however, equally well from ETP_H as from ETP. The results presented show that exposure of particles to pH 8·5 does not influence their potential to yield soluble NADH–ubiquinone reductase and suggest that the absence of ubiquinone reductase activity in the dehydrogenase is due to lack of exposure to heat–acid–ethanol, and not to snake-venom enzymes or alkaline pH. The reason why Sanadi *et al.* (1965) were unable to extract ubiquinone reductase from venom-treated particles is discussed elsewhere (Salach, Tisdale, Singer & Bader, 1967).

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The Tocopherols of the Blue-Green Algae

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Henninger, Bhagavan & Crane (1965) examined the lipids of the blue-green alga *Anacystis nidulans* and found two naphthaquinones and one plastoquinone, all of which differed from quinones previously isolated from other organisms. The plastoquinone resembled plastoquinone-9 (where the numeral indicates the number of isoprene units in the side chain) although it was slightly more polar on thin layers of silica gel. Of the two naphthaquinones, the less polar compound (of similar polarity to phylloquinone) was considered to be a hydroxy derivative of phylloquinone whereas the other was considerably more polar. No α-tocopherolquinone or tocopherols could be detected. As a result of these findings it was suggested that the quinones of the blue-green algae are different from those that are found to be characteristic of higher plants.

However, Carr & Hallaway (1966) found α-tocopherolquinone in four species of blue-green algae other than *Anacystis nidulans*. Allen, Franke & Hirayama (1967) re-examined the lipid of *Anacystis nidulans* and identified the three quinones present as phylloquinone, plastoquinone-9 and a hydroxy derivative of phylloquinone (the polar naphthaquinone described by Henninger *et al.* 1965). They were unable to detect any tocopherols or tocopherolquinones. Independent work by H. M. Hallaway and N. G. Carr (personal communication) has led to the same identification of the three quinones.

This lack of tocopherols in *Anacystis nidulans* was further confirmed by Hirayama (1967), who suggested that in view of the absence of tocopherols from other procaryotic micro-organisms, the photosynthetic bacteria, these compounds may be necessary for the maintenance of chloroplast structure.

As *Anacystis nidulans* was unusual among the blue-green algae in lacking α-tocopherolquinone, the occurrence of tocopherols in a number of species was investigated.

Anabaena variabilis, *Nostoc muscorum*, *Anacystis nidulans* and *Fremyella diplosiphon* (1429/1 Cambridge culture collection) were each grown autotrophically in 10l. batches of medium C of Kratz & Myers (1954) with the addition of sodium hydrogen carbonate (0·05%). At the end of the exponential phase the algal cells were harvested in a Sharples centrifuge. The lipids were extracted by homogenization of the cell paste with ice-cold methanol in a mechanical Potter-Elvehjem homogenizer until no

further chlorophyll could be extracted. The lipid was transferred to diethyl ether, the methanol being washed out with aq. 5% (w/v) ammonium sulphate. The ethereal extract was washed twice with ammonium sulphate solution and finally with water before being dried over anhydrous sodium sulphate. The dried extract was evaporated to dryness *in vacuo* at room temperature in a rotary evaporator. The lipid was applied to a column of silicic acid–Celite (6:3, w/w) and the non-polar lipid, containing any tocopherols, separated from the chlorophyll and other polar lipids by elution with 25% (v/v) diethyl ether in light petroleum (b.p. 40–60°). The eluted lipid was again evaporated to dryness *in vacuo* and chromatographed on thin layers of silica gel G with chloroform, with authentic tocopherols as markers.

Ultraviolet-absorbing compounds were located by their quenching of fluorescence in ultraviolet light after the whole of the plate had been sprayed with 0·01% sodium fluorescein in ethanol. The tocopherols were located by then spraying the edges of the plate with Emmerie–Engel reagent and the relevant compounds were removed from the centre of the plate and eluted with ether. Any Emmerie–Engel-positive compounds so obtained were rechromatographed with 20% (v/v) di-isopropyl ether in light petroleum (b.p. 40–60°), re-eluted and their absorption spectra determined after evaporation to dryness and dissolution in cyclohexane.

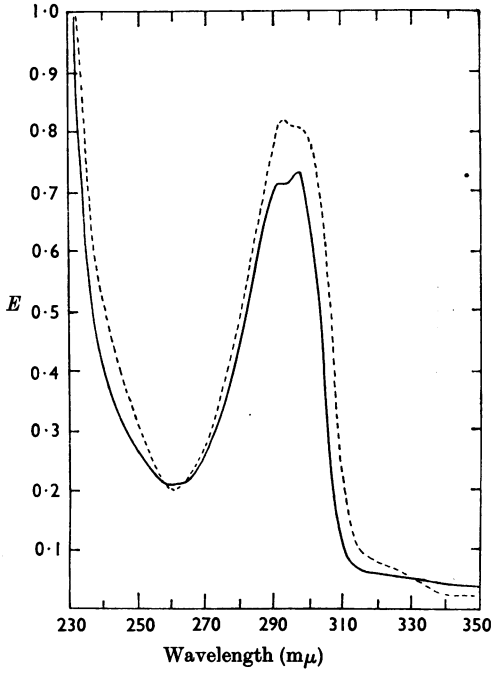

Fig. 1. Absorption spectra of Emmerie–Engel-positive material from *Anabaena variabilis*. ——, Material of polarity identical with that of α-tocopherol; – – – –, material of polarity identical with that of β-tocopherol.

On chromatography, the lipid from *N. muscorum* showed only one Emmerie–Engel-positive compound of R_F on silica gel identical with that of α-tocopherol with both chloroform (0·65) and 20% (v/v) di-isopropyl ether in light petroleum (0·73); thus it was inseparable from α-tocopherol in the system of Pennock, Hemming & Kerr (1964). Moreover, the compound had the same absorption spectrum as authentic α-tocopherol ($\lambda_{max.}$ 292 and 298mμ).

In contrast, the lipid from *Anabaena variabilis* contained an Emmerie–Engel-positive compound of similar polarity to β-tocopherol, with R_F values 0·44 in chloroform and 0·68 with 20% (v/v) di-isopropyl ether in light petroleum. On elution this material had an absorption spectrum identical with that of β-tocopherol ($\lambda_{max.}$ 293 and 299mμ) (Fig. 1). However, it was difficult to determine whether α-tocopherol was also present, as one of the major carotenoids of this organism, echinenone, has a similar polarity on silica gel. However, chromatography on polyamide layers with methanol–ethyl methyl ketone–water (20:10:3, by vol.) separated an Emmerie–Engel-positive compound (R_F 0·50) from the echinenone (R_F 0·24). On elution, this compound was found to have the same absorption spectrum as α-tocopherol and could not be separated from the authentic compound by chromatography (Fig. 1).

The lipid from *F. diplosiphon* contained a large amount of echinenone, but after chromatography on polyamide only one tocopherol, namely α-tocopherol, was found.

Chromatography of the lipid from *Anacystis nidulans* showed the presence of a small amount of an Emmerie–Engel-positive compound. This compound was found to have the same polarity in chloroform (R_F 0·58) and 20% di-isopropyl ether in light petroleum (R_F 0·70) as plastochromanol. However, not enough was present to enable its absorption spectrum to be determined.

Summarizing these findings, *Anabaena variabilis* has both α- and β-tocopherol, *Nostoc muscorum* and *Fremyella diplosiphon* have only α-tocopherol, whereas *Anacystis nidulans* has no tocopherols, but does have low concentrations of plastochromanol.

Apart from *Anacystis nidulans* all the blue–green algae examined appear to be similar to other organisms that evolve oxygen during photosynthesis in having tocopherols and α-tocopherolquinone. This appears to rule out a role for the tocopherols in the maintenance of chloroplast structure (Hirayama, 1967).

Since *Anacystis nidulans* is capable of carrying out a normal photosynthesis despite its lack of α-tocopherolquinone, an obligatory role of α-tocopherolquinone in oxygen-producing photosynthesis must be seriously questioned. However, perhaps the function of α-tocopherolquinone in this organism is taken over by the hydroxyphylloquinone, which is not present in the other species of blue–green algae examined.

The suggestion of Henninger *et al.* (1965) that the quinones and related compounds of the blue–green algae are different from those characteristic of higher plants appears now to be based on one species, *Anacystis nidulans*, which is probably atypical.

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A Hybrid Protein–Polysaccharide of Keratan Sulphate and Chondroitin Sulphate from Pig Laryngeal Cartilage

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Chondroitin sulphate–protein complexes from cartilage contain variable amounts of glucosamine and galactose attributable to keratan sulphate, which may be an integral part of the complex macromolecule (Partridge & Elsden, 1961; Seno, Meyer, Anderson & Hoffman, 1965). The present paper describes the isolation of an antigenically distinct protein consisting of a single antigen that contained keratan sulphate together with considerable amounts of uronic acid and galactosamine that may represent a hybrid proteoglycan containing both keratan sulphate and chondroitin sulphate.

Proteoglycans of chondroitin 4-sulphate from pig laryngeal cartilage have been fractionated by gel filtration on 6% agarose (Tsiganos & Muir, 1965) and electrophoresis (Muir & Jacobs, 1967) into distinct antigenic components differing in size, amino acid composition and glucosamine and galactose contents. The largest constituents, possessing all the antigens of the starting material, contain considerably more glucosamine and galactose than the smallest, which possess a single-species cross-reacting antigen (C. P. Tsiganos & H. Muir, unpublished work). The moiety that contained glucosamine was therefore isolated after exhaustive hyaluronidase digestion of a proteoglycan preparation obtained by extracting cartilage for a total of 72hr. essentially as described by Muir (1958).

A 1·5g. sample of the above preparation dissolved in 0·1M-acetate buffer, pH5, containing NaCl (0·15M) (Hoffman, Meyer & Linker, 1956) was digested under toluene with 70000units of hyaluronidase (EC 3.2.1.35) added in three portions over 3 days. Insoluble material was removed by centrifuging and the supernatant treated with an equal volume of saturated aqueous 9-aminoacridine hydrochloride. After standing overnight at 4° the precipitated hyaluronidase-resistant material was centrifuged off, and the supernatant was saved and stored at 4°. The precipitate was converted into the soluble sodium salt by suspending it in acetate buffer and shaking with Zeo-Karb 225 8% DVB (Na^+ form), which took up the aminoacridine. The hyaluronidase digestion and precipitation of resistant polyanionic material with aminoacridine was repeated first with 15000units of hyaluronidase for 24hr. followed by 20000units of purified enzyme for 24hr. It was re-isolated and further digested with 80000 units of highly purified enzyme for 24hr. After the final precipitation with amino-