Molecular Weight of the Adrenocorticotropic Hormone

BY ELLEN BURTNER

Diffusion and sedimentation experiments have been performed with dilute solutions of the adrenocorticotropic hormone which has been prepared and described by Li, Simpson and Evans of the Institute of Experimental Biology, Berkeley, California.[1] In making up the solutions 20 mg. of the purified preparation was dissolved in 3 cc. of phosphate buffer of *p*H 7.0 and containing sodium chloride to give ionic strength 0.28, and dialyzed against the buffer salt system. Two sedimentation velocity experiments were performed in the standard Svedberg oil-turbine high velocity ultracentrifuge, and one diffusion experiment was carried out in a Lamm cell. The position of the peak in the former, and the spreading of the peak in the latter, at the end of successive time intervals, were observed by the Lamm scale line displacement method.

The sedimentation constant, s_{20}, was calculated by using the procedure described by Svedberg and Pedersen.[2] While the individual values of s_{20} calculated at half-hour intervals during each experiment vary somewhat, the two averaged values agree to within 5%. Variations between the results in individual experiments may be due to the fact that the material is light and thus travels only a short distance in each time interval, making the error in the determination of the change of position of the boundary an appreciable fraction of the total distance traveled. In the scale line displacement *vs.* distance in cell diagrams, the curves obtained were not unlike those characteristic of a single substance.

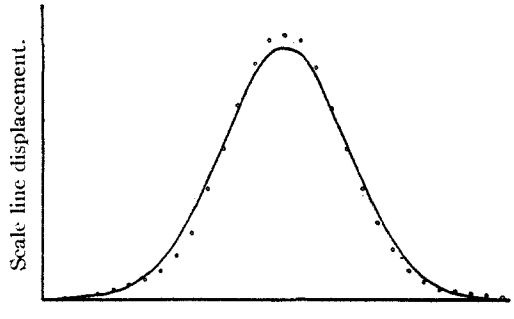

Fig. 1.

(1) C. H. Li, M. E. Simpson and H. M. Evans, *Science*, **96**, 450 (1942).

(2) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, Oxford, 1940.

The diffusion constant was calculated from the equation $D = \sigma^2/2t$, where σ is the standard deviation of the curve obtained by plotting the scale line displacement against position in the cell. The value was then adjusted to give the diffusion constant for a hypothetical process taking place in pure water at 20°. Comparison of the experimental curve, when reduced to normal coordinates, with the Gaussian error curve, gave evidence of some inhomogeneity. In the figure the solid line represents the ideal diffusion curve of a single substance, and the circles are the experimental points.

The molecular weight was calculated by using the familiar equation $M = RTs/D(1-V\rho)$. The partial specific volume was not determined because a sufficient amount of the hormone was not available. In the absence of information to the contrary, it was assumed to be 0.75. The frictional ratio, f/f_0, was obtained by using the equations $f = M(1 - V\rho)/s$ and $f_0 = 6\pi\eta N(3MV)^{1/3}/(4\pi N)^{1/3}$. It gives as an approximate value 3:1 for the ratio of major to minor axis of the assumed unhydrated ellipsoidal molecule.

Experimental data, with calculations, are summarized to form Table I. For reasons given above the magnitude of the uncertainty involved in these figures is relatively high. (Probable error in molecular weight is ± 10%.)

TABLE I

MOLECULAR KINETIC DATA FOR THE ADRENOCORTICOTROPIC HORMONE (FRICTIONAL AND AXIAL RATIOS COMPUTED FOR UNHYDRATED ELLIPSOIDAL MOLECULE)

s_{20} (in S)	D_{20} (in 10^{-7} sq. cm./sec.)	M	f/f_0	a/b
2.08	10.4	20,000	1.1	3

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RECEIVED FEBRUARY 25, 1943

Tribenzylsulfonium Hydrogen Sulfate and Hydroxide

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It has been found that concentrated sulfuric acid brings about the cleavage of dibenzyl sulfide at moderate temperatures. Unlike the well known reaction of ethers and sulfuric acid, however, the products isolated are not benzyl mercaptan and benzyl hydrogen sulfate, although they are likely intermediates in the reaction. The principal product obtained is tribenzylsulfonium hydrogen