the steroid (Bruckdorfer, Graham & Green, 1968). To obtain information on the binding of sterol molecules in natural membranes and lipid bilayers, this process has been used to introduce steroids containing a probe group in different parts of the molecule into liposomes and erythrocyte membranes. The $\alpha\beta$ -unsaturated oxo group can be introduced into the steroid skeleton at various points with relatively small effects on their general shape and conformation. The $n-\pi^*$ transition of this chromophore has a Cotton effect at about 350 nm that is solvent-sensitive and can be used to assess the nature of the environment in which it is located.

Optical-rotatory-dispersion curves of 4,6-cholestadien-3-one (I), 3-hydroxy-3-cholesten-2-one (II), 3-hydroxy-5-cholesten-7-one (III) and 3-hydroxy-5,8(14)-cholestadien-15-one (IV) were recorded in a number of solvents of different polarity over the range 290–520 nm. Each was then dispersed with egg lecithin (phosphatidylcholine) in water to form liposomes. These were sonicated, centrifuged and filtered through a 450 nm membrane filter to give optically clear preparations. The dispersions contained the following molar proportions of steroid to lecithin: compound (I), 1:1; compound (II), 0.64:1; compound (III), 1:1; compound (IV), 0.57:1. Electron micrographs of negatively stained preparations showed the typical liposome structure (Bangham & Horne, 1964). Optical-rotatory-dispersion curves were recorded for each dispersion, and it was found that for compounds (I) and (II) the curves resembled those obtained in polar solvents such as ethanol; for compound (III) the curve resembled that obtained in a less-polar solvent such as triethyl phosphate, and for compound (IV) the curve resembled that obtained in non-polar solvents such as cyclohexane.

Erythrocyte 'ghosts' and liposomes were incubated together for 16 h. Compound (II) did not exchange at all with membrane cholesterol, but the others replaced 50–80% of the endogenous sterol. After sonication, centrifugation and filtration the optical-rotatory-dispersion curves were recorded. Some spectral shifts occurred because of light-scattering, but the curves were very similar to those given by the liposomes. Osmotic studies on whole erythrocytes containing the test steroids showed that they were no more fragile than the controls, so incorporation of the steroid molecules apparently caused no significant distortion of the membrane structure.

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Aggregated States of Chromatophore Membrane Protein from *Rhodopseudomonas spheroides*

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We are interested in the structure and physico-chemical properties of the membrane proteins of photosynthetic organelles. Previous studies (Lees, Mapp, Redmond & Rogers, 1970; Keylock, Kirk & Rogers, 1970) have reported results for chloroplast thylakoid proteins from *Phaseolus vulgaris* and *Euglena gracilis* respectively, and the investigations reported in the present communication concern similar studies on chromatophore membrane protein from *Rhodopseudomonas spheroides* N.C.I.B. 8253.

Pigmented organisms were grown anaerobically in the light in the medium S described by Lascelles (1956). Harvested cells were disrupted in the French pressure cell, and chromatophores were isolated by sucrose-density-gradient centrifugation (Gorchein, Neuberger & Tait, 1968). Chromatophores were ruptured by ultrasonication and washed extensively with water to remove soluble components. Subsequent acetone precipitation and repeated washing with acetone and finally ether yielded the insoluble, virtually lipid-free, preparation termed chromatophore membrane protein.

This protein, dissolved in buffers containing sodium dodecyl sulphate, migrated as a single symmetrical boundary in sedimentation-velocity studies in the analytical ultracentrifuge. However, under various conditions of protein and sodium dodecyl sulphate concentration and buffer composition the single boundary differed in sedimentation coefficient. For example, in 0.1% sodium dodecyl sulphate the sedimentation pattern was characteristic of dissociation of aggregated species to smaller subunits. Above protein concentrations of 4.0 mg/ml the extrapolated $s_{20,w}^0$ value was in the range 6–7 S, whereas below this protein concentration values extrapolated to intercept the $s_{20,w}^0$ axis at 3.3 S. Irreversible dissociation to the 3.3 S species could be achieved by treatment for a short time with 6 M-urea.

In higher (1.0%) sodium dodecyl sulphate concentrations the membrane protein migrated at approx. 1.6 S at protein concentrations greater than 4.0 mg/ml. Below this concentration sedimentation coefficients decreased with protein concentration to a value (0.8–1.0 S) characteristic of sodium dodecyl sulphate micelles.

These findings are similar to those obtained for chloroplast thylakoid proteins from *E. gracilis* (Keylock *et al.* 1970) and barley (W. J. Owen, D. N. Lees, A. Perry & L. J. Rogers, unpublished

work) but differ somewhat from those obtained for *Ph. vulgaris* (Lees *et al.* 1970), and are quite different to those for mitochondrial structural protein from rat liver (Awbery, Lees & Rogers, 1970).

N-Terminal-end-group analysis by the fluorodinitrobenzene method of Sanger (1945) as modified by Levy & Li (1955) yielded a single terminal residue, identified by two-dimensional t.l.c. (Brenner, Niederweiser & Pataki, 1965) as histidine. Extremely small quantities of terminal aspartic acid and an unidentified residue were also present.

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Inhibition of Photosynthetic Electron Transport by 1,1,1-Trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT) at a Site in the Intermediate Electron-Transport Chain

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Delaney, Owen & Rogers (1971) have reported the identification of a site of inhibition of photosynthetic electron transport by DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane] before photosystem 2. However, the observation (Lawler & Rogers, 1968; Owen, Rogers & Hayes, 1970) that DDT affects cyclic photophosphorylation, a photosynthetic activity that does not involve photosystem 2, indicates there is probably also inhibition at some other site. From recent studies this second site of inhibition by DDT appears to be located in the intermediate electron-transport chain linking the two photosystems.

This site was identified from studies of electron donation to photosystem 1 by 2,6-dichlorophenol-indophenol-ascorbate, *NNN'N'*-tetramethyl-*p*-phenylenediamine-ascorbate or 2,3,5,6-tetramethyl-

p-phenylenediamine (diaminodurene)-ascorbate couples. Electron donation was measured in the oxygen electrode by oxygen consumption with paraquat or diquat as electron acceptor from photosystem 1 (Izawa, Connolly, Winget & Good, 1966; Böhme & Trebst, 1969). Electron donation from water through photosystem 2 was inhibited by *N*-(3,4-dichlorophenyl)-*N'N'*-dimethylurea.

Chloroplasts were isolated from DDT-treated susceptible barley some 3 days after spraying with DDT, when Hill activity, as measured by 2,6-dichlorophenol-indophenol or ferricyanide photo-reduction, was inhibited some 50%. This inhibition is a measure of the effect of DDT at the site before photosystem 2 (Delaney *et al.* 1971).

In such chloroplasts electron donation from 2,6-dichlorophenol-indophenol was the same as in chloroplasts isolated from untreated plants; however, when *NNN'N'*-tetramethyl-*p*-phenylenediamine or 2,3,5,6-tetramethyl-*p*-phenylenediamine was used as electron donor electron transport was significantly lower. Under comparable conditions electron donation from 2,3,5,6-tetramethyl-*p*-phenylenediamine was less than that from *NNN'N'*-tetramethyl-*p*-phenylenediamine. These results indicate that there is a site of inhibition by DDT in the intermediate electron-transport chain before the site of electron donation from 2,6-dichlorophenol-indophenol but after the sites of electron donation from *NNN'N'*-tetramethyl-*p*-phenylenediamine or 2,3,5,6-tetramethyl-*p*-phenylenediamine.

Chloroplasts from DDT-treated resistant barley showed no difference, compared with chloroplasts from untreated barley, in any of these photosynthetic activities.

When chloroplasts were isolated from susceptible barley and then treated with 400 µg of DDT/mg of chlorophyll for 60 min no inhibition of electron donation from 2,6-dichlorophenol-indophenol, *NNN'N'*-tetramethyl-*p*-phenylenediamine or 2,3,5,6-tetramethyl-*p*-phenylenediamine to diquat was shown even under conditions where Hill activity as measured by 2,6-dichlorophenol-indophenol photo-reduction was 45% inhibited. In previous studies of other photosynthetic activities (Lawler & Rogers, 1968; Owen *et al.* 1970; Delaney *et al.* 1971) results have been similar for either type of DDT treatment; some possible reasons for the anomalous results in the case of electron donors to the intermediate electron-transport chain will be discussed.

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