Acknowledgments.—This work was supported by research grant RG-2350 from the National Institutes of Health, U. S. Public Health Service; and by grant G-1805 from the National Science Foundation.

IOWA CITY, IOWA

[CONTRIBUTION FROM THE ROCKEFELLER INSTITUTE]

Application of the Archibald Principle for the Ultracentrifugal Determination of the Molecular Weight in Urea Solutions of Histone Fractions from Calf Thymus

BY RODES TRAUTMAN[1] AND CHARLES F. CRAMPTON

RECEIVED JANUARY 12, 1959

The Archibald principle has been employed for the determination of molecular weights by a method in which knowledge of the initial concentration of the solute is not essential. A least squares method is given by which data from various exposures in Archibald measurements may be combined so that a single value for the molecular weight may be obtained from a series of experiments. Analysis of the data for the known reference protein ribonuclease, at about 1% concentration in acetate buffer, *p*H 5.5, verified both the linearity of the function plotted as the boundary separated from the meniscus and the applicability of the method. Ribonuclease was also centrifuged in *p*H 5.5 acetate buffer containing 6 *M* urea. The data indicated that the non-ideality activity coefficient correction term for ribonuclease in urea is reasonably small. Two possibly heterogeneous, aggregating proteins were analyzed to determine the molecular weight *M*. Data for calf thymus histone Fraction A indicated that it did not dissociate in 6 *M* urea and that $M = 10{,}000 \pm 2000$. Data for calf thymus histone Fraction B indicated that it dissociated in half in 6 *M* urea and that for the monomer $M = 16{,}000 \pm 1600$. The minimal molecular weights calculated from amino acid analyses were higher than these values, especially for histone fraction A, and the most probable explanation is that histone Fractions A and B both contain mixtures of proteins of different composition.

The investigations reported here were undertaken to determine the molecular weight in solutions of the chromatographically purified histone Fractions A and B from calf thymus.[3] These fractions have reproducible amino acid compositions, and, if they are assumed to be homogeneous, the analytical data would require minimal molecular weights of 20,600 and 19,700, respectively. In order to determine the molecular weights an ultracentrifugal method that utilizes an extension of the Archibald principle[4] was employed. The method was verified by the use of ribonuclease, the molecular weight of which is accurately known.[5,6] During the course of this work there appeared a complete review by Luck, *et al.*,[7] in which were described the molecular weights and association properties of histones very similar to some of those reported here.

The original exposition of the Archibald principle was in terms of the sedimentation and diffusion coefficients *s* and *D*, respectively, and the concentration at the limits of the solution column. The principle states that since no dissolved molecules can flow out of the cell through either end, the processes of outward settling and inward diffusion balance exactly. Klainer and Kegeles[8] were first able to shift the analysis from one requiring *c* to one requiring the initial concentration c^0, when measurements of the concentration gradient in a sector cell were made. Then, recently, Kegeles, Klainer and Salem[9] have gone one step further and apparently have succeeded in removing any explicit expression of *s* or *D*, so that only the anhydrous molecular weight *M* and c^0 appear, in addition to the usual partial specific volume, density and activity coefficient terms.

Since a molecular weight itself is independent of the concentration and since the velocity ultracentrifuge yields sedimentation and concentration information almost independently, it would seem that c^0 also could be removed from the concept and application of the Archibald principle. One proposal along this line, made by Ginsburg, Appel and Schachman,[10] combines the data for the top and bottom limits of the solution column in any one exposure. The method to be described here, in a way, is an extension of this notion, but instead utilizes the data at different *times* at the same limit to determine both *M* and c^0. Looked at another way, this analysis indicates how all the data from one run or a series of runs can be averaged to yield a single value of the molecular weight, either with or without using the initial concentration.

Theory

Prior to complete separation of the boundary region from the meniscus, the Archibald principle, as presented by Kegeles, Klainer and Salem[9] states that at the axial limit of the solution, r_a, the concentration gradient will adjust itself as the concentration falls so that

$$M(1 - \bar{V}\rho)\omega^2 r_a - (\partial\mu/\partial r)_{T,P,r_a} = 0 \qquad (1)$$

where *M*, $\bar{V}$ and μ are the (anhydrous) molecular weight, partial specific volume and molar chemical

(1) U. S. Dept. of Agriculture, Plum Island Animal Disease Laboratory, Greenport, New York. A preliminary report was presented before the Division of Biological Chemistry at the 130th National Meeting of the American Chemical Society, Atlantic City, September, 1956. Data on the modifications of the Archibald method proposed elsewhere[2] have not yet appeared, and hence the control data on ribonuclease serve to substantiate the technique involved.

(2) R. Trautman, *J. Phys. Chem.*, **60**, 1211 (1956).

(3) C. F. Crampton, W. H. Stein and S. Moore, *J. Biol. Chem.*, **225**, 363 (1957).

(4) W. J. Archibald, *J. Phys. Colloid Chem.*, **51**, 1204 (1947).

(5) C. H. W. Hirs, W. H. Stein and S. Moore, *J. Biol. Chem.*, **221**, 151 (1956).

(6) S. M. Klainer and G. Kegeles, *Archiv. Biochem. Biophys.*, **63**, 247 (1956).

(7) J. M. Luck, H. A. Cook, N. T. Eldredge, M. I. Haley, D. W. Kupke and P. S. Rasmussen, *Arch. Bicchem. Biophys.*, **65**, 449 (1956).

(8) S. M. Klainer and G. Kegeles, *J. Phys. Chem.*, **59**, 952 (1955).

(9) G. Kegeles, S. M. Klainer and W. J. Salem, *ibid.*, **61**, 1286 (1957).

(10) A. Ginsburg, P. Appel and H. K. Schachman, *Archiv. Biochem. Biophys.*, **65**, 545 (1956).

potential, respectively, of the solute component, ω, T and P are the constant angular velocity, absolute temperature and pressure at r_a. This can be rearranged to

$$M_{app}(1 - \bar{V}\rho) \equiv \frac{RT(\partial c/\partial r)_{ra}}{\omega^2 r_a c_a} = \frac{M(1 - \bar{V}\rho)}{1 + (\partial \ln y/\partial \ln c)} \quad (2)$$

where M_{app} is an apparent molecular weight calculated as though the activity coefficient y were unity and R is the gas constant. It should be noted that the concentration can be expressed in any units and, further, if index of refraction or optical density are used to determine the concentration, the appropriate value of y can be chosen to allow for conversion to a mass concentration scale.

The substitution of the initial concentration c^0 for the concentration c_a is done through[6]

$$(c_a - c^0) = -\int_{ra}^{rp} \left(\frac{r}{r_a}\right)^2 \left(\frac{\partial c}{\partial r}\right) dr \quad (3)$$

where r is a point in the plateau region of the cell free from concentration gradients of the solute. Consider this rearrangement of equation 2 where

$$q_a \equiv \frac{RT(\partial c/\partial r)_{ra}}{\omega^2 r_a} = M_{app}(1 - \bar{V}\rho)[(c_a - c^0) + c^0] \quad (4)$$

$(c_a - c^0)$ is to be determined from equation 3. A plot of q_a *vs.* $(c_a - c^0)$ should yield $M_{app}(1 - \bar{V}\rho)$ for its slope and $-c^0$ for its intercept on the $(c_a - c^0)$ axis.

Similarly, the relationship for the peripheral limit of the solution column at r_b is

$$q_b \equiv \frac{RT(\partial c/\partial r)_{rb}}{\omega^2 r_b} = M_{app}(1 - \bar{V}\rho)[(c_b - c^0) + c^0] \quad (5)$$

where[6]

$$(c_b - c^0) = \left(\frac{r_a}{r_b}\right)^2 \int_{rp}^{rb} \left(\frac{r}{r_a}\right)^2 \left(\frac{\partial c}{\partial r}\right) dr \quad (6)$$

These two relationships of equations 4 and 5 are shown in a single graph as Fig. 1. Start at the point E, marked also as $t = 0$, on the vertical axis which is the ordinate scale for q_a plotted on the left and q_b on the right-hand side. Proceeding downward and to the left through the points A_1, A_2 and finally to D, the line gives the dependence of q_a on $(c^0 - c_a)$ as the peak forms and separates from the meniscus as indicated by the insert figures. Qualitatively, this is the dependence of the height of the schlieren pattern at the meniscus on the area under the curve. Similarly, proceeding upwards and to the right from E, the line gives the variation, as the run proceeds, of the height of the pattern at the bottom of the cell, calculated as q_b, with the area under the curve, calculated as $(c_b - c^0)$. It should be noted that runs at all speeds will start at the point E. For low speed runs only A_1, for example, may be reached before the plateau is gone. In higher speed runs the range is from E to D; however, the higher the speed the greater are the difficulties of measurement at the beginning, and so data cannot be obtained near E. The composite of several runs at various speeds will cover the entire region and should fall on the same line. The slope of this line is $M_{app}(1 - \bar{V}\rho)$ from equations 4 and 5.

The method of calculation of Kegeles, *et al.*,[6,8,9] when c^0 is known consists of calculating the slope

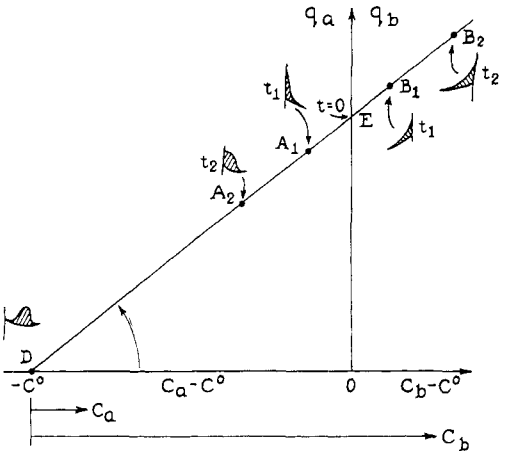


Fig. 1.—Linear method of applying the Archibald principle. See text for meaning of symbols. Insert figures represent schlieren patterns for the various points marked. Slope of line is $M_{app}(1 - \bar{V}\rho)$.

from $\overline{DA_1}$, then $\overline{DA_2}$, etc. The method of Ginsburg, *et al.*,[10] mentioned above, when c^0 is not known, consists of calculating the slope from $\overline{A_1B_1}$, $\overline{A_2B_2}$, etc. The method proposed here is to use all the points that have been obtained, say A_2, A_1, B_1, B_2, etc., finding the single best slope, either graphically or by the method of least squares. The intercept on the axis of abscissa yields, then, the best value of c^0, whether or not experimental points at D are available to use in calculating the least squares line. In Fig. 1 the concentrations c_a and c_b are shown for reference as the lowest scales from $-c^0$, but it should be noted that the data are obtained and plotted from the origin as $(c_a - c^0)$ or $(c_b - c^0)$ directly.

The method indicated in Fig. 1 for handling the data from one or a series of runs involves knowing the limits r_a and/or r_b. The procedure used to locate these limits is described in the Experimental section. It should be noted here that, by knowing r_a, some property in addition to M_{app}, namely c^0, can be determined. This is in contrast to the method in which c^0 is known and $(\partial c/\partial r)/rc$ is calculated for all values of r, and thence r_a and M_{app} are determined from the common intersection.

Recently Ehrenberg[11] has given a method of plate reading using the area integral $\int(\partial c/\partial r)dr$ instead of $\int(r/r_a)^2(\partial c/\partial r)dr$. The method requires a synthetic boundary cell run extending the same length of time as the main Archibald type run and necessitates two measurements of area for each point. In principle Ehrenberg's method calculates $M_{app}(1 - \bar{V}\rho)$ from the slope of a line parallel to the chord $\overline{DA_1}$ but lying to the right of it. Since methods have been given[2] for facilitating the determination of $\int(r/r_a)^2(\partial c/\partial r)dr$, it is not necessary to avoid this integral. However, there is a more fundamental reason why the synthetic boundary cell was not used in these experiments. With biological materials which are not available in crystalline form or cannot be dialyzed, it becomes difficult to make up the proper solvent to use for overlay in the

(11) A. Ehrenberg, *Acta Chem. Scand.*, **11**, 1257 (1957).

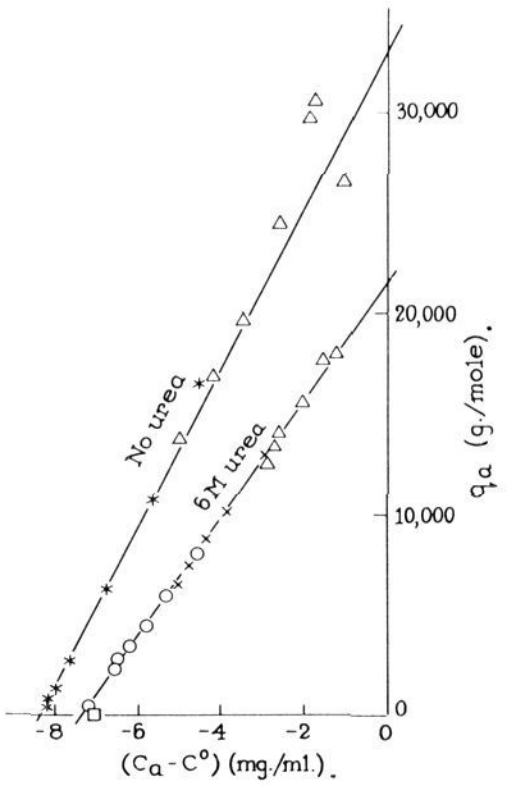


Fig. 2.—*Linear plot for ribonuclease.* Γ/2 0.1, *p*H 5.5 acetate buffer both with (right-hand line) and without (left-hand line) 6 *M* urea: △, 25,980 r.p.m.; *, 52,640 r.p.m., O, 59,780 r.p.m.; □, av. of 3 values at 59,780 r.p.m., 7.07, 7.17, 7.19 mg./ml.

cup. This is especially true when crystalline urea is added to say 1 ml. of the sample to make a 6 *M* solution. Here the solvent corresponds to about 36% area in contrast to about 1% for the protein and must be sufficiently precise to avoid inverted density and/or superimposed gradients.

The urea was chosen to promote disaggregation. Unfolding of the monomer unit,[12] should it occur, will not affect the Archibald method, which is independent of shape. However, there may be complications due to a variation of $\bar{V}$[13a] or the activity coefficient that would possibly occur when the solvent[13b] is changed.

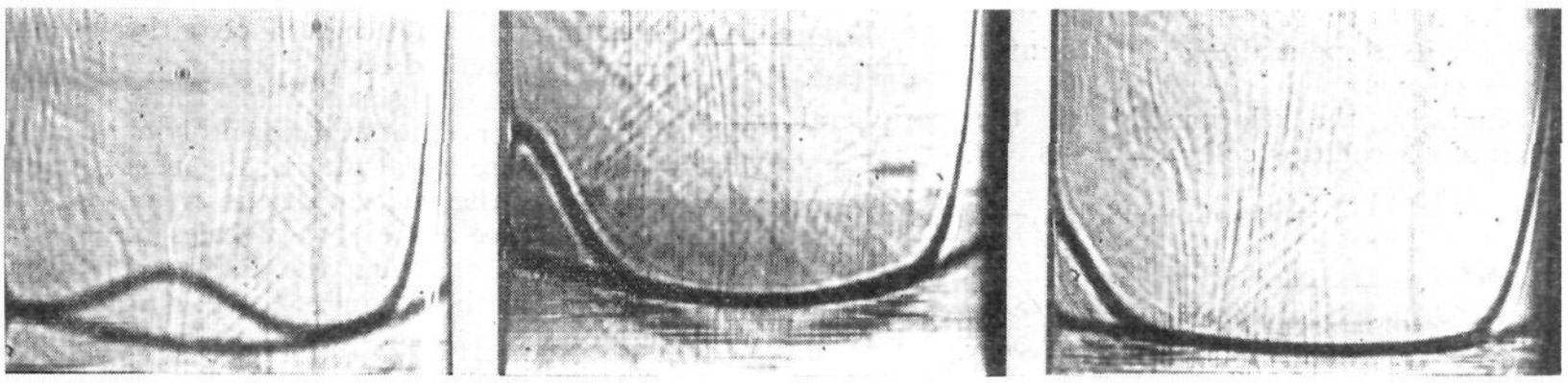
Experimental

Ultracentrifugation.—A Spinco Model E ultracentrifuge[14] equipped with phaseplate schlieren optics was used and double sector 12 mm. cells were employed. The photographic plates were measured on a two dimensional microcomparator and the data calculated according to procedures described elsewhere[2] which involve evaluating the integral of equation 2 as the summation of ordinates equally spaced on a radius-cubed scale. Direct recording of the measurements from the microcomparator, and calculation of q_a and $(c_a - c^0)$, were accomplished with a Tetractys.[15]

The cell image conjugate to the meniscus consists of a central shadow flanked by sharply defined fringes. The position of the meniscus was taken as the bisector of the chord joining the first fringe, say, on either side of the central shadow. This was facilitated by locating the light source on the optical axis of the lower collimating lens by means of the reflected image from a mirror quartz and using the central shadow as the fringe bisector.[16] In the earlier experiments, before it was realized that a 0.1 mm. error in r_a on the photographic plate contributed about a 10% error in the molecular weight, and before the optical system was aligned, it was necessary to determine r_a at the common intersection of approximate $(\partial c/\partial r)/rc$ curves.

Materials.—The specimen of crystalline ribonuclease (RNAse) was Armour Lot. No. 381059, for which structural studies have been established[5] a formula weight of 13,683. The preparation and the amino acid composition of histone Fractions A and B already have been described.[3] The preparations examined in the ultracentrifuge were 24 and 22d of Table II and 24d of Table III of reference 3. The proteins were dissolved in aqueous sodium acetate, *p*H 5.5, ionic strength 0.1. Where indicated, this solvent also contained 6 *M* urea. The respective densities of these solvents, *measured pycnometrically are given in* Table I.

Partial Specific Volume.—The experimental value[12] of 0.692 was used for ribonuclease. It is not far from the value calculated from the amino acid composition, 0.709, by standard methods recently applied to ribonuclease by Schachman.[17] This method was used to calculate $\bar{V}$ for histone Fractions A and B yielding 0.765 and 0.746, respectively.



A B C

Fig. 3.—Ribonuclease in 6 *M* urea: A, 59,780 r.p.m., 418 min.; B, 39,460 r.p.m., 160 min.; C, 25,980 r.p.m., 154 min.; double sector centerpiece, 60° phaseplate angle.

Results

Ribonuclease.—In Fig. 2 is given the q_a *vs.* $(c_a - c^0)$ plot for ribonuclease in the acetate buffer (the left-hand line). Two speeds were used, 25,980 and 52,640 r.p.m. The lowest point at the lower speed was reached in 160 minutes, and the point corresponding to D of Fig. 1 was reached at the higher speed also in about 160 minutes. The least square line through these 15 points yields $M_{app}(1 - \bar{V}\rho_1) = 4270$ g./mole ± 5% and $c^0 = 8.2$ mg./ml. ± 2%. The corresponding data for ribonuclease in the 6 *M* urea solvent are shown as the right-hand line in Fig. 2. The least square line in this case gives $M_{app}(1 - \bar{V}\rho_2) = 2980 \pm 1\%$ and $c^0 = 7.2 \pm 0.4\%$. In Fig. 3 are shown representative schlieren patterns of the type measured. These data indicate that the q_a *vs.* $(c_a - c^0)$ plot is linear for a single solute and that even with a curved baseline the Archibald principle can be applied.

(12) W. F. Harrington and J. A. Schellman, *Compt. rend. lab. Carlsberg, ser. chim.*, **30**, 21 (1956).

(13) (a) P. A. Charlwood, THIS JOURNAL, **79**, 776 (1957); (b) S. Katz and H. K. Schachman, *Biochem. Biophys. Acta*, **18**, 28 (1955).

(14) Spinco Division, Beckman Instruments, Inc., Palo Alto, California.

(15) Olivetti Corporation of America, New York, N. Y.

(16) R. Trautman, *Biochim. Biophys. Acta*, **28**, 417 (1958).

(17) H. K. Schachman, "Methods in Enzymol.," **IV**, 32 (1957).

TABLE I

MOLECULAR WEIGHT CALCULATIONS FROM DATA OBTAINED BY ANALYZING VELOCITY ULTRACENTRIFUGE EXPERIMENTS AS INDICATED IN FIG. 1

Solvent[a]	Quantity[b]	RNAse	Histone Fractions A	Histone Fractions B
	$\bar{V}$	0.692	0.765	0.746
No urea	c^0	8.2 (2%)	9.7 (1%)	8.6 (1%)
1.0031	$M_{app}(1 - \bar{V}\rho)$	4270 (5%)	2290 (2%)	7940 (4%)
	M_{app}	14000	9850	31500
	c^0	7.2 (0.4%)	7.2 (3%)	7.3 (3%)
6 *M* urea	$M_{app}(1 - \bar{V}\rho)$	2980 (1%)	2010 (6%)	2940 (6%)
1.1014	M_{app}	*12500*	*12800*	*16500*
	$\partial \ln y/\partial \ln c$	+0.09 (12%)	−0.23 (20%)	−0.04 (130%)
Probable mol. wt.	Centrifuge[c]	14000 ± 700	10000 ± 2000	16000 ± 1600
	Amino acid[d]	13683	20600	19700

[a] Acetate buffer, *p*H 5.5, Γ/2 0.1 present in both solvents; numerical entry is D^{20}_4 in g./ml. [b] $\bar{V}$ for RNAse from literature 12; histones from amino acid composition. c^0 and $M_{app}(1 - \bar{V}\rho)$ are the intercept in mg./ml. and slope in g./mole, respectively, of the least squares line as in Fig. 1; numbers in parentheses are the standard deviations in per cent. M_{app} in g./mole is calculated from $M_{app}(1 - \bar{V}\rho)$ and is assumed to carry the same standard deviation. $\partial \ln y/\partial \ln c$ is calculated from equation 2 using M for RNAse, histone Fractions A and B, respectively: 13,683, 9850, 31500/2; standard deviation calculated from standard deviation of $M_{app}(1 - \bar{V}\rho)$ by multiplying by $(1 + \partial \ln c/\partial \ln y)$. [c] The values most consistent with the data in the two solvents. [d] Minimum values from integral numbers of the least prevalent amino acids.

The values of $M_{app}(1 - \bar{V}\rho)$ are shown plotted against ρ in Fig. 4 as points marked α and β. The values of $1/\bar{V}$ and anhydrous molecular weight M, 1/0.692 and 13,700, respectively, are intercepts determining the dotted line. The point α is on the line indicating that the activity coefficient term of equation 2 is small and thus also checking this Archibald procedure. Point β is not far off. If

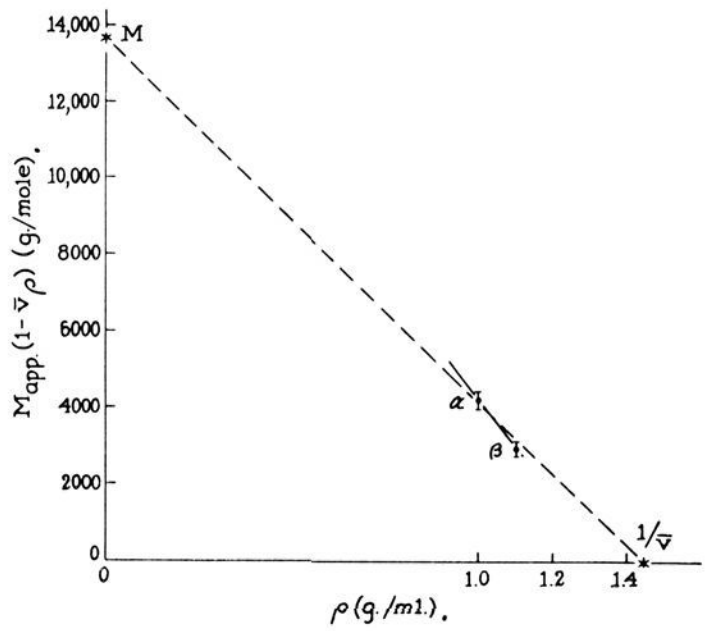


Fig. 4.—Summary molecular weight plot for ribonuclease. Vertical size of points indicate ± one standard deviation; α, no urea; β, 6 *M* urea; ordinate is slope from linear Archibald plot, Fig. 2; *M* and $1/\bar{V}$ known as 13,683 and 1/0.692, respectively.

each point is considered separately to determine a line through a precise $\bar{V}$, then the intercept at $\rho = 0$ is M_{app} with the same error as given for $M_{app}(1 - \bar{V}\rho)$. These values are listed in Table I. Further, the activity coefficient term was calculated from equation 2 and the known molecular weights giving the value 0.09 for the urea solvent, as indicated in the table. The values of $\partial \ln y/\partial \ln c$ and the deviations of M_{app} from the 13,683 figure indicate what the limits of the method might be when applied to the unknown histones.

Histones.—In Fig. 5 are shown representative schlieren patterns for the histone Fractions A and B. The times have been chosen so that, if there were no change in shape or aggregation, the distance moved in the 6 *M* urea would be the same as in the absence of the urea. By inspection it can be seen that histone Fraction A perhaps is the same size in the two solvents, but histone Fraction B probably dissociates in the urea. Measurement of the patterns as for ribonuclease yields the data given in Table I. Using just the experiments without urea the molecular weight of histone Fractions A is 9850. However, the experiment in urea yielded a higher value, for M_{app} with a negative value for the activity correction term (using the value of 9850 for *M* in its calculation). This is not at all like the behavior of the ribonuclease. Since the smallest value of *M* is desired for comparison with the amino acid composition, the probable value of 10,000 is chosen, and a large error of 20% is included to allow for the anomalous results in urea.

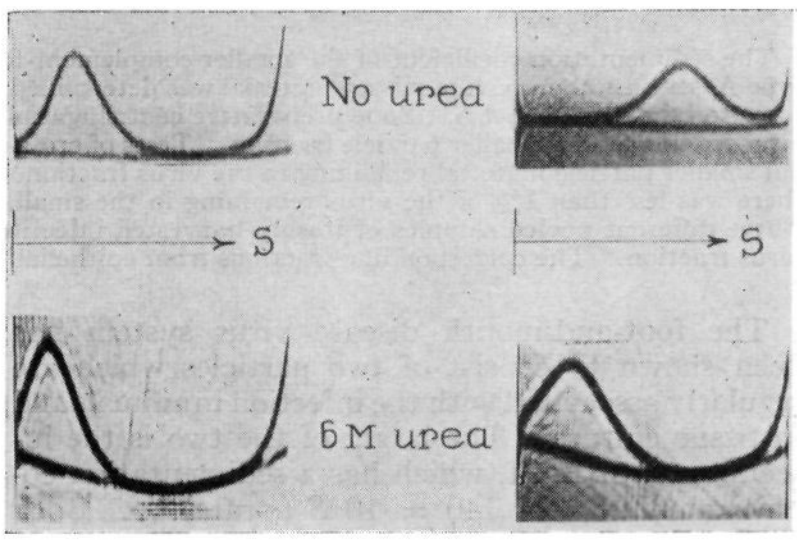


Fig. 5.—Histone fractions: left-hand column, histone Fraction A; right-hand column, histone Fraction B.

Histone Fraction B, on the other hand, shows a drop in M_{app} from 31,500 to 16,500 going to the urea solvent. If half of the 31,500 is taken for *M*, then the activity coefficient term of −0.04 is within experimental error of the value for the ribonuclease. Hence, the probable value of 16,000 with only a 10% error is chosen for the molecular weight.

It would appear that A is presently heterogeneous with respect to amino acid composition and that B may be much less so. On the other hand, both Fractions A and B are not grossly heterogeneous with respect to molecular weight of the monomer kinetic unit, since both gave symmetrical, but broad, peaks on prolonged centrifugation in 6 M urea. More significantly, the statistics of Table I indicate that there was no curvature attributable to heterogeneity of the q_a *vs.* $(c_a - c^0)$ plots since the standard deviation of the slope is low.

Discussion

There may be some advantage in using the Archibald principle in various solvents on a material with a known large activity coefficient term to see if a correct value for the term can be determined. In cases where the correction is small or known, the use of higher density solvents to slow down the sedimentation may extend the usefulness of the Archibald principle to higher molecular weight classes which yield patterns too steep to measure accurately even at the lowest speeds and concentrations now practical.

The data of Fig. 2 are taken from the same experiments reported elsewhere[16] to show the variation of $(\partial c/\partial r)/rc$ with r and to locate the position of r_a from the common intersection of such curves. It was of interest to know whether such information also could be obtained from a q_a *vs.* $(c_a - c^0)$ plot, where the prime represents an incorrect choice of the position of r_a. Treating r_a as a variable, the data were analyzed to see what value minimized the quadratic term in the least squares polynominal. Actually, the very slight curvature reversed as r_a was varied by 0.1 mm. on either side of the geometrical value of r_a. This is interpreted to mean that the q_a *vs.* $(c_a - c^0)$ representation does not sacrifice the ability to determine r_a, if desired. Also, it indicates one cause of non-linearity in the plot. But this curvature is so slight as to be normally undetectable; hence the wrong choice of r_a will still introduce the same systematic error in M_{app} as it does in the $(\partial c/\partial r)/rc$ method—about 10%/0.1 mm. on the plate. On the other hand, polydispersity of molecular weight will yield a large curvature.[2] This has recently been discussed by Erlander and Foster.[18]

The values of M found here for the chromatographic histone Fractions appear significantly greater than those reported by Luck, *et al.*,[7] especially for histone fraction B. Perhaps less degradation is involved in the isolation.

Acknowledgments.—The authors wish to acknowledge with thanks the interest and encouragement of Drs. S. Moore and W. H. Stein.

(18) S. R. Erlander and J. F. Foster, Abstracts of the 133rd and 134th National Meetings, American Chemical Society, April, 1958, and Sept., 1958, Division of Biological Chemistry.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE PLUM ISLAND ANIMAL DISEASE LABORATORY, ANIMAL DISEASE AND PARASITE RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

Partition by Zone Ultracentrifugation of the Two Complement-Fixing Particles in the Foot-and-Mouth Disease Virus System[1]

BY RODES TRAUTMAN, MILTON SAVAN AND SYDNEY S. BREESE, JR.

RECEIVED JANUARY 31, 1959

The sedimentation coefficient of the smaller complement-fixing (CF) particle of the foot-and-mouth disease virus system, type A, strain 119, in bovine-passed material was determined by assay of moving boundary fractions from a swinging bucket rotor to be $s_{20,w} = 14 \pm 1$ S. Zone preparative centrifugation of the velocity type was used to separate infectious fluids into a virus fraction and a smaller particle fraction. Tests of cross-contamination revealed that there was less than 5% of the original smaller particle material remaining in the virus fraction as determined by CF activity measurement. Correspondingly, there was less than 1% of the virus remaining in the smaller particle fraction as determined by infectivity measurement. Three different pooled samples of freshly harvested infectious vesicular fluid showed half of the CF activity to be in the virus fraction. The corresponding fractions from epithelial extracts showed only one fourth.

The foot-and-mouth disease virus system has been shown to consist of two particles which are regularly associated with the infection in animals and in tissue culture. The larger of the two is the infectious virus itself, which has a sedimentation coefficient of $s_{20,w} = 140 \pm 10$ S (s-rate).[2-5] Both particles exhibit complement-fixing (CF) activity. Recent information on the s-rate of the smaller particle and the partition of total CF particles has been reported by Bradish, *et al.*[6] They reported the s-rate to be 7.8 S and assigned 50–100% of the CF activity to this smaller particle in preparations obtained from infected guinea pig, mouse and bovine sources.

The purpose of the work reported here was to apply zone centrifugation for the quantitative separation of the two sizes of antigens. Such a fractionation would enable direct measurement on crude biological fluids of the partition of the CF activity as well as provide the two antigens free from each other for subsequent experiments. Before investigating the zone method, it was necessary to redetermine the s-rate of the smaller CF particle since

(1) This material was presented before the Division of Biological Chemistry at the 134th National Meeting of the American Chemical Society, Sept. 1958.

(2) A. Randrup, "Physico-chemical and Immunological Studies on the Virus of Foot-and-Mouth Disease and Its Host Tissue Microsomes," E. Munksgaard, Copenhagen, 1957.

(3) H. L. Bachrach, *Am. J. Vet. Res.*, **13**, 13 (1952).

(4) G. Pyl, *Arch. Exp. Vet. Med. (Reims)*, **10**, (3), 358 (1956).

(5) S. S. Breese, Jr., R. Trautman and H. L. Bachrach, *Biochemica et Biophysica Acta*.

(6) C. J. Bradish, J. B. Brooksby, J. F. Dillon and M. Norambuena, *Proc. Roy. Soc. (London)*, **B140**, 107 (1952).