of incubation were not long enough for the attainment of a constant ratio of concentrations (intracellular/extracellular), the effect of insulin and fasting on the final equilibrium could not be determined.

These results indicate that future concepts of insulin action must be broadened to include the stimulation of both sugar and amino acid penetration. Further studies of amino acid penetration and intracellular-concentrating processes and the influence of dietary and hormonal factors thereon are currently in progress.

Departments of Medicine and Biochemistry, DAVID M. KIPNIS*

Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, Mo. (U.S.A.) MATTHEW W. NOALL

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* John and Mary Markle Scholar in Medical Science.

Cytochrome reactions in *Chromatium*

Spectrophotometric investigations[1,2] of *Rhodospirillum rubrum* have indicated that cytochromes are involved in electron transport during bacterial photosynthesis. The present study of the purple sulfur bacterium *Chromatium*, strain *D*, substantiates this concept.

The bacteria were grown at 29° under anaerobic conditions in a liquid inorganic medium containing sulfide, thiosulfate, and bicarbonate as substrates. Tungsten lamps furnished illumination. The bacterial cultures were examined after most of the sulfur had disappeared from the cells. A double-beam spectrophotometer[3] was used to record absorption changes upon irradiation of a sample with near infrared ($\lambda > 700$ mμ) light; a split-beam spectrophotometer[4] was used to obtain the difference spectra of pairs of samples in the dark.

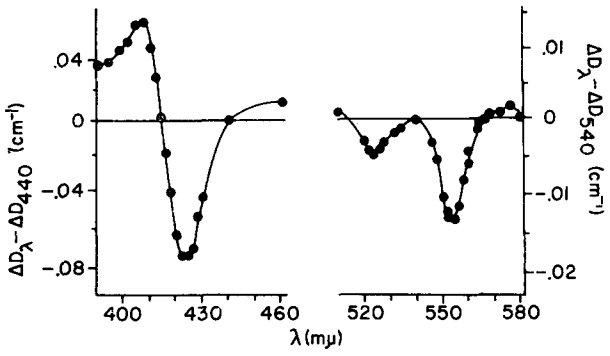

Fig. 1. Absorption-spectrum changes upon irradiation under anaerobic conditions. The trough locations are 423, 524, and 553 mμ. The ratio $(\Delta D_{423} - \Delta D_{440})/(\Delta D_{553} - \Delta D_{540})$ is approximately 4.5. Two different cultures were used for the two spectral intervals shown.

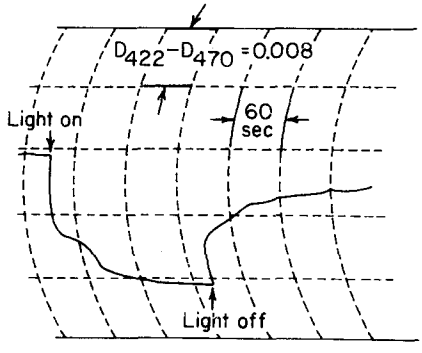

Fig. 2. Kinetics of the anaerobic light effect. The change in absorption at 422 mμ *minus* the change at 470 mμ has been recorded.

When anaerobic suspensions were irradiated with near infrared, the change in absorption spectrum for the region 390 to 580 mμ indicated the oxidation of cytochrome (Fig. 1). The kinetics of the light-on transition were diphasic, and the kinetics of the light-off transition were triphasic (Fig. 2). The differences between the spectra of these various phases showed that more than one cytochrome species was involved in the total light reaction.

The addition of oxygen in the dark caused the partial oxidation of the cytochrome system. However, the changes in absorption upon aeration were only 70 to 90% as great as the changes upon irradiation. The addition of phenylmercuric acetate (30 to 60 μM) to aerobic suspensions resulted in even further oxidation of the cytochrome system by oxygen, whereas KCN (3 mM) partially inhibited the reaction(s) with oxygen.

Irradiation of aerobic bacteria caused absorption-spectrum changes due to further oxidation of cytochromes. The kinetics of the light-on reaction were diphasic. In cooled samples (3 to 4°), a fast and a slow phase were clearly distinguished. The spectra of the two phases show that two distinct cytochromes were oxidized. The spectrum of the total change is the same as that of the first phase of the light-off transition under anaerobic conditions.

On the basis of the kinetic and spectral evidence four cytochromes are postulated: *C 422*, *C 423.5*, *C 426*, and *C 430*. The numerical designations indicate the γ-peaks of the reduced-*minus*-oxidized difference spectra. The corresponding α-peaks are located at 555, 553, 552, and 560 mμ respectively. The ratio of γ- to α-peak is 5 ± 1 for each cytochrome except *C 430* for which this ratio is roughly 10. *C 422* and *C 430* are involved in both anaerobic and aerobic light effects, whereas *C 423.5* and *C 426* are involved in the anaerobic light effect and in reactions with oxygen. *C 426* is a CO-binding pigment[2] and presumably reacts directly with molecular oxygen. The two hemoproteins isolated by NEWTON AND KAMEN[5] from acetone powders of *Chromatium* appear to be *C 423.5* and *C 426*.

The steady-state oxidation level of the cytochrome system in anaerobic bacteria during irradiation was measured over an intensity range covering 4.7 powers of ten. At very low intensities irradiation caused the oxidation of only *C 423.5*. As the intensity was increased, *C 426* became oxidized also; and at the highest intensity (where the light-off transition was triphasic) all four components were oxidized. The relative intensities (I/I_{max}) required for half-maximum oxidation of *C 423.5*, *C 426*, and the combination of *C 422* and *C 430* were respectively $1.3 \cdot 10^{-1}$, $6 \cdot 10^{-2}$, and $3 \cdot 10^{-1}$.

It has been possible to estimate the quantum requirement for a partial reaction in photosynthesis, the oxidation of cytochrome *423.5* in the intact cell. The initial rate of absorption change at 423 mμ upon irradiation of anaerobic bacteria was measured at several intensities of 589 mμ light provided by a sodium lamp in conjunction with two Wratten 23A filters (to remove other emission lines) and a Corning 038 filter (to remove near infrared). The intensity of irradiation was monitored by a phototube which had been calibrated spectrophotometrically by using a solution of carboxymyoglobin as an actinometer[6]. The fraction of the actinic beam absorbed by each bacterial sample was measured by means of an integrating sphere. The average rate of 589 mμ light absorption could thus be calculated in einsteins·l^{-1}·sec^{-1}. The initial rate of cytochrome *423.5* oxidation upon irradiation was calculated on the assumption that $\Delta\varepsilon_\alpha = 20$ cm^{-1} mM^{-1}, a typical value for cytochromes of type *b* and *c*[7]. From the γ to α ratio, $\Delta\varepsilon_{423} \simeq 100$ cm^{-1} mM^{-1}. The initial rate of cytochrome oxidation was measured over a range of intensities up to $2 \cdot 10^{-7}$ millieinsteins·cm^{-2}·sec^{-1}. Over this range the initial rate was proportional to the rate of light absorption, and the quantum requirement was calculated to be two quanta/electron (seven samples, 21 to 27% absorption of actinic light). During irradiation the cytochrome system appears to be the main pathway of electron transfer from reduced sulfur compounds to the photooxidant, since oxidizing equivalents can be transfered to this system with a quantum efficiency as high as that for the overall photosynthetic process[8].

A more detailed account of this work will be published later.

Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. (U.S.A.)

JOHN M. OLSON*
BRITTON CHANCE

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