Elution of cholesterol and esters from paper chromatograms

After the separation the dried chromatogram was out into strips 1 cm wide. The strips were eluated with chloroform. After standing for 24 h at room temperature with occasional stirring the Liebermann-Burchard reaction was carried out in the eluates. From the values obtained it is possible to calculate the ratio of free cholesterol/esters. The results are in good agreement with those obtained in the quantitative methods[5] (Fig. 2).

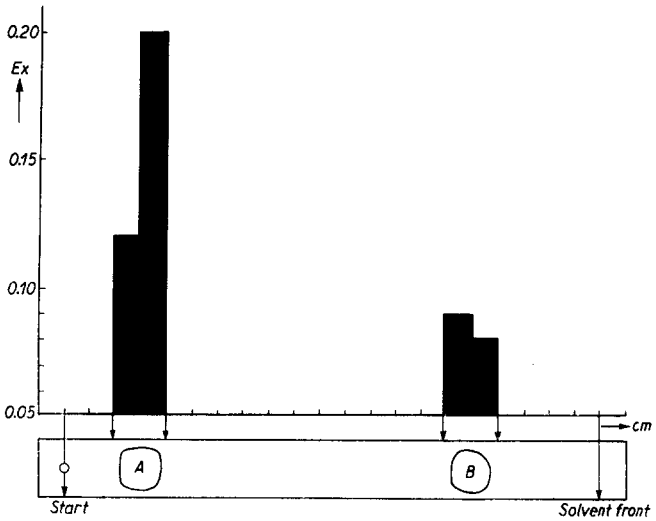

Fig. 2. Elution of cholesterol esters and free cholesterol of human blood serum from paper chromatogram. A: cholesterol esters; B: free cholesterol.

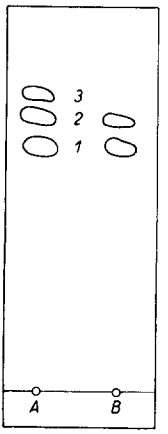

Fig. 3. Paper chromatogram of higher fatty acids in cholesterol esters of serum. Ascending chromatography as described. A: Standards (from bottom of scheme upwards): 1, palmitic and oleic acid; 2, linoleic; 3, linolenic. B: Higher acids of cholesterol esters of human blood serum: 1, palmitic and oleic acid; 2, linoleic.

Paper chromatography of higher fatty acids isolated from cholesterol esters was carried out on Whatman's paper No. 3 impregnated with 10% paraffin oil in ether (v/v) and acetic acid as mobile phase. The spots are developed by the method of KAUFMANN AND NITSCH[6]. As shown in Fig. 3 it can be postulated that cholesterol esters of human blood serum probably contain palmitic, oleic and linoleic acid.

Further experiments are in progress and will be published later.

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Reversible changes in bacteriochlorophyll in purple bacteria upon illumination

Illumination was found to bring about a reversible change in the absorption spectrum of a suspension of purple bacteria. The change was measured by means of a sensitive differential spectrophotometer. We constructed this spectrophotometer—previously described[1]—for the measurement of small changes in the absorption spectrum of light-scattering suspensions upon illumination.

The absorption vessel was a glass cylinder of 5 cm length and 2 cm diameter, sealed at

both ends with glass plates. An opening over the length of the cylindrical wall was covered with a glass plate, after the vessel was filled to the rim. Since this cover was not completely air-tight, the suspensions, which were not able to respire vigorously, were possibly not anaerobic. A weak monochromatic beam of variable wavelength for measuring the changes in absorption traversed the length of the vessel. The actinic beam was incident on the side wall of the vessel. Illumination of the suspension with a broad band in the blue caused a decrease in absorption at 880 mμ, which was completely reversed in darkness. Fig. 1 shows that, for a suspension of *Rhodospirillum rubrum* in water, the absorption decreased roughly linearly with light intensity up to a certain saturation value, while, for bacteria suspended in a solution of 0.5 % peptone and 0.5 % NaCl, a change in absorption occurred only at intensities at which photosynthesis presumably was saturated.

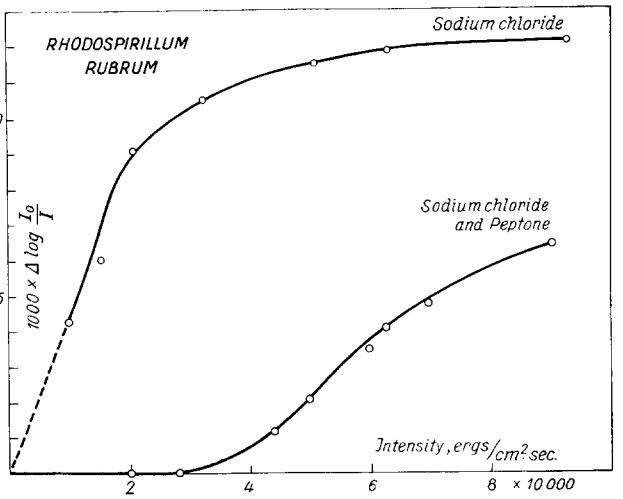

Fig. 1. The decrease in absorption at 880 mμ for *Rhodospirillum rubrum* strain 4 is plotted as a function of the intensity of the exciting light, which occurred in a band between 400 and 550 mμ. The bacteria were centrifuged and suspended in tap water containing 0.5 % NaCl and 0.5 % peptone plus 0.5 % NaCl respectively.

The changes were measured at various wavelengths at a constant intensity of actinic light, somewhat lower than needed to attain saturation. These changes, when plotted as a function of the wavelength, determine a difference spectrum (Fig. 2). The long wavelength peak of this spectrum occurs at approximately the same wavelength as the maximum of the type of bacteriochlorophyll called B 890[1] and thus probably is caused by a decrease of B 890 absorption upon irradiation. This decrease can be considered to be caused either by complete disappearence at the maximum of a small fraction of the bacteriochlorophyll or by a small change in the absorption spectrum of the total amount. If the absorption spectrum of a small fraction disappears completely, then the decrease in absorption of *Rhodospirillum* at 810 mμ is too great to be caused exclusively by disappearance of B 890. Since previous evidence[1] suggested that the infrared absorption of *Rhodospirillum* was caused by B 890 only, the changes were thought to be caused by a small change in an appreciable fraction of B 890. Very recently, however, THOMAS, GOEDHEER AND KOMEN[2] found unexpectedly that treatment with acid decreased the absorption at 800 mμ somewhat more than that of the major maximum. This suggested that part of the absorption at 800 mμ was caused by a trace of another pigment, perhaps B 800. Thus part of the decrease at 810 mμ might be caused by a disappearance of the minor absorption maximum of a small fraction of B 890 and of the major maximum of a fraction of B 800. The observation of a pronounced decrease at 810 mμ in the absorption spectrum of *Chromatium* (Fig. 2), a species which contains a larger amount of B 800 than *Rhodospirillum*, supports the suggestion that the peak at 810 mμ is partly caused by B 800. The difference spectrum in the visible of *Rhodospirillum rubrum* suspended in aerobic water showed that illumination produced an absorption band at about 430 mμ[4]. Also in anaerobic peptone, but at actinic intensities far above that needed for saturation of photosynthesis, an increase in absorption was found at 430 mμ which was superimposed upon a decrease in absorption which occurred already at lower intensities and indicated oxidation of a cytochrome.

Since the increases in absorption at 790 and 430 mμ upon illumination are most pronounced in oxidizing media[4,5], these increases are presumably caused by an oxidation. This oxidation might be the removal of the two hydrogen atoms from the fourth pyrrole nucleus of bacteriochlorophyll. Treatment of a methanol solution of bacteriochlorophyll with ferric chloride oxidized bacteriochlorophyll to a chlorophyllous pigment—possibly bacterioviridin—with major absorption peaks in ether at 434 and 676 mμ (*cf.*[6]). The blue maximum corresponds fairly well with the maximum of the difference spectrum, but the red maximum differs about 120 mμ from the infrared peak in illuminated bacteria. Such a shift might well be caused by the solvent: a shift of 120 mμ was observed in the infrared maximum of B 890 in *Chromatium* upon extraction with ether[3], while the near ultraviolet maximum is less affected. The absorption maximum of bacterioviridin in green bacteria is about 747 mμ, corresponding to a shift of 70 mμ (*cf.*[7]).

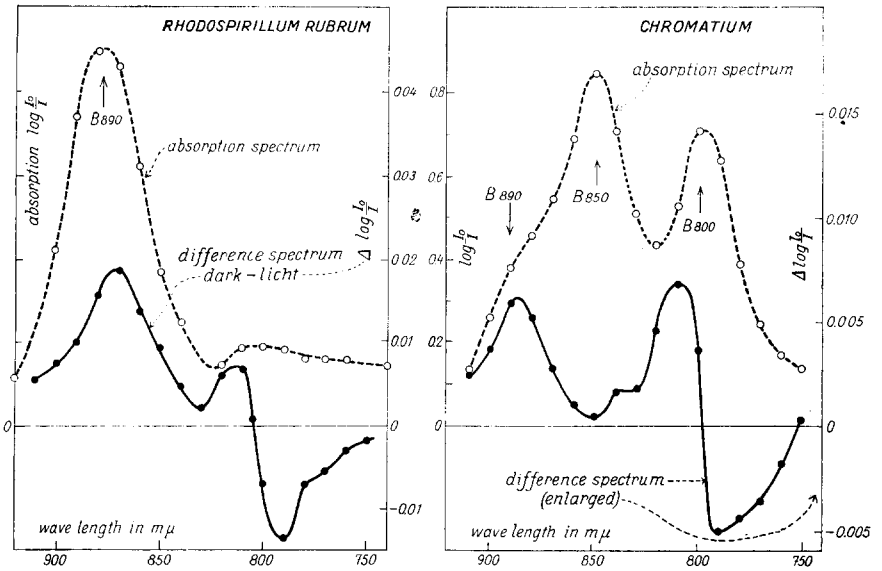

Fig. 2. Absorption and difference spectra for *Rhodospirillum rubrum* strain 4 suspended in tap water, and for an aqueous extract[3] of *Chromatium* strain D. The difference spectra are enlarged 10 and 50 times with respect to the absorption spectra. The spectral changes were brought about by illumination with an intensity of $3 \cdot 10^4$ ergs/cm^2 sec in the region 400–550 mμ. The location of the main infrared maxima of the various bacteriochlorophyll types is indicated by arrows.

The change in bacteriochlorophyll in illuminated bacteria was so far observed only when the cytochrome pigment was already in the oxidized state[4]. The reduced cytochrome may react so fast with oxidized bacteriochlorophyll that the concentration of oxidized bacteriochlorophyll is too small to be observable.

These observations suggest the following reactions:

Excited bacteriochlorophyll + X $\longrightarrow$ oxidized bacteriochlorophyll (bacterioviridine?) + XH (photosynthetic reductant).

Oxidized bacteriochlorophyll + reduced cytochrome + proton(s) $\longrightarrow$ bacteriochlorophyll + oxidized cytochrome.

It should, however, be pointed out, that this is not the only possible interpretation.

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