

The following technique has been used for overcoming some difficulties and stabilizing a reproducible method.

A combination of a freeze-drying process and homogenization in isotonic sucrose solution permits a concentration of proteins suitable for paper electrophoresis to be obtained without denaturation of the proteins. The livers removed from the exsanguinated animals were frozen within 15 min. after death and dried *in vacuo*. The gently powdered dried material was suspended in isotonic sucrose solution containing 0.2 per cent of versene, and homogenized with a Potter-Elvehjem glass homogenizer. The isolation of cellular fractions was then carried out by differential centrifugation<sup>4</sup>. The final supernatant containing the soluble cytoplasmic proteins was clear and transparent with pH 6.8 and a protein concentration, depending upon the initial dilution, of about 4 per cent.

Immediately after preparation, 0.02 ml. of this solution was allowed to run overnight on strips of Whatman No. 1 filter paper in an electrophoretic apparatus similar to that described for serum proteins<sup>5</sup> (potential gradient 15 V. per cm.).

A series of experiments was carried out to find a suitable buffer solution which would differentiate the velocity of migration of the various protein fractions. The only suitable solution was found to be a borate buffer of pH 8.6 and  $\mu = 0.18$ .

A clear resolution of five major protein components was obtained and developed by staining the strips with naphthalene black (Fig. 1c). Figs. 1a and b show the less satisfactory separations with a veronal and a phosphate buffer of pH 8.6 and  $\mu = 0.1$ .

An important point on which the success of the separation depends is the freshness of the prepared material. The ageing of the protein solutions was investigated and it was found that a noticeable alteration occurs within twenty-four hours, and within forty-eight hours the proteins migrate as a single band with no separation (Fig. 1d).

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<sup>2</sup> Henning, N., Kinzler, H., Demling, L., and Mannuss, E., *Klin. Wschr.*, **30**, 390 (1952).

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<sup>5</sup> Grassman, W., Knedel, M., and Hannig, K., *Dtsch. Med. Wschr.*, **76**, 333 (1951).

### A Crystalline Chlorophyll-Protein Complex from *Chlamydomonas*

THE isolation of a crystalline chlorophyll-protein complex from clover was recently reported and illustrated by Takashima<sup>1</sup>. Spikes, Lumry and Anderson<sup>2</sup> repeated the crystallization of this complex from thirty species of plants. We have also obtained material with a very similar appearance from many green leaves; for example, clover, *Trifolium repens*; cocksfoot, *Dactylis glomerata*; bracken fronds, *Pteridium aquilinum*. In addition, an apparently crystalline chlorophyll-protein complex has been obtained from a species of alga, *Chlamydomonas*.

*Chlamydomonas dorsoventralis* (Culture Collection of Algae, Cambridge) was grown in an inorganic salt medium<sup>3</sup> aerated with 5 per cent carbon dioxide - air under artificial illumination. The cells were harvested, suspended in a small volume of medium and an equal volume of  $\alpha$ -picoline added. The suspension was then centrifuged and dioxan added to the clear green supernatant to a final concentration of 20 per cent. On leaving overnight at 0° C., microscopic green crystals of the chlorophyll-protein complex separated in rosettes. These were recrystallized from  $\alpha$ -picoline and dioxan mixture. The material formed roughly equidimensional clusters, each appearing to have grown dendritically from a centre.

Chlorophyll was extracted from the complex by organic solvents, and the absorption spectrum of a solution of the pigment in ether indicated the presence of chlorophylls *a* and *b*. The usual amino-acids were detected by paper chromatography in a hydrolysate of the complex.

Similar crystals were also obtained from a species of marine *Chlamydomonas* kindly provided by Dr. C. P. Spencer. In contrast, the above procedure failed to extract any coloured material from intact cells of *Chlorella vulgaris*, grown with either carbon dioxide or glucose as source of carbon.

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### Separation and Estimation of Chitosamine and Chondrosamine in Complex Hydrolysates

THE estimation of hexosamines in hydrolysates of complex biological materials, containing both protein and carbohydrate parts, has for some years been recognized as one of the more important analytical problems of biochemistry. The separation and identification of the two hexosamines, chitosamine (glucosamine) and chondrosamine (galactosamine), known to occur in the animal organism, is likewise difficult. The use of ion-exchange resins offers a convenient technique for separating the hexosamines from each other and from other substances.

Moore and Stein<sup>1</sup> report the position of a hexosamine peak using elution chromatography with both 100-cm. and 15-cm. columns of 'Dowex 50' resin. Partridge and Elsdon<sup>2</sup> have separated chitosamine from chondrosamine on 100-cm. columns of 'Dowex 50'; the peaks emerge in the region 250-350 fractions from the beginning of the run where the citrate buffer of pH 4.25 is emerging. Unfortunately, the hexosamine peaks overlap some of the amino-acid peaks, necessitating the analysis of alternate fractions by the ninhydrin<sup>3</sup> and the Elson and Morgan<sup>4</sup> methods, respectively, to enable a distinction to be made. In addition, we have found the position of the peaks