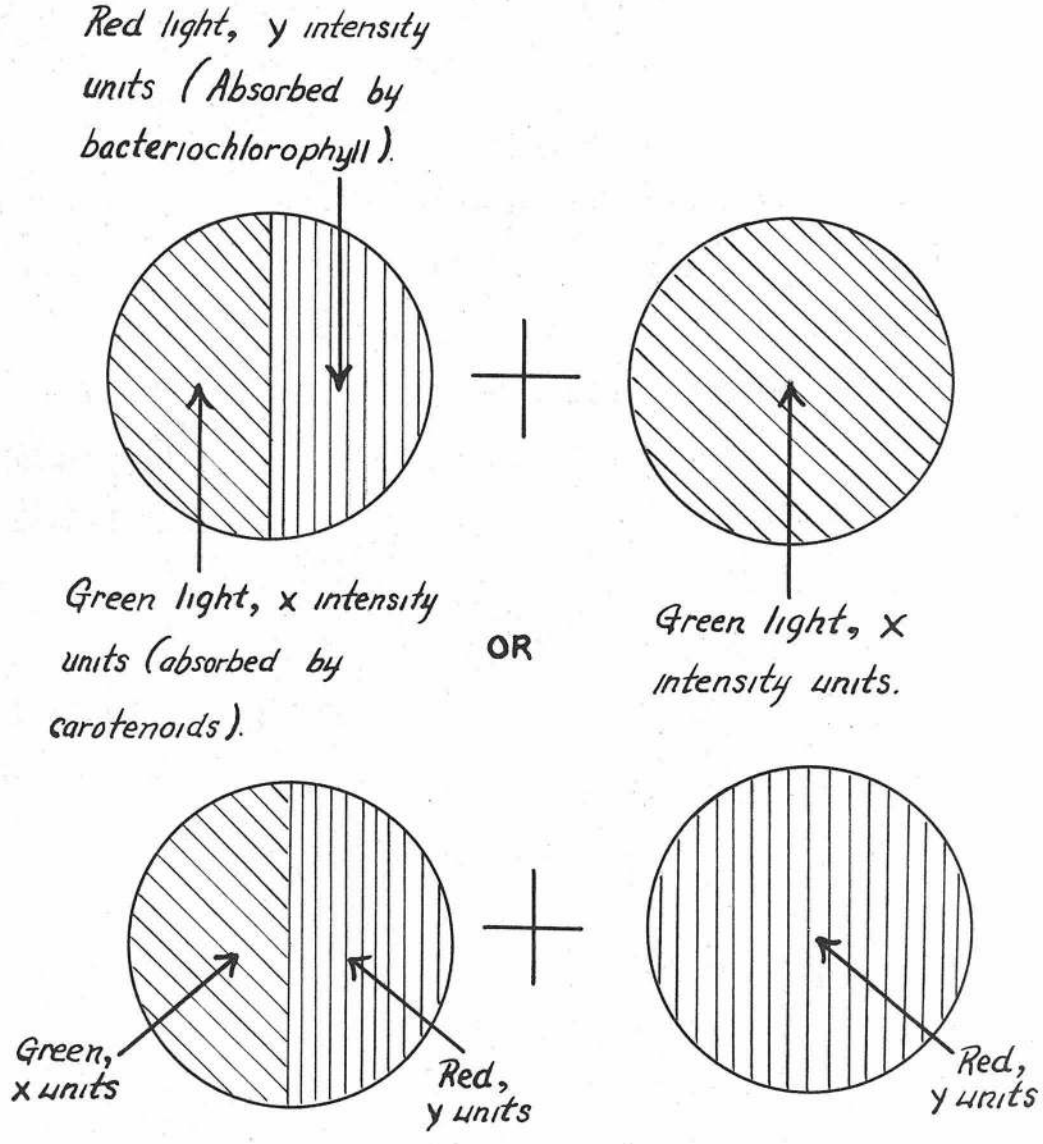
The influence of temperature was investigated at 10°C and at 25°C with the aid of a microthermostat built along the lines of one described by Thomas and Nijenhuis (6). This device, shown schematically in Fig. 10, was essentially a double-bottomed microscope slide through which water of controlled temperature could circulate. The room temperature remained between 25°C and 26°C throughout the period of these investigations; the temperature in the bacterial culture was calibrated against the temperature of the circulating water with the help of a six junction copper-constantan thermopile built for the purpose.

The influence of oxygen on the matching intensities in the divided field was observed both by introducing



MICROTHERMOSTAT

Fig. 10



(EXPLANATION IN TEXT)

Fig. 11

air bubbles into the observation cells and by noticing the results of accidental air leaks which occurred frequently (these microscopic leaks were easily detected by their attendant chemotactic accumulation patterns).

To see whether or not the relative sensitivities of the bacteriochlorophyll and carotenoid systems varied with the intensity of illumination, the intensity in each half of the divided field was raised or lowered by the same factor and the effect on the match was observed.

The influence of prior illumination with white light was examined using regimes ranging from three hours' dark adaptation to one hour of strong light adaptation (ten inches from a 100 watt lamp).

Finally, the effect of simultaneous uniform illumination of the preparation during exposure to the divided field was investigated. This procedure amounted to augmenting the illumination in each half of the field by the same amount. The superimposed illumination was filtered and adjusted in intensity in such a way that it was equal to the light already present in one half or the other of the divided field as shown in Fig. 11. An examination of the stability of the intensity match under these conditions amounted to a test of the additivity, in their phototactic effects, of quantities of light absorbed by different pigment systems.

c. Observations and Discussion: The results of all

of the investigations just described were negative; the match of intensities which suppressed phototactic accumulations did not vary by more than 2% under any of the changing conditions which were applied. The two pigment systems, bacteriochlorophyll and carotenoid, were either stable in their phototactic activity under these changes or were influenced in the same way and to the same degree; in either case no distorting influence on the action spectrum should be expected.

To interpret the present action spectrum and the one obtained by Manten we are left, then, with the possibility discussed in the foregoing section that the blue sensitivity is largely due to a highly active minor carotenoid which varies markedly in concentration from one strain of R. rubrum to another. Alternative to this view is the possibility that one or both of the action spectra was vitiated by an effect which we have not considered.

IV. WEBER LAW ADHERENCE

a. Method: The importance of checking Schrammeck's findings (4) of the remarkable Weber Law adherence displayed by the phototaxis of R. rubrum has been discussed in the first section. The essential experimental procedure used in performing this check was the same as that used by Schrammeck; the only significant refinement was the use of photography in making observations of the phototactic accumulation patterns. The bacteria, prepared for observation in the same manner as was done in the action spectrum measurements, were exposed to a divided field of illumination one half of which was slightly brighter than the other (both halves were illuminated with white light from the same source). Following a three minute exposure the preparation was photographed under uniform illumination to record the pattern of bacterial accumulation which persisted where the boundary of the divided field had been. The appearance of this accumulation pattern was thus studied in its dependence upon the intensities I_o and I in the two halves of the field. It was found that below some value of $I_o - I$ (hereafter denoted ΔI) there was no perceptible accumulation pattern. As ΔI was increased the first manifestation of phototaxis appeared in the form shown in Fig. 12a; the boundary divid-

(threshold)
Lower half darker
a

Lower half darker
b

Upper half darker
c

Accumulation Patterns in Divided Field
of Illumination
x2

Fig. 12

ing the halves of the split field was bordered on the darker side by a narrow zone of reduced bacterial density. As ΔI was increased further a zone of increased bacterial density became apparent on the brighter side of the boundary (Fig. 12b). Further increase in ΔI caused both zones to become wider and more pronounced until the extreme response was attained in which one half of the field was uniformly much denser than the other half (Fig. 12c). The criterion chosen in defining the phototactic threshold was the first positive appearance of an accumulation pattern.

In the course of a typical experiment the intensity I_o in one half of the field was maintained at 290 erg/cm^{2}sec while the intensity I in the other half was given successive values, less than I_o, until a definite phototactic accumulation pattern could be observed. The intensity I was then set equal to 290 erg/cm^{2}sec and the intensity I_o was reduced in successive stages until a phototactic pattern appeared. Photographs were taken of the bacterial response to all settings of I and I_o, and the threshold values of $(I_o-I)/I_o$ and of $(I-I_o)/I$ were observed and averaged. The data for this experiment are shown in Table II. The threshold values of $(I_o-I)/I_o$ and $(I-I_o)/I$ are seen to be .02 and .04 respectively. Averaging these, we say that $(\Delta I/I_o)_t$, the threshold value of $\Delta I/I_o$, equals .03 when I_o equals 290 erg/cm^{2}sec. The difference between the two values .02 and .04 probably arose because

I_o erg/cm^{2}sec	I erg/cm^{2}sec	$\frac{I_o - I}{I_o}$	$\frac{I - I_o}{I}$	Photo-taxis
290	290	0		-
290	287	.01		-
290	284	.02		+(faint)
290	281	.03		+
287	290		.01	-
284	290		.02	-
281	290		.03	-
278	290		.04	+(faint)
275	290		.05	+

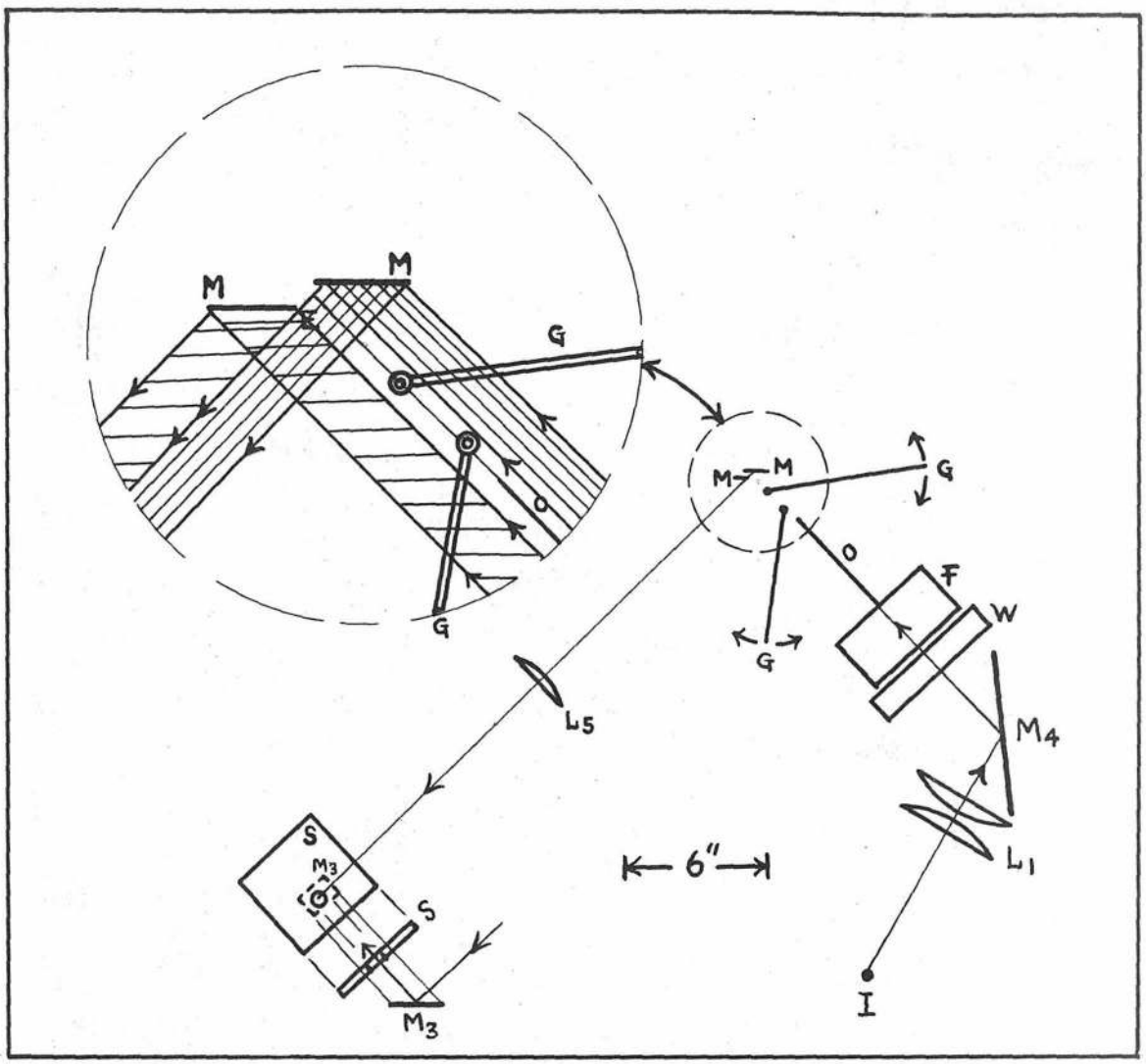
Table II.

the two halves of the field were not perfectly matched when each was set at 290 erg/cm^{2}sec; the averaging eliminated this source of error from the final result.

The threshold having been determined for one value of I_o, the entire process was repeated at another level of intensity. The average preparation could be used for four or five hours before its sensitivity began to deteriorate; in this time it was possible to cover three or four values of I_o twice.

Adherence to the Weber Law would require that the threshold value of $\Delta I/I_o$ be constant as I_o is varied; the results of this investigation were summarized in a plot of $(\Delta I/I_o)_t$ against I_o.

b. Instrumentation: The optical arrangement providing a split field of illumination is shown schematically in Fig. 13. It was adapted to the layout of the dismantled monochromator, so that the components I, L_1, L_5, M_3, and S were the same as those shown in Fig. 6, and in the same locations (the 500 watt lamp, I, was replaced by a 750 watt lamp). After leaving L_1 the collimated beam was deflected in an appropriate direction by the mirror M_4; it was next sent through a cooling cell W through which water was circulated. In penetrating this cell the light beam passed through 3/4 inch of water and 1/4 inch of lucite with the result that most of the radiation above 1000 mμ was absorbed.



DIVIDED FIELD ILLUMINATOR

Fig. 13

After leaving the cell W the beam entered a filter housing F; upon emerging from F it was divided into a right and a left half by a partition O. The right and left halves of the beam were rendered uniform by a ground glass plate contained in F, and their intensities could be reduced equally by attenuating screens, also contained in F. Screens occupying half the width of the beam, placed in the forward end of F so that their inner edges touched the partition O, served to attenuate one half of the divided beam relative to the other. The relative intensities of the two halves were controlled with greater delicacy by the intervention of two glass plates G; each plate could be rotated from a position normal to the beam to a position which formed an angle of 17.50° with the direction of the beam. The resulting transmission by either plate ranged smoothly from 86% (at normal incidence) to 59% (at 17.5° to the beam). At this point in the system two uniform beams of light had been formed which could be controlled accurately in intensity, independently of each other. It was desired next to bring these beams into juxtaposition, separated by a sharp boundary, at the microscope stage. This was done by means of the offset mirrors M; their spacing was such that the central region dividing the two halves of the light beam was removed and the uniform portions of the divided beam were brought together. At the inner edge E of the forward mirror the

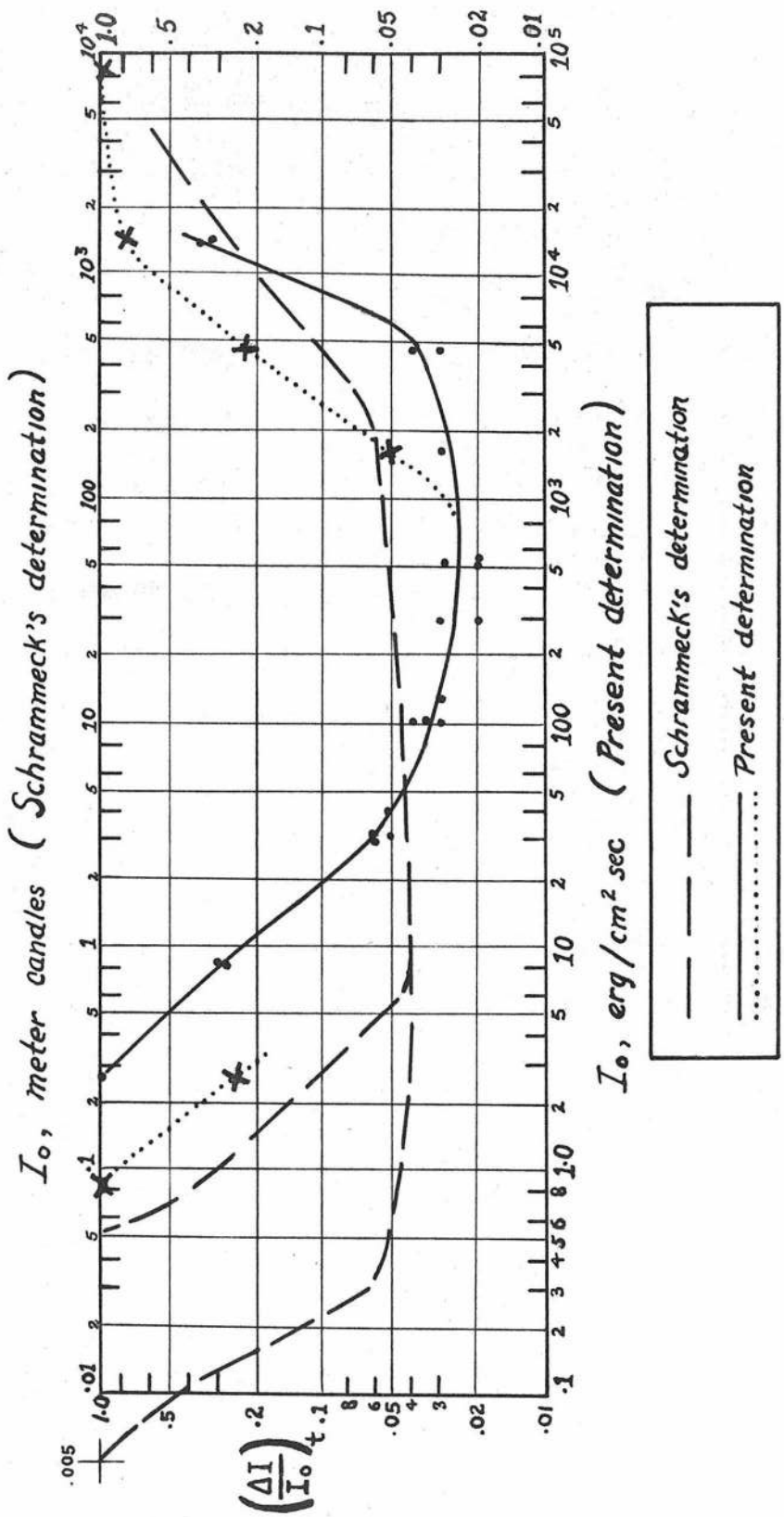
two halves of the beam were in close contact with each other without appreciable gap or overlap; by imaging this edge at the microscope stage a sharp boundary was obtained separating the halves of a divided field of illumination.

The bacterial accumulation patterns were photographed with a Zeiss Contax camera, kindly lent by Prof. J. Weigle, which was mounted above the microscope stage. The camera was used in conjunction with a short-focus adapting lens, so that the photographic image of the bacterial preparation was nearly the size of the preparation itself.

c. Observations and Discussion: Before referring to a plot of $(\Delta I/I_o)_t$ against I_o it would be well to discuss the manner in which the plot was obtained. In most cases the same bacterial preparation could be used for comparative threshold measurements at three or four values of I_o. In the middle intensity range, from about 100 to 1000 erg/cm^{2}sec, there was little difference in $(\Delta I/I_o)_t$ from one preparation to the next. At lower intensities, at which the threshold value of $\Delta I/I_o$ began to rise, greater differences in sensitivity between different preparations became apparent. These differences became most pronounced at the absolute threshold intensity, at which $(\Delta I/I_o)_t$ was equal to unity (half of the divided field was totally dark). This threshold intensity for discrimination between light and darkness varied about a mean value by a factor

of about three from one preparation of bacteria to another. At intensities higher than the middle range the value of $(\Delta I/I_o)_t$ rose as light saturation set in; here again different preparations began to vary widely in their phototactic sensitivities. As in the case of the absolute threshold intensity, the intensity at which saturation began to appear varied by a factor of about three around a mean value for different preparations. It was observed that a high value of the saturating intensity was correlated with a high value of the absolute threshold intensity; differences in sensitivity of the bacterial preparations appeared to be equivalent to changes in the scale of intensity, suggesting that the differences arose from variations in the quantum efficiency of the overall phototactic process.

In constructing a plot of $(\Delta I/I_o)_t$ vs. I_o it was necessary to fit together the results of experiments on different preparations of bacteria having different phototactic sensitivities; the outcome of this construction is presented in Fig. 14 along with a plot of the results obtained by Schrammeck (the individual points representing Schrammeck's data, about 100 in all, are not shown; they exhibit about the same degree of scatter as the points of the present investigation). The data of three experiments in the middle intensity range were grouped together. A curve (solid line) drawn through these points was then extended through points



PHOTOTACTIC DISCRIMINATION THRESHOLD VS. INTENSITY OF ILLUMINATION

Fig. 14

representing two experiments, on preparations having approximately equal sensitivities, covering the lower range of intensities. The same curve was extended in the higher range of intensities through the data of two experiments which entered the saturating intensity region. The preparations used in these two experiments were observed to have the same absolute threshold, within about 20%, as the preparations used in the two experiments which covered the low intensity range. The complete curve, then, represents data on preparations having approximately equal sensitivities (equal absolute thresholds, equal saturating intensities, and the same threshold $\Delta I/I_o$ in the middle intensity range). An incomplete curve representing the data of one experiment is also plotted (dotted line). The preparation used in this experiment was unusually sensitive, having saturating and absolute threshold intensities which were lower by a factor of about 4 than the corresponding intensities for the average preparation. The incomplete curve representing the response of this unusually sensitive preparation is seen to be shifted to the left, without serious distortion, from the principal curve; it would coincide approximately with the principal curve if its values of I_o were all raised by a factor of 4.

The curve representing Schrammeck's data (dashed line) shows two branches at the low intensity end; Schrammeck

actually obtained data for four branches of which these two represent the extremes of sensitivity. In the region of saturation two sets of data were obtained by Schrammeck, one for Rhodospirillum rubrum and one for a Chromatium species. The results were in sufficiently close agreement to be represented by a single curve at the high intensity end of the plot. Throughout his experiments Schrammeck used both Rhodospirillum and Chromatium; no consistent differences in the phototactic sensitivities of these types were observed.

The obvious conclusion to be drawn from Fig. 14 is that the Weber Law adherence reported by Schrammeck was not corroborated in the present investigation. Schrammeck found that the threshold value of $\Delta I/I_o$ remained sensibly constant (between .04 and .06) over a five thousandfold range of I_o extending from .04 to 200 meter candles. The data of the present investigation indicate that $(\Delta I/I_o)_t$ remains approximately constant (between .025 and .05) over a range of I_o of only a hundredfold.

In considering these discrepant results we should note first that Schrammeck did not report observations, on the same preparation of bacteria, of both the absolute threshold intensity and the saturating intensity. The preparations used in exploring the region of saturation may have had absolute threshold intensities as low as .005 m.c. or as high as .05 m.c. If an absolute threshold

of .05 mc was held in common with the saturating intensity shown, it would be expected that a preparation having an absolute threshold of .005 m.c. would display saturation at one tenth the saturating intensity shown. While the range of I_o yielding Weber Law adherence would then be curtailed by a factor of 10, the remaining five hundredfold range of fair Weber Law adherence would still stand in marked contrast to the hundredfold range of approximate Weber Law adherence observed in the present investigation.

It may be argued that the present investigation did not reveal fully the capacity for Weber Law adherence latent in the bacteria. An abnormally high absolute threshold might result from an insensitive observational procedure. This condition might be expected to yield a sensitivity curve in which the rising portion in the low intensity region was shifted closer to the rising portion in the region of saturation, crowding the flat middle portion out of the picture. It would behoove us, then, to examine critically the manner in which such a sensitivity curve would be distorted by various influences.

Before discussing the variation in phototactic sensitivity of a preparation of bacteria, let us consider in detail the events leading to the appearance of a phototactic accumulation pattern. Confining our attention for the moment to a single bacterium, we find that a decrease in the intensity of light impinging on the

organism has some probability of eliciting from it a phototactic response (a reversal of swimming direction). The greater and the more abrupt the decrease, the greater is the probability that a response will occur. Let us consider that the decrease of illumination generates in the bacterium an effect E which tends to bring about a phototactic response. We shall make no effort to define E objectively; it is introduced merely as a symbol of the strength of the phototactic stimulus. We say, then, that the probability P of a phototactic response is for each bacterium a function of E which increases monotonically with E.

When a preparation of purple bacteria is exposed to a divided field of illumination, the formation of a noticeable accumulation pattern depends primarily upon the frequency of individual phototactic responses at the boundary and upon the sensitivity of the observational procedure. The frequency of responses depends upon the mean speed of locomotion of the bacteria, upon the mean probability P(E) of response when crossing the boundary, and upon the population density of the culture. The population density, having no direct connection with the degree of activity of the bacteria, will be classed, for convenience, as a factor in the sensitivity of observation. For an observational procedure having a given sensitivity, applied to a preparation of bacteria having a given mean swimming speed, the appearance of a noticeable accumulation pattern will then require that the mean response probability P(E) be greater than a critical (threshold)

value.

Having introduced some specific variables into the process of phototactic accumulation, let us see how the plot of $(\Delta I/I_o)_t$ vs. I_o might be altered in form by changes in these variables.

A change in the quantum efficiency of the overall phototactic process would be equivalent to a change in the scale of intensity; on a logarithmic plot the curve would simply be shifted as a whole to the right or left (this effect has been observed and described earlier in this section).

A decrease in the sensitivity of the observational procedure would necessitate a greater mean value of the response probability P(E) for the generation of a threshold accumulation pattern. An increase in the required mean P(E) would also result from a decrease in the mean swimming speed of the bacteria; if half the bacteria were bereft of their motility the response probability of the remainder would have to be doubled in order for the gross accumulation pattern to be unaffected. Both of these influences, necessitating a greater mean value of P(E) for a threshold observation, require in turn a greater value of E (a stronger stimulus). A third factor, the responsiveness of a bacterium, can be defined by the relationship between strength of

stimulus E and response probability (P(E). The greater the value of P(E) corresponding to a given value of E, the more responsive is the bacterium. A decrease in the mean responsiveness of the bacteria in a preparation would then necessitate a greater value of E for the attainment of a threshold mean value of P(E).

Having seen that the factors of crude observation, low motility, and poor responsiveness all necessitate a stronger stimulus for the generation of a perceptible phototactic accumulation pattern, let us see what the strength of stimulus E means in terms of ΔI and I_o. Certainly a stronger stimulus is always obtained by increasing ΔI, regardless of the value of I_o. At any given value of I_o, then, an increase in E would correspond to an increase in $\Delta I/I_o$. When $I = 0$ and $\Delta I = I_o - I = I_o$, an increase of E can be obtained only by increasing I_o. Thus the requirement of a stronger stimulus for an observable response corresponds to an increase in the absolute threshold intensity and to an increase in $(\Delta I/I_o)_t$ at all higher values of I_o, including the region of saturation. The intensity at which saturation sets in, presumably determined by the relative rates of some critical light and dark processes in the bacteria, would not be affected by the requirement of a stronger stimulus for an observable response. Thus we

see that the factors of insensitive observation, poor motility, and low responsiveness would alter a logarithmic plot of $(\Delta I/I_o)_t$ vs. I_o by shifting the absolute threshold intensity closer to the saturating intensity and by raising the general level of $(\Delta I/I_o)_t$ throughout the curve. Referring to the curves of Fig. 14, we see that the one representing the present determination actually attains lower values of $(\Delta I/I_o)_t$ than does the one representing Schrammeck's experiments; for this reason we can assume confidently that the poor Weber Law adherence found in the present investigation was not caused by an abnormally high absolute threshold resulting from crude observations or from sluggish bacteria.

The possibility remains that the saturating intensity might have been abnormally low in the present investigation, causing the rising part of the plot at high intensities to encroach upon the rising part near the absolute threshold.

To place our discussion of the phototactic saturating intensity on a concrete basis, let us assume with Manten that the phototactic stimulus is mediated through the initial photosynthetic reaction pathways of the organism. The validity of this assumption is supported strongly by the finding of Thomas (29) that the phototactic and photosynthetic action spectra of R. rubrum (strain-S-4)

are in reasonable agreement and by the observations of Thomas and Nijenhuis (6) on the agreement between the saturating intensities for phototaxis and for photosynthesis under various conditions.

The saturating intensity for phototaxis (and for photosynthesis) depends then upon the efficiency of the photoreceptive and initial photochemical processes and upon the rate of a subsequent dark process which becomes limiting when the products of the light reaction are formed too rapidly to be accommodated by the dark reaction. A reduced photochemical efficiency, equivalent to a change in the intensity scale, shifts the phototactic sensitivity curve without altering its shape, as we have already seen. A reduction in the rate of the limiting dark process, however, will lower the saturating intensity without affecting the absolute threshold intensity or the threshold value of $\Delta I/I_o$ below saturation; as a result the phototactic sensitivity curve will show an earlier onset of saturation but will remain unaltered below the saturating region.

Since the disagreement between the present phototactic sensitivity curve and that of Schrammeck might have arisen from such an effect it would be well to consider this possibility in a more quantitative manner. The difference in rate of the limiting dark process which would resolve the disagreement should be estimated, and the possibility that such a difference might have existed

should be considered. The ratio of saturating intensity to absolute threshold intensity was about 5000/2.5, or 2000, in the present case; in Schrammeck's determination it was between 4000 and 40,000 depending upon which value of the absolute threshold intensity corresponded to the single value of saturating intensity shown. It could be expected, then, that a twofold to twentyfold increase in the rate of the limiting dark process in the present investigation would have led to a sensitivity curve agreeing essentially with that obtained by Schrammeck.

The most obvious factor which would influence the rate of a "dark" chemical reaction is temperature. It was observed both in the present investigation and by Thomas and Nijenhuis (6) that a decrease in temperature did cause a marked decrease in the saturating intensity, amounting to about a twofold reduction per 10°C. The temperature of the bacteria in the present investigation was between 25°C and 27°C; in Schrammeck's experiments it was maintained at about 20°C. This difference in temperature would introduce a factor of roughly 1.5 in the relative dark reaction rates, necessitating a threefold to thirtyfold change by factors other than temperature for the resolution of the conflicting data.

Aside from its dependence upon temperature, the rate of the limiting dark reaction might depend upon a number

of factors such as the nature of the substrate acting as hydrogen donor and the presence of substances inactivating or reacting competitively with a critical enzyme. It is even possible that the limiting dark reaction may not be the same for different strains of R. rubrum or for the same strain under different culture conditions. If a particular strain grown under certain conditions is deficient in some enzyme, the reaction involving that enzyme may replace some other reaction in limiting the photosynthetic rate at high intensities of illumination.

In view of these possibilities it is not unlikely that the limiting dark reaction rate might differ by a factor of more than three between different strains of purple bacteria grown under different conditions. Weighing against this likelihood is the fact that Schrammeck observed the same saturating intensity for the phototaxis of members of two different genera of purple bacteria, Rhodospirillum and Chromatium. It is possible, of course, that the agreement between these unrepeated observations may have been coincidental. We may conclude, at any rate, that the results of the present investigation and those of Schrammeck are reconcilable in a plausible manner on the basis of differing dark reaction limitations. We are then led to the further conclusion that the present investigation did not reveal the greatest capacity for Weber Law adherence latent in the phototactic response

of Rhodospirillum rubrum.

It is tempting to speculate that in other cases than the present one (in the case of visual intensity discrimination by humans, for example) a close adherence to the Weber Law might be masked by the early onset of saturating influences, and that under some conditions a determination of response vs. stimulating intensity might reveal a much higher saturating intensity and a closer Weber Law adherence than has been observed to date.

On the other hand,the results of the present investigation on R. rubrum might be considered to weigh against the prevalence of Weber Law adherence, contradicting as they do one of the few known cases of convincing adherence to this so-called law.

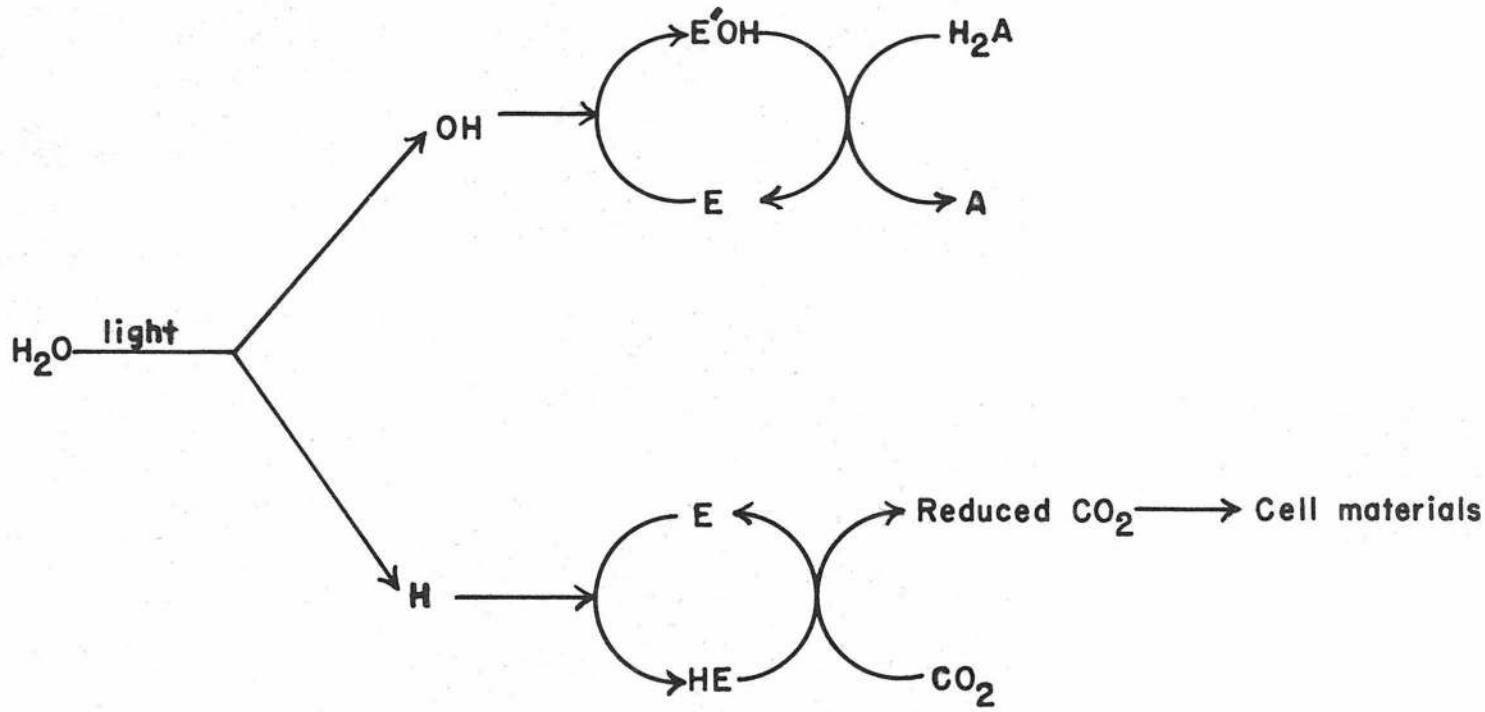
Should further experiments reveal a more widespread occurrence of close adherence to the Weber Law, its significance as a fundamental property of an irritable cell would still be open to question. It may be that a simple mechanism casing Weber Law adherence is embodied in the structure of a cell, and that its discovery would be of considerable value in elucidating the problem of irritability. On the other hand, the approximate Weber Law adherence which has been observed in biological systems may be the result of a superposition of many physico-chemical factors which modify the relation between stimulus and response. Such a structure could

develop as a result of many evolutionary modifications and could vary widely in its detailed nature from one type of living system to another. If this is so, the search for a cause of Weber Law adherence would be pointless and fruitless.

V. CHEMOTAXIS FOR OXYGEN

a. Introduction: It was mentioned in the first section that the chemotaxis of R. rubrum for oxygen was studied in an attempt to gain some insight into the relationship between the tactic behavior of the purple bacteria and their metabolic activities. Before we consider the results of these studies let us examine more closely the mechanism of bacterial photosynthesis, proposed by van Niel (25), which was outlined briefly in the first section.

The primary photochemical act is assumed, as in the case of plant photosynthesis, to be a photolysis of water giving rise to a reducing fragment and an oxidizing fragment ("photoperoxide"). The reducing fragment is carried enzymatically to CO_2 or higher intermediates, bringing about their reduction. The oxidizing fragment is carried enzymatically to a suitable hydrogen donor which is oxidized. The hydrogen donor or some of its oxidation products might serve also as a source of reducible material for synthesis of cell materials, in which case additional CO_2 or its equivalent need not be supplied. Nevertheless, the primary function of the hydrogen donor is assumed to be the removal of the photoperoxide, the accumulation of which would block



SCHEME OF BACTERIAL PHOTOSYNTHESIS AFTER VAN NIEL (25)

Fig. 15

the photosynthetic process. The scheme we have just described is illustrated (after van Niel) in Fig. 15, where H and OH are the reducing fragment and the photoperoxide respectively, E and E' are enzymes, and H_2A is the hydrogen donor.

The energy of the absorbed light is assumed, then, to be used in forming a reduced enzyme HE, capable of reducing CO_2, and an oxidized enzyme E'OH which can oxidize H_2A. In the case of plant photosynthesis water can serve as the hydrogen donor H_2A. This can be regarded as a consequence of the greater energy of light quanta absorbed by bacteriochlorophyll when compared with quanta absorbed by chlorophyll; the greatest absorption by the former pigment takes place at 680 $m\mu$, compared with 875 $m\mu$ for the latter pigment.

The reduction of CO_2 and the concomitant oxidation of H_2A can take place, with most strains of R. rubrum, at the expense of energy other than absorbed light. In the dark, in the presence of molecular oxygen, the reduction of O_2 can serve as a source of energy for a chemosynthetic metabolism in which CO_2 is reduced to cell materials. This process is more wasteful of the hydrogen donor than is the photosynthetic process; in the latter case the oxidation of a certain amount of

H_2A can lead to an equivalent reduction of CO_2, while in the former case a large amount of H_2A is expended in the exergonic reduction of O_2.

b. Observations: Bearing in mind the foregoing metabolic scheme, let us turn to some observations on the tactic behavior of R. rubrum toward changes in oxygen concentration.

Dense suspensions of the bacteria were placed in the same observation chambers which were used in phototactic studies; gradients of oxygen concentration were established within these chambers and the ensuing chemotactic accumulation patterns were observed. Simultaneous observations of phototactic accumulation patterns could be made by illuminating the preparations concurrently. The methods of realizing oxygen gradients within the observation chambers were crude but suitable for the demonstration of some unequivocal results. A slight rise in the oxygen concentration near the periphery of a chamber, resulting from the slow leakage of air through the vaseline seal, could be utilized. A more pronounced oxygen gradient was attained by the introduction of an air bubble into the chamber.

In order to assess the observations of chemotaxis resulting from these oxygen gradients we should consider two possible sources of anomaly: the changing nature of

the gradients after their causes have been established, and the existence of chemotaxis for substances other than oxygen. Factors involved in the first of these difficulties are the transport of oxygen through the observation chamber and the uptake of oxygen by the bacteria (it has been shown repeatedly that purple bacteria do not liberate oxygen under any circumstances). A rapid transport of oxygen can be expected, since the process of diffusion is supplemented by convection resulting from the motility of a dense population of bacteria. One would anticipate, then, that any oxygen introduced at one region in the chamber would soon be distributed fairly uniformly throughout the chamber. An estimate of the rapidity of this process will be gained later in this section. Offsetting the leveling effect of transport is the utilization of oxygen by the bacteria under certain conditions. The ability of R. rubrum to reduce O_2 in the dark has already been mentioned; this oxygen uptake is suppressed completely, as far as manometric measurements reveal, when the bacteria are photosynthesizing under light-saturated conditions (30). It need not be inferred that a low rate of photosynthesis will block the aerobic chemosynthetic process entirely, but under conditions in which both types of metabolism are possible the photosynthetic mode is highly favored. In the dark, then, one can

expect that utilization of O_2 by the bacteria will tend to preserve gradients of oxygen between its source and other regions of the observation chamber; in the light this compensating influence need not be expected.

Turning to the possibility of chemotaxis for substances other than oxygen, it has been noted by Mölisch (2) and others that purple bacteria respond tactically to a wide variety of ions and organic substances. More to the point, evidence for a tactic avoidance of metabolic products of the bacteria has been observed repeatedly (1, 2, present investigation). The particular observation which has been interpreted in this way has been the appearance of an inverse "after-image" following the development of a phototactic accumulation pattern. If phototactic bacteria are caused to accumulate in a spot of light, and if the spot of light is then removed, the bacteria do not merely redistribute themselves uniformly; a spot of reduced bacterial density develops where the spot of light and of bacterial accumulation had been. As the inferred local concentration of metabolites becomes dissipated the spot of negative accumulation vanishes. This dissipation usually requires about five minutes for its completion under the conditions prevailing in the present investigation; in connection with an earlier paragraph we can

estimate that an unsustained gradient of oxygen in the observation chamber will be dissipated in a similar length of time.

We have seen that a tendency toward reversal of bacterial accumulation patterns, attributable to a negative chemotaxis for accumulated metabolites, must be taken into account in evaluating chemotactic studies. Another possibility which must be anticipated is the existence of chemotactic effects caused by nitrogen or by vaseline, since local concentrations of these substances in the observation chamber were not avoided. This possibility was ruled out by placing the observation chamber, with and without a vaseline seal, in a larger chamber containing either air, oxygen, or nitrogen, and by placing blobs of vaseline in preparations of bacteria which were otherwise free of vaseline. In no case was a chemotactic effect observed which could be attributed to anything but air or oxygen.

In summary of the last few paragraphs, two factors should be recognized in an appraisal of the present studies of the chemotaxis for oxygen of R. rubrum. First, the oxygen gradients can be expected to be more pronounced in preparations kept in the dark than in illuminated preparations. Second, the local accretion of metabolites can be expected to cause a reversal of bacterial accumulation patterns when the primary cause

20' dark

a

a + 5' light

b

b + 25' dark

c

PHOTOSYNTHETICALLY GROWN BACTERIA

45' light + 15' dark

d

d + 3' strong light

e

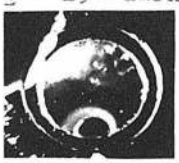
75' light

f

45' dark + 35' light

g

g + 15' dark



h

h + 15' light

i

CHEMOSYNTHETICALLY GROWN BACTERIA

Chemotactic Accumulation Patterns
x2

Fig. 16

of taxis is removed or diminished.

Let us turn now to a statement and an evaluation of the results of these investigations of chemotaxis for oxygen. The principal line of investigation was conducted in the following manner: a dense suspension of R. rubrum was placed in an observation chamber of the type already described and was kept in the dark for one hour; at the end of that time the bacteria were in a state of uniform distribution throughout the chamber. An air bubble was then introduced into the chamber, the operation being performed in total darkness. After a given regime of illumination and darkness following the introduction of the air bubble the preparation was observed and photographed. Observations were made on cultures grown anaerobically in the light and on cultures grown aerobically in the dark. Reproduction of the bacteria in the observation chamber, under conditions differing from the growth conditions of the culture, was minimized by using fully developed cultures in all experiments.

The basic results of these experiments are shown in the selection of photographs displayed in Fig. 16, in which the light and dark regime following the introduction of the air bubble is written above each photograph. In photograph b, for example, the preparation

was kept in the dark for twenty minutes and then illuminated for five minutes prior to its observation. Illumination was provided by a 60 watt lamp two feet from the preparation; the strong light referred to in photograph e consisted of a 100 watt lamp six inches from the preparation. In all photographs the air bubble appears as a dark spot in the lower portion of the chamber.

The chemotactic behavior of photosynthetically grown R. rubrum is illustrated in Figs. 16 a, b, and c. These three photographs, all of the same preparation of bacteria, were taken at successive stages in the regime of darkness and illumination following the introduction of the air bubble. Fig. 16a reveals a negative chemotaxis for oxygen (avoidance of the region near the air bubble) on the part of bacteria which cannot be photosynthesizing. Fig. 16b shows that this negative chemotaxis becomes much more pronounced under conditions which permit photosynthesis. This is the type of response which has been observed repeatedly by Molisch (2) and others. In Fig. 16 c we see that a dark period following a light period causes the chemotactic accumulation pattern to become obliterated; this effect can be attributed to the negative chemotaxis for metabolites which we have already discussed.

When R. rubrum is grown aerobically in the dark it acquires a tactic response to oxygen which is in striking

opposition to the negative chemotaxis displayed by the photosynthetically grown bacteria. In Fig. 16 d we see a well defined, pronounced accumulation of bacteria in the vicinity of the air bubble. This unmistakeable positive chemotaxis was never observed with light-grown bacteria; with the dark-grown bacteria it materialized both in the dark and in the light, the pattern tending to be more diffuse in the light (see Figs. 16 g, h, and i, representing the same preparation at successive times). A few minutes' exposure to very strong illumination eradicated the chemotactic accumulation pattern, as can be seen in Fig. 16 e (same preparation as in Fig. 16 d). Following a prolonged exposure to light, of the order of an hour, an accumulation pattern representing negative chemotaxis began to develop in addition to the positive chemotactic pattern. This effect is seen clearly in Fig. 16 f; it is not as well developed in Figs. 16 g, h, and i.

The foregoing results can be summarized as follows:

<u>Photosynthetically grown bacteria</u>:

1. Negative chemotaxis for O_2 in dark.
2. More pronounced negative chemotaxis for O_2 in light.
3. Positive chemotaxis for O_2 absent, even after prolonged dark adaptation.

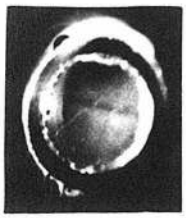
<u>Chemosynthetically grown bacteria</u>:

1. Positive chemotaxis for O_2 in dark and in light.

2. Accumulation pattern more diffuse in dark than in light.
3. Pattern obliterated by strong illumination.
4. Negative chemotaxis for O_2 appears after prolonged light adaptation.

Let us now examine the results of another line of investigation, based upon the slow leakage of air through the vaseline seal of the observation chamber. This leakage did not cause a perceptible chemotactic effect with dark-grown bacteria, nor could it be detected with light-grown bacteria as long as the illumination of the preparation was below the region of saturation for phototaxis. However, in the course of investigating the Weber Law adherence of the phototactic response it was noticed that the onset of phototactic saturation was usually accompanied by a pronounced withdrawal of bacteria from the periphery of the observation chamber. This effect, which arose in about three fourths of the preparations examined, appeared at the lowest intensity which produced a detectable saturation of the phototactic sensitivity; it vanished again when the intensity was reduced to a point just below the onset of saturation. The shape of this withdrawal pattern (Fig. 17b) suggest strongly that it represents a chemotactic response to air leaking through the vaseline seal at various points in

the periphery of the chamber. When the saturating intensity for phototaxis was reduced by lowering the temperature of the preparation it was found that the chemotactic pattern again made its first appearance at the onset of saturation. Finally, it was observed that the chemotactic pattern vanished again at an intensity well above the onset of saturation. This last effect was not recorded photographically; it was observed in an experiment involving intensities of 4600, 14,000, and 82,000 erg/cm^{2}sec. The lowest of these intensities was insufficient for phototactic saturation, the middle one produced a definite saturation, and the highest was effectively in the region of complete saturation where $(\Delta I/I_o)_t$ is nearly unity (see Fig. 14). A chemotactic pattern of the type shown in Fig. 17b appeared regularly when the preparation was illuminated with 14,000 erg/cm^{2}sec and vanished regularly at the higher and lower intensities. After six observations at each of these three intensities the chemotactic pattern began to develop more sluggishly and after about half an hour's uninterrupted illumination it could no longer be observed. This "wearing off" under continued illumination, which probably resulted from a dissipation of the small oxygen gradient, may have been responsible for the failure of a chemotactic pattern to develop in

I_o = 4600 erg/cm^{2}sec  $\Delta I/I_o$ = .07

a

I_o = 14,000 erg/cm^{2}sec  $\Delta I/I_o$ = .20

b

I_o = 4600 erg/cm^{2}sec  $\Delta I/I_o$ = .07

c

Chemotaxis at Phototactic Saturation
x2

Fig. 17

approximately one fourth of the cases observed.

From the observations just described it can reasonably be inferred that the negative chemotaxis for oxygen of photosynthetically grown R. rubrum shows a marked rise in sensitivity at intensities of illumination corresponding to the onset of phototactic saturation. In comparison, the chemotactic response at higher and at lower intensities is much less sensitive.

c. Discussion: Evidence has already been cited concerning the action spectra and saturating intensities for photosynthesis and phototaxis, which supports the notion of Manten(5) that the phototactic response of R. rubrum is mediated through or associated with an abrupt decrease in the rate of its photosynthetic metabolism. A reasonable corollary to this idea is that any factor which inhibits the photosynthetic rate can be expected to elicit a tactic response. Furthermore, it might be expected that when R. rubrum is growing chemosynthetically by reducing oxygen it will respond tactically to a factor which decreases the rate of chemosynthesis. A chemotaxis for oxygen may, then, be the result of an influence of oxygen upon the photosynthetic or chemosynthetic rate; the observations at hand will be discussed from the point of view of support or contradiction of this hypothesis.

It has been observed (31) that under illumination which is above saturation for photosynthesis and in the presence of oxygen the metabolism of R. rubrum is strictly photosynthetic; no perceptible uptake of O_2 takes place. Evidence is lacking as to whether the chemosynthetic metabolism is wholly or partly suppressed at lower intensities of illumination, nor has the possibility of a difference in this respect between light-grown and dark-grown bacteria been investigated. The related question of whether the presence of oxygen suppresses the rate of photosynthesis has not been investigated with purple bacteria. It has long been known, however, that excessive concentrations of oxygen inhibit the rate of photosynthesis in plants. Furthermore, the photosynthetic development of cultures of R. rubrum is generally inhibited by oxygen (24). Fresh isolates of this organism are usually unable to grow in the presence of minute amounts of oxygen; only after a protracted period of adaptation involving numerous culture transfers is a tolerance of oxygen acquired. It is probable, then, that oxygen exerts a suppressive effect on the rate of photosynthesis of R. rubrum. If we accept this assumption, most of the chemotactic effects which we have mentioned can be correlated with an influence of oxygen on the photosynthetic or chemosynthetic rate in such a way that a tactic response

accompanies a decrease in synthetic rate.

In the dark a decrease of oxygen concentration should lead to a decrease in the rate of chemosynthesis in R. rubrum; if we associate a tactic response with this decrease in rate we should anticipate the positive chemotaxis for oxygen which has been observed in the dark with dark-grown bacteria. The disappearance of the pattern of positive chemotaxis under strong illumination is in accord with the complete suppression of chemosynthetic activity by illumination which is intense enough for photosynthetic saturation.

At lower intensities of illumination the type of metabolism predominating among these dark-grown bacteria is open to speculation. The development of a pronounced pattern of negative chemotaxis, in addition to the positive chemotactic pattern, after an hour's light adaptation of dark-grown bacteria reveals that a change has taken place in a significant fraction of the bacterial population (it should be remembered here that extensive cell division in one hour is unlikely in a fully developed culture). The most likely change under light adaptation is a transition from a chemosynthetic metabolism to a photosynthetic metabolism; the inference is that without prolonged light adaptation the metabolism of dark-grown bacteria at moderate intensities of illumination is chemosynthetic. The positive chemotaxis for

oxygen of dark-grown R. rubrum under moderate illumination thus remains in accord with an association between tactic response and decreased rate of synthesis. The fact that the pattern of positive chemotaxis is more diffuse in the light than in the dark can be attributed to a partial suppression of chemosynthesis by moderate illumination. This suppression of chemosynthesis could act on the pattern in two ways: the tactic sensitivity of the bacteria to oxygen could be diminished, and the oxygen gradient could become weaker because of reduced O_2 uptake by the bacteria.

Turning to the chemotactic behavior of photosynthetically grown R. rubrum, the pronounced negative chemotaxis for oxygen under illumination supports our association of tactic response with decrease in synthetic rate if we assume that oxygen suppresses the rate of photosynthesis. Such an association is contradicted flatly, however, by the appearance of a negative chemotaxis for oxygen in the dark. This negative chemotaxis, displayed by bacteria which could not have been photosynthesizing, was observed many times with preparations which had been kept in total darkness for a period extending from an hour prior to the introduction of the air bubble to several minutes afterward. In every case the chemotactic pattern was present the instant the preparation was illuminated for observation. The motility of the bacteria

after more than an hour's darkness, evidenced by their ability to form a tactic accumulation pattern, must be ascribed to chemosynthetic activity. We are confronted then with a tactic response of R. rubrum which cannot be associated with a decrease in rate of synthesis unless we make the awkward assumption that the chemosynthetic rate of light-grown rubrum is greater at very low concentrations of oxygen than at moderate concentrations. Rather than resort to such a desperate attempt to retrieve an hypothesis, let us abandon the idea of a strict association between tactic behavior and rate of synthesis. We shall retain the assumption that the photosynthetic rate of R. rubrum is inhibited by the presence of oxygen.

The basic observations on R. rubrum which must be reconciled are:

1. Action spectra and saturating intensities for photosynthesis and for phototaxis are in agreement.

2. Photosynthetically grown cultures exhibit, in darkness, a negative chemotaxis for oxygen which becomes much more pronounced under illumination.

3. Chemosynthetically grown cultures exhibit a positive chemotaxis for oxygen both in darkness and under moderate illumination.

4. Chemosynthetically grown bacteria can be

induced by light adaptation to exhibit a negative chemotaxis for oxygen.

These observations can all be reconciled if we make the following assumptions:

1. In its qualitative aspects the tactic behavior of R. rubrum is governed not by the immediate metabolic activity of the organism but by its latent metabolic capacity. If the bacterium is capable of photosynthesis it will respond tactically to any environmental change which would lower the potential photosynthetic rate.

2. A secondary influence is exerted on the tactic behavior by the type and rate of synthetic activity actually taking place in the bacterium, a decrease in the rate of synthesis being associated with a tendency toward tactic response. This secondary influence can modify the tactic response quantitatively but cannot supersede the latent photosynthetic capacity of the organism in dictating tactic behavior.

3. If the bacterium is incapable of photosynthesis (we assume this to be the case with dark-grown bacteria which have not been light-adapted) its tactic behavior is associated both qualitatively and quantitatively with the secondary influence of a change in the actual chemosynthetic rate.

The first of these assumptions accounts for the observations relating phototaxis and photosynthesis; it

also accounts for the negative chemotaxis for oxygen which light-grown bacteria display in the dark if we retain the assumption that oxygen inhibits the photosynthetic rate. The second assumption accounts for the fact that the negative chemotaxis for oxygen of light-grown bacteria becomes more pronounced if the bacteria are illuminated. The third assumption in conjunction with the second accounts for the positive chemotaxis for oxygen displayed by dark-grown bacteria. Finally, the gradual development of a negative chemotaxis for oxygen in an illuminated culture of dark-grown bacteria is accounted for by the first and third assumptions.

The foregoing treatment is speculative but it effects the reconciliation of a variety of observations with a minimal number of independent assumptions.

The essential feature of our treatment is the rejection of the hypothesis that the tactic response of R. rubrum is associated primarily and invariably with a decrease in the rate of its synthetic metabolism. The necessity of the rejection becomes apparent when we recall the observation on which it is based: on one hand we have dark-grown purple bacteria, metabolizing in the dark (chemosynthetically) and exhibiting a positive chemotaxis for oxygen. On the other hand we have light-grown purple bacteria, also metabolizing chemosynthetically but displaying a negative chemotaxis for oxygen. It

seems highly unlikely that an increase in oxygen concentration would cause a decrease of the chemosynthetic rate in one case and an increase in the other.

An effect which remains to be discussed is the greatly enhanced sensitivity to oxygen of light-grown R. rubrum under illumination of an intensity which corresponds to the onset of phototactic saturation (Fig. 17). In keeping with our treatment we shall consider this effect in terms of an influence of oxygen on the photosynthetic rate; in other words, we shall infer that the assumed inhibitory influence of oxygen on photosynthesis is much more pronounced at intensities of illumination corresponding to the onset of photosynthetic saturation than at higher or lower intensities. To interpret this inferred effect we must speculate in detail on the role of oxygen in the photosynthetic process.

Several ways in which oxygen could interact with the system to suppress the photosynthetic rate could be conceived. One of the possibilities will be described by way of illustration of how an enhanced effect at the onset of saturation might be explained.

We assume that an increase in oxygen concentration is equivalent to an increase in the concentration of the photolytic oxidizing fragment OH. An increased

concentration of photoperoxide or its equivalent then inhibits the photosynthetic process by a back reaction of OH with H to produce water, leading to a diversion of H from the pathway which leads to CO_2 reduction. The observation which we wish to interpret can be accounted for by this mechanism.

At low intensities of illumination the rate of photosynthesis is limited by the small rate of cleavage of water into H and OH. The reducing enzyme is almost entirely in its oxidized form E. With this combination of a high concentration of E and low concentrations of H and OH the probability is small that H will react with OH instead of with E. In order to effect a significant diversion of H from the path leading to CO_2 reduction, the concentration of OH or its equivalent must attain a value comparable with the high concentration of E. Hence the sensitivity of the photosynthetic rate to changes in oxygen concentration will be relatively low.

A change of light intensity leads to a change in the concentration of H; at light saturation the photosynthetic rate is insensitive to such a change. Accordingly a large diversion of H into a reaction with OH will not influence the photosynthetic rate appreciably under light saturation.

At intensities of illumination corresponding to the first onset of saturation the balance between

concentrations of H, OH, and E becomes more delicate. H is being formed at a sufficiently high rate that the concentration of E is lowered to a level comparable with the concentration of OH. The diversion of H into a reaction with OH is comparable in rate to the reaction of H with E, and a change in the concentration of OH or its equivalent can be expected to alter this partition markedly, bringing about a significant change in the photosynthetic rate. Thus the enhanced sensitivity to oxygen displayed by R. rubrum at intensities corresponding to the onset of saturation can be explained.

Let us turn now from this example of a workable theory to what can be said with assurance. We are in a position to locate, relative to each other, two points in the chain of reactions which comprise the photosynthetic process. One of these points is the dark process which limits the photosynthetic rate under light saturation (the limiting process need not be the same for all growth conditions and strains of R. rubrum). The other point is the earliest process which is affected by oxygen (earliest refers to the position of the process in the chain of events beginning with light absorption and ending with synthesis of cell materials). The point of earliest interaction with oxygen must precede the point of rate limitation under saturation; if this were not

true the attainment of saturation could have no influence on the sensitivity of the response to oxygen.

A number of experiments suggest themselves which would support or contradict the assumptions which we have made. The influence of oxygen on the photosynthetic rate of R. rubrum should be investigated. The influence of moderate illumination of the chemosynthetic rate has yet to be determined. The capacity for photosynthesis of dark-grown R. rubrum and its variation with light adaptation should be determined; the question of whether cell division is necessary for effective light adaptation should be settled rigorously. An observation of chemotactic and phototactic effects should be attempted with the simultaneous determination of type and rate of synthetic activity; this should be done both with light-grown and with dark-grown bacteria. Finally, a variety of agents capable of evoking a tactic response in R. rubrum should be sought and studied in their effects on the metabolism of the bacteria. Particularly useful would be agents, such as oxygen, which act differently upon the photosynthetic and chemosynthetic processes. The need for more work with the dark-grown bacteria should be emphasized; comparatively little is known about their activities and capabilities. It is hoped that such experiments will throw considerable light on the relationships between the metabolic activities of R. rubrum and its tactic behavior.

VI. SUMMARY

In conclusion let us review the principal results which have emerged from the present investigation and outline a course of future experimentation which should enhance our understanding of the phenomenon of irritability.

A redetermination of the phototactic action spectrum of Rhodospirillum rubrum has yielded evidence that spirilloxanthin, the predominant non-chlorophyllous pigment of this organism, absorbs light which is active in promoting a phototactic response. The most recent evidence prior to this investigation had indicated that phototactically active light is absorbed only by the bacteriochlorophyll and by some of the less abundant red pigments.

Studies of the chemotaxis for oxygen of R. rubrum have revealed a distinct difference in this respect between bacteria grown photosynthetically and bacteria grown aerobically in the dark. Under conditions in which both types of bacteria are metabolizing aerobically in the dark the former react to oxygen with a negative chemotaxis while the latter react with a positive chemotaxis. As a result of this observation the hypothesis of a direct association between a tactic response and a decrease in the rate of synthetic activity of R. rubrum has been abandoned. The

relationship between tactic response and rate of synthesis has been tentatively reformulated in such a way that the latent capacity for photosynthesis, rather than the immediate metabolic activity, is taken as the primary associate of the tactic behavior.

A number of critical points in the relationship between pigments, metabolism, and tactic behavior remain to be investigated. Several of these are listed at the end of the preceding section. In addition to these, a spectrum of photosynthetic growth of R. rubrum (rate vs. wave length) should be determined. The significance of previous observations on the growth of R. rubrum in a spectrum is open to question because of the great difference in intensity which ordinarily prevails between the blue and the near infra-red portions of a spectrum. Also an attempt should be made to investigate the extent to which different strains of R. rubrum, and cultures of the same strain grown under different conditions, vary in their pigment content. If such variations are significant they must be taken into account with care in the interpretation of spectral observations.

Studies of the pigments and metabolic activities of purple bacteria are interesting in themselves and have thrown considerable light, in the hands of van Niel and others, on the mechanism of photosynthesis. The

underlying purpose of the present investigation, however, has been the elucidation of mechanisms involved in biological irritability, and the experiments on phototaxis and chemotaxis have been made from the point of view of enhancing our knowledge of the structure of an irritable system. The investigation of the adherence of the phototactic sensitivity to the Weber Law has been in keeping with this basic purpose. The degree of Weber Law adherence observed was much less striking than that which had been reported earlier (4) for the same system, and the possibility has been considered that an extensive Weber Law adherence might be masked in many cases by an early onset of saturating influences which arise at high intensities of stimulation. In this connection it might be of value to investigate the responses of other irritable systems to changes in the intensity of stimulation, paying particular attention to the variation, with changes in external conditions, of absolute threshold and of saturating intensity.

The main attack, through the phototaxis of R. rubrum, on the problem of irritability should consist of a thorough quantitative investigation of the phototactic response in its relationship to the nature of the light stimulus. At present there exist only scattered pieces of information on this subject. The fundamental response is, of course, a reversal of the direction of swimming of

a bacterium following an abrupt decrease in its illumination. A pulse of darkness which interrupts a period of illumination will evoke a phototactic response provided that the pulse is of sufficient duration; a brief examination during the course of the present investigation revealed that pulses shorter than about one tenth of a second were usually insufficient to elicit a response. Two such subliminal pulses can be summed, if closely spaced in time, to yield an above-threshold stimulus (3). A momentary increase in illuminating intensity will also elicit a phototactic response provided that its duration is longer than about one fifth of a second (present investigation). The response always occurs after the decrease of intensity which terminates the pulse. An effect of accommodation can be observed; an abrupt decrease of intensity is much more effective in promoting phototaxis than is an equal decrease which takes place gradually (3, present investigation). A refractory condition is evident in R. rubrum for about half a second following a phototactic response; during this interval the bacterium is highly resistant to phototactic stimulation (present investigation). Finally, an appearance of rhythmically repeated responses can be observed if the illumination of R. rubrum is reduced and maintained at its reduced level (2, present investigation). Under this condition the bacterium will reverse its swimming direction repeatedly; the frequency of these reversals declines gradually until (after about

one minute) the response vanishes. Such behavior is strikingly reminiscent of the action potentials which represent the "off" response in an optic nerve fiber.

It is hoped that forthcoming studies will place the observations of the last paragraph on a quantitative basis, and that a better idea will be gained of the generality, or lack thereof, of similar observations which have been made on the responses of nerve fibers.

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