Methods for synthesis of several acetals are given and some of their physical constants have been determined.

MADISON, WISCONSIN

THE OSMOMETRIC METHOD OF DETERMINING THE MOLECULAR WEIGHTS OF PROTEINS

BY GILBERT ADAIR[1]

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In a recent paper Svedberg and Nichols[2] state that the osmotic pressure method of measuring the molecular weights of proteins is rendered quite uncertain by the Donnan equilibrium. Svedberg and Fåhraeus[3] stated that conflicting results, obtained by different investigators of the molecular weight of hemoglobin, were partly due to the difficulty of measuring osmotic pressures against semipermeable membranes and partly due to the Donnan effect.

An explanation of these difficulties has been suggested.[4] The conflicting results referred to by Svedberg had been obtained with hemoglobin equilibrated with water of unknown hydrogen-ion concentration. New measurements[4] showed that deviations from the isoelectric point led to abnormally high osmotic pressures on account of the excess of ions inside the membrane.

Unfortunately, Svedberg and Fåhraeus did not refer to any of the papers on the osmotic pressure of hemoglobin published in 1924 and 1925, which showed that the membranes gave true equilibria,[4,5,6] and the molecular weights corrected for the ion distribution effects were the same as the figure they obtained by the ultra-centrifugal method, namely, 62,000–71,000 for purified horse hemoglobin.

The osmometric data for the hemoglobins of various species have been given in the papers referred to as follows: hemoglobin of man, the horse and the sheep (solvent $N/10$ NaCl, etc.);[4] hemoglobin of man and the ox (solvent salts of red corpuscle);[6] hemoglobin of the horse and the sheep (solvent, distilled water).[4] All the molecular weights agreed to within 10% of 66,800, which is four times the equivalent calculated from iron analyses.

The agreement of the results with different salt solutions affords a check on the accuracy of the membrane equilibrium corrections in the case of hemoglobin. The empirical correction formula[5] which was pro-

[1] Fellow of King's College, Cambridge, England.

[2] Svedberg and Nichols, THIS JOURNAL, **48**, 3081 (1926).

[3] Svedberg and Fåhraeus, *ibid.*, **48**, 430 (1926).

[4] Adair, *Proc. Roy. Soc.* (*London*), **109A**, 292 (1925).

[5] Adair, *Proc. Camb. Phil. Soc.* (*Biol.*), **1**, 75 (1924).

[6] Adair, *Proc. Roy. Soc.* (*London*), **108A**, 627 (1925).

visionally suggested (because Donnan's assumptions concerning osmotic pressures apply only to ideal solutions) can be now withdrawn in favor of methods based on a new theoretical treatment of membrane equilibria.[7]

The corrections for hemoglobin were fairly accurate, but I had made no experiments on other proteins and the available data were difficult to interpret. Later work has shown that some of the provisional estimates[5] require revision. The average mass of the protein particles in serum must be raised from 80,000 to about 100,000.

The data for egg albumin in concentrated ammonium sulfate solutions cannot yet be interpreted with confidence, but it is probable that 43,000 is nearer the truth than the earlier figure 66,000 which is now withdrawn.

Sørensen, on the basis of certain assumptions concerning the ionization of ammonium albuminate, formerly considered doubtful, deduced that the molecular weight of salt-free egg albumin would be about 34,000, an estimate recently confirmed by the centrifugal experiments of Svedberg and Nichols. Critics of the osmometric method might object that the influence of traces of alkali dissolved from the glass, and the hydrogen ions given off by the protein, might increase the osmotic pressure and the free energy of the protein and diminish the apparent molecular weight, but even in the case of acidic proteins like egg albumin, dissolved in distilled water, the hydrogen ions are unlikely to cause errors exceeding 20%, and it seems reasonable to claim that the agreement of the results obtained by different methods supports the view that the osmometer gives at least approximately correct results under suitable conditions.

Although the unequal distribution of ions is often advanced as a theoretical objection to the osmometric method, it is advantageous from the practical point of view because it facilitates the study of the activity coefficients of diffusible ions in the presence of proteins, an important point when the protein systems are studied as a whole.

CAMBRIDGE, ENGLAND

[7] Adair, *Proc. Roy. Soc.* (*London*), (in press).