scanner unit, have been performed with 620, 350 and 280nm light. At all wavelengths sedimentation-velocity measurements gave identical results. Because of the magnitude of the extinction coefficient of the 620nm absorption band of phycocyanin, measurements could be made at concentrations as low as 0.005mg/ml. The monomer is not the only species present above concentrations of 0.01mg/ml. It is possible to stimulate disaggregation to monomer only by employing a pH of 3.9. Band sedimentation and conventional sedimentation-velocity measurements indicate that hexamer is the first aggregate formed. The aggregation process is analogous to a micellar process. The behaviour is consistent with a structural role for phycocyanin in addition to its known function as an energy-transfer pigment. Fluorescence measurements also indicate that the largest aggregates have the highest fluorescence efficiency (Lee & Berns, 1968*a*). The aggregation process in phycocyanin may be a prototype for membrane-forming systems.

This work was supported in part by Grant GB-7519 from the National Science Foundation.

Berns, D. S. (1967). *Pl. Physiol., Lancaster*, **42**, 1569.
Berns, D. S. (1970). *Biochem. biophys. Res. Commun.* **38**, 65.
Kao, O. & Berns, D. S. (1968). *Biochem. biophys. Res. Commun.* **33**, 457.
Lee, J. J. & Berns, D. S. (1968*a*). *Biochem. J.* **110**, 457.
Lee, J. J. & Berns, D. S. (1968*b*). *Biochem. J.* **110**, 465.
Scott, E. & Berns, D. S. (1965). *Biochemistry, Easton*, **4**, 2597.

Structure of Phycocyanobilin

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Phycocyanobilin, the prosthetic group of the algal biliprotein C-phycocyanin, is a linear tetrapyrrolic compound. An extensive study by Ó hEocha (1963) revealed that three different phycobilins can be obtained, depending on the conditions of the hydrolysis of the protein. The three different phycobilins were designated phycobilins 630, 655 and 608. Phycobilin 630 is considered to be the native form (hence the 'real' phycocyanobilin), whereas phycobilins 655 and 608 are derivatives obtained after prolonged treatment with concentrated acid. In view of its u.v. spectrum phycocyanobilin should be structurally intermediate between mesobiliverdin and mesobiliviolin.

A structure for phycocyanobilin has been proposed based on the information obtained from mass and n.m.r. spectra (Cole, Chapman & Siegelman, 1967, 1968; Crespi, Boucher, Norman, Katz & Dougherty, 1967; Crespi, Smith & Katz, 1968). According to these workers the molecule has a molecular weight of 586, or more precisely a molecular weight of 614 for its dimethyl ester.

Our results (B. L. Schram & H. H. Kroes, unpublished work) indicate that the acid form of phycocyanobilin has a molecular weight of 588 and that the dimethyl ester has a molecular weight of 614 (2 less than expected for a dimethyl ester of a dibasic acid with molecular weight 588).

A close look at the mass spectrum of the acid reveals several properties inconsistent with the structure previously proposed. We therefore present a new structure for phycocyanobilin that has two hydrogen atoms more and that has an internal hydrogen bond.

The new structure can better explain the chemical properties of phycocyanobilin. In the first place, breaking of the internal hydrogen bond, e.g. by esterification or treatment with concentrated acid, causes the molecule to be converted into a more stable dehydrogenated form. The structure given by Cole *et al.* (1967, 1968) will be that of phycobilin 655, since it is the stable form of the ester. It also explains the irreversibility of the transition of phycobilin 630 into phycobilin 655. The n.m.r. spectra are obtained with a solution of phycocyanobilin in trifluoroacetic acid or in deuterated pyridine; the molecule is probably converted readily into its dehydrogenated form in these solvents and the two extra hydrogen atoms will not be observed.

Phycobilin 608 has a violinoid structure and is isomeric with phycobilin 655. A tentative structure will be given, based on an analysis of the electronegative properties of the individual pyrrole rings in phycobilin 655.

Cole, W. J., Chapman, D. J. & Siegelman, H. W. (1967). *J. Am. chem. Soc.* **89**, 3643.
Cole, W. J., Chapman, D. J. & Siegelman, H. W. (1968). *Biochemistry, Easton*, **7**, 2929.
Crespi, H. L., Boucher, L. J., Norman, G. D., Katz, J. J. & Dougherty, R. C. (1967). *J. Am. chem. Soc.* **89**, 3642.
Crespi, H. L., Smith, U. & Katz, J. J. (1968). *Biochemistry, Easton*, **7**, 2232.
Ó hEocha, C. (1963). *Biochemistry, Easton*, **2**, 375.

Some Aspects of the Localization of Phycobiliproteins in the Rhodophyceae and the Cyanophyceae

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The phycobiliproteins observed in the Rhodophyceae appear in general in the form of globules (*Porphyridium cruentum, Rhodomela subfusca, Antithamnion glanduliferum*) but sometimes in the form

of thin discs (*Porphyridium aerugineum*; Gantt, Edwards & Conti, 1968). The phycobiliproteins of *Rhodochorton Rothii* are also of the latter type.

In *Batrachospermum virgatum* (Giraud & Lichtle, 1970) a different arrangement can be observed. The phycobiliproteins appear in the form of parallel cylinders 300–350 Å in diameter. This appearance can be modified to a series of V-shapes when the orientation of the lamellar surfaces on section is changed.

This arrangement is not found in the related genus *Lemanea*, in which typical globules are visible.

Gantt & Conti (1968) have shown that in the Cyanophyceae the phycobiliprotein globules may face each other between two lamellae (*Tolypothrix tenuis*). In *Chroococcus minutus* and in *Oscillatoria brevis* the globular accumulations form 'bridges' between lamellae and the zone has a reticulated appearance.

Gantt, E. & Conti, S. F. (1968). *J. Bact.* **97**, 1486.
Gantt, E., Edwards, M. R. & Conti, S. F. (1968). *J. Phycol.* **4**, 65.
Giraud, G. & Lichtle, C. (1970). *J. Phycol.* (in the Press).

Study of Porphyrins Present in Hepatoma Tissue

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Acquired photosensitizing porphyria arising from a benign liver tumour has been described by Tio, Leijnse, Jarrett & Rimington (1957). A similar case, but involving a malignant tumour, has been described by Thompson, Nicholson, Farnan, Whitmore & Williams (1970). Porphyrins from the liver of the latter patient were compared with those from normal human liver.

Porphyrins from the tumour were separated into a fraction F_1, extractable into ethereal acetic acid but not extractable therefrom into 3% sodium acetate, a fraction F_2 extracted from the ethereal acetic acid into 3% sodium acetate and then into butan-1-ol, and a fraction F_3 strongly adherent to the residual tissue but removed by conversion of the porphyrins into methyl esters with methanolic 5% sulphuric acid, dilution with water and extraction into chloroform.

Countercurrent analysis (Smith, Belcher & Mahler, 1970) of fraction F_1 showed that the peak 1, which normally contains only coproporphyrin, contained also two-carboxyl and three-carboxyl aetio-type hydrophilic porphyrins with Soret maxima (1.5M-HCl) at 405 and 407 nm respectively. These were possibly hydroxylated and were reduced (Pd/H_2) to a porphyrin with a Soret maximum at 402nm. Peak II contained two-carboxyl and three-carboxyl porphyrins spectroscopically identical with those found in normal liver and with Soret maxima at 405 and 405.5nm respectively. Peak III contained only protoporphyrin.

Radioautography of the abnormal F_1 porphyrins after treatment with 1-fluoro-2,4-dinitro[U-^{14}C]-benzene showed the three-carboxyl material of peaks I and II to be radioactive. Inertness of these products to Ag_2SO_4 indicated an abnormal protein binding (Rimington, Lockwood & Belcher, 1968) or the presence other fluorodinitrobenzene-reactive groups.

Fraction F_2, purified on Sephadex G-25 (Rimington & Belcher, 1967) and resolved on lutidine t.l.c., gave two-carboxyl and three-carboxyl porphyrins with Soret maxima (1.5M-HCl) at 405 and 405.5nm respectively. Hydrolysis and chromatography of the fraction F_3 esters also showed the presence of two-carboxyl and three-carboxyl porphyrins.

Rimington, C. & Belcher, R. V. (1967). *J. Chromat.* **28**, 112.
Rimington, C., Lockwood, W. & Belcher, R. V. (1968). *Clin. Sci.* **35**, 211.
Smith, S. G., Belcher, R. V. & Mahler, R. (1970). *Biochem. J.* **118**, 38P.
Thompson, R. P. H., Nicholson, D. C., Farnan, T., Whitmore, D. N. & Williams, R. (1970). *Gastroenterology* (in the Press).
Tio, T. H., Leijnse, B., Jarrett, A. & Rimington, C. (1957). *Clin. Sci.* **16**, 517.

Specificity of Biliverdin Reductase

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The specificity of the enzymic reduction of biliverdin to bilirubin has been in dispute ever since the first demonstration of the activity in mammalian tissues by Lemberg & Wyndham (1936). These authors attributed the activity to non-specific side effects of a variety of reductase activities. More recently Singleton & Laster (1965) found hepatic biliverdin reductase activity to be mainly NADH-dependent with a variable proportion of NADPH-dependent activity, whereas Tenhunen, Ross, Marver, & Schmid (1970) considered the activity to be entirely NADPH-dependent.

By using the general assay procedures of the above authors, we confirm the presence of both NADH- and NADPH-dependent biliverdin reductase activity in guinea-pig liver extracts. However,