

It is concluded that under the conditions described there is no simple exchange between inorganic phosphate and the phosphate groups of either glucose-1-phosphate, adenylic acid, 2,3-diphosphoglyceric acid or adenosine triphosphate. Incorporation of phosphorus-32 into these compounds in experiments with tissue in these circumstances must be considered to be a result of metabolic reactions.

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<sup>1</sup> Hevesy, G., and Aten, A. H. W., jun., *Kgl. Danske Videnskab. Selskab Biol. Medd.*, **14**, 5 (1939).

<sup>2</sup> Perrier, C., and Segrè, E., *Ricerca Sci.*, **9**, 628 (1938), quoted by Hevesy, G., "Radioactive Indicators", 85 (Interscience, New York, 1948).

<sup>3</sup> Gourley, D. R. H., *Fed. Proc.*, **10**, 300 (1951).

<sup>4</sup> Sacks, J., *J. Biol. Chem.*, **181**, 655 (1949).

<sup>5</sup> Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, **66**, 375 (1925).

### Sedimentation and Diffusion of Soluble Fibroin

AQUEOUS solutions of fibroin were prepared by dissolving degummed silk in cupri-ethylenediamine, neutralizing, and dialysing against distilled water. In order to obtain solutions as examined by Coleman and Howitt<sup>1</sup>, part of the fibroin was precipitated by adding hydrochloric acid, and the part remaining in solution was neutralized and further dialysed.

Measurements of sedimentation velocity on this solution, brought to 0.2 M in potassium chloride in order to eliminate charge effects, were made in an ultracentrifuge, using a field of 150,000 g and observing by the Lamm scale method. Diffusion measurements, with 0.2 M potassium chloride throughout the system, were made in a Neurath diffusion cell<sup>2</sup> at 20° C., also with the scale method; diffusion constants were evaluated by the area-height method. Since soluble fibroin is unstable, freshly-prepared solutions were used.

The sedimentation peak remained single in all the runs, and the diffusion curve was always symmetrical, sometimes being slightly leptokurtic compared with a Gaussian curve. For different preparations of soluble fibroin at the same concentration we often found widely differing diffusion constants; there were smaller differences among the sedimentation constants. Thus the sedimentation constant  $s$  varied between 2.3 and 2.8 ( $\times 10^{-13}$  sec.) at a concentration of 5 gm./litre; and the diffusion constant  $D_A$ , measured over a range of concentration from 1.2 to 7.8 gm./litre, varied (at random with the concentration) between 3.1 and 1.6 ( $\times 10^{-7}$  cm.<sup>2</sup> sec.<sup>-1</sup>) in different preparations. From a total of five sedimentation runs and eleven diffusion experiments on twelve fibroin preparations, the average results were:

$$s = 2.6 \times 10^{-13} \text{ and } D_A = 2.5 \times 10^{-7} \text{ at } 20^\circ \text{C.}$$

We did not find the suppression of widening of the sedimentation peak that is associated with an increase of sedimentation constant on dilution; therefore sedimentation constant probably does not vary much with the concentration in any one preparation of soluble fibroin. Similarly, the symmetry of the diffusion curve suggests that, for any one preparation, Fick's law is valid. We may therefore calculate molecular weights from these results without

gross error. From the apparent specific volume of degummed silk in water (0.704), we assume the partial specific volume  $V$  is 0.7.

The extremes of molecular weight, corresponding to the lowest sedimentation constant and the highest value of  $D_A$  (and conversely), are 60,000 and 150,000. The average molecular weight, corresponding to the average sedimentation constant and the average  $D_A$ , is 84,000. These are all well above the molecular weight of 33,000 reported by Coleman and Howitt for soluble fibroin prepared in the same way. The great variation of molecular weight that we find between different preparations of soluble fibroin shows that a more precise control is necessary either in dissolving the silk (where degradation may occur), or in the dialysis, or in the partial precipitation by hydrochloric acid. (This precipitation may conceivably be a molecular-weight fractionation.) Until this control is attained, we have clearly no knowledge of any unique 'molecular weight of fibroin'.

The average sedimentation constant and  $D_A$  correspond to a frictional ratio ( $f/f_0$ ) of 3. The fibroin molecule in these solutions is therefore not a compact unhydrated sphere. Assuming no hydration, we could fit the average experimental results by a compact rigid ellipsoid or other non-spherical shape, or by a random coil<sup>3</sup> of great flexibility. The experimental results, however, do not justify a detailed calculation.

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<sup>2</sup> Neurath, H., *Chem. Rev.*, **30**, 367 (1942).

<sup>3</sup> Kuhn, H., *J. Coll. Sci.*, **5**, 331 (1950).

### Reversible Photo-Bleaching of Chlorophyll in Rigid Solvents

CHLOROPHYLL solutions undergo a reversible 'bleaching' when strongly irradiated<sup>1,2</sup>. Possible explanations for the effect have postulated the formation of a long-lived excited state of the dye followed by a dismutation reaction between excited and normal dye molecules<sup>3,3</sup>, or a redox reaction between the excited dye and solvent<sup>4</sup>. To date, work on the photo-bleaching reaction has been carried out at room temperature in fluid solvents, and has dealt with changes in the red absorption band.

We have obtained a rough measurement of the reversible spectral changes produced by irradiation of chlorophyll-*a* in a rigid solvent at liquid nitrogen temperature. The chlorophyll was prepared according to the method of Zscheile and Comar<sup>5</sup>, and made up to  $1.2 \times 10^{-5}$  M in a solvent consisting of ether, isopentane and ethanol in 8:3:5 volume ratio. Solutions were contained in a 'Pyrex' cell of 6 mm. square cross-section and were carefully degassed on the vacuum line. The cell fitted snugly into a hole in a cylindrical dural metal-block thermostat placed in an unsilvered 'Pyrex' Dewar vessel, and supported within the Dewar vessel by a metal extension rod which dipped into liquid nitrogen. A small hole drilled through the block allowed a beam of white light to be passed through the solution immediately behind the front face of the cell. The emergent light then passed through a constant-deviation spectro-