Alkaloids in Germinating Seeds of *Atropa belladonna*

During recent work on alkaloid biogenesis in *Atropa belladonna*, the results of which will be published elsewhere, we used James's method for demonstrating alkaloids in solanaceous meristems[1]. This method is based on the formation of a red precipitate around a piece of plant tissue as a result of the diffusion of alkaloid in a potassium iodide – iodine solution (Bouchardat's reagent).

James tacitly assumes that the alkaloids demonstrated in germinating seeds are tropane alkaloids; but as Bouchardat's reagent is non-specific, there is insufficient proof for this.

Paper chromatography[2] of extracts of germinating seeds and seedlings of *A. belladonna* showed that the alkaloid which appears first is not hyoscyamine, or scopolamine, apoatropine, belladonnine or tropine; but an unidentified, strongly basic alkaloid, which probably is bellaradine, first isolated by King and Ware from Bulgarian *A. belladonna* root[3].

Afterwards, scopolamine and then hyoscyamine appear.

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[1] James, W. O., *Nature*, **158**, 377 (1946).
[2] Munier, R., and Macheboeuf, M., *Bull. Soc. Chim. Biol.*, **32**, 192 (1950).
[3] King, H., and Ware, L. L., *J. Chem. Soc.*, 331 (1941).

Reversible Photo-oxidation of a Cytochrome Pigment in Photosynthesizing *Rhodospirillum rubrum*

The investigation reported here is a continuation of experiments[1] on reversible changes in light absorption in purple bacteria induced by irradiation. It presents evidence which indicates that, in living bacteria in the absence of oxygen and in the presence of substrate, a cytochrome pigment is oxidized by illumination and is reduced in the dark. The bacteria used were vigorously growing, one or two days old, *Rhodospirillum rubrum*, strains 1 and 4. The growth medium for strain 1 was tap-water containing 1 per cent peptone, and for strain 4 tap-water with 1 per cent peptone and 0·5 per cent sodium chloride.

The apparatus for measuring the changes in absorption was a modification of the sensitive differential spectrophotometer described earlier[1]. The change in absorption was brought about by illuminating a bacterial suspension with a beam of variable intensity, and the change was measured by means of a monochromatic scanning beam the wave-length of which could be varied. This scanning beam was at right angles to the actinic beam. It was too weak to cause a change in absorption. The source of actinic radiation was a tungsten lamp with a filter transmitting beyond 670 mμ.

Irradiation of a suspension of *Rhodospirillum rubrum* strain 1 in its growth medium under anaerobic conditions produced marked changes in absorption at about 430 mμ at rather low actinic intensities. The original absorption was restored in the dark. These changes were complete in the time of the order of a second. The changes in absorption coefficients at different wave-lengths of a bacterial suspension produced by a constant actinic beam are plotted in Fig. 1. This curve we call the spectrum of the change in absorption. If the bacteria were suspended in 0·03 *M* sodium acetate plus 0·05 *M* phosphate buffer *p*H 6·8, under anaerobic conditions, instead of in peptone, a similar spectrum was produced by illumination, as measurements at a few wave-lengths indicated. A similar spectrum of the change in absorption was found for the more anaerobic strain 4 of *Rhodospirillum rubrum*, when measured in its growth medium.

At high actinic intensities a different spectrum of the change in absorption is found, the shape of which depends upon the intensity. This spectrum probably is a combination of the spectrum of Fig. 1 with another spectrum. The infra-red part of this other spectrum has been published earlier[1]. The changes in absorption in the infra-red are probably due to changes in bacteriochlorophyll absorption.

The spectrum of the change in absorption of the *Rhodospirillum* suspension (Fig. 1) has a positive maximum at about 427 mμ and a negative one at about 409 mμ. Except for a shift of the maxima of about 7 mμ to longer wave-lengths, the spectrum of the change in absorption of the bacteria is similar to the spectrum obtained[2] by subtracting the spectrum of reduced cytochrome *c* from that of oxidized cytochrome *c* (Fig. 1, dotted line). This indicates that a cytochrome pigment is oxidized by a light reaction and is reduced by a dark reaction, as indicated by the restoration of the original absorption in the dark.

Van Niel[3] provided evidence that the last step or steps of the photosynthetic dehydrogenation of the substrate in *Rhodospirillum rubrum* are the same as the last steps in the dehydrogenation of the substrate in the dark by respiration. The respiratory dehydrogenation presumably proceeds by way of a cytochrome system[4]. A cytochrome has been extracted[5,6] from *Rhodospirillum rubrum* strain 1 similar in reducing properties to cytochromes *c*, but, unlike *c*, not oxidizable by cytochrome oxidase from pig heart[6].

Although it is not yet possible to tell whether this cytochrome is identical with the cytochrome (Fig. 1) that is oxidized by irradiation, we may say that our experiments indicate that the photosynthetic oxidation of the substrate in *Rhodospirillum rubrum* is mediated by a cytochrome pigment.

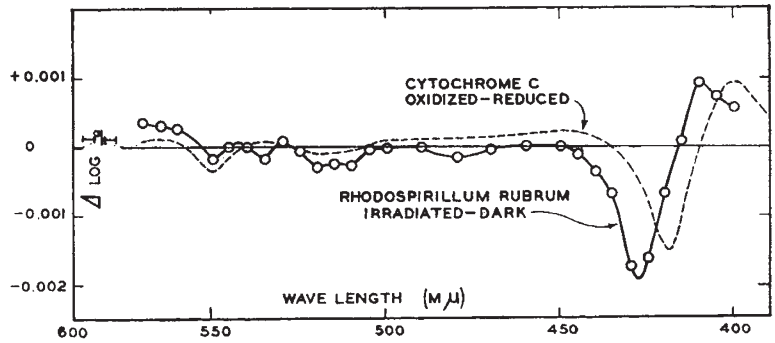

Fig. 1. Spectrum of the change in absorption of a suspension of the non-sulphur purple bacterium *Rhodospirillum rubrum* strain 1 produced by a low actinic light intensity. Difference of absorption coefficient in the light and in the dark is plotted as a function of the wave-length of the scanning beam. The absorption coefficient of the suspension at 880 mμ, corrected for scattering, was 0·9. The figure shows also the difference of absorption spectra of pure oxidized and reduced cytochrome *c* according to Lundegårdh (ref. 2)