picric acid in methanol, giving a precipitate of a crystalline picrate, m.p. 190° (lit.[3] 190°).

1-Methylisoquinoline (V) was obtained by the dehydrogenation of IV in the presence of palladium–charcoal in tetralin or directly from III by a simultaneous decarboxylation and dehydrogenation, following the procedure reported previously[2] for an analogous compound. The crude base was converted to the picrate, m.p. 208–210° (lit.[4] 209–210°) and the sulfate, m.p. 248–250° (lit.[5] 246–247°).

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Concentration Effect on Sedimentation Rate and its Use in Estimating Molecular Weights[1]

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Concentration Effect on Sedimentation Rate.—Many studies have been made relating sedimentation rate to the concentration of the sedimenting substance. The results have been found in almost all cases to follow the equation

$$S_0/S = 1 + Ac \qquad (1)$$

where S is the rate at concentration c, S_0 is the rate extrapolated to zero concentration, and A is an experimentally determined constant which may be called the specific sedimentation slope. This relation is in agreement with theoretical considerations of Burgers[2] as demonstrated by Schachman and Kauzmann,[3] and of Powell and Eyring.[4]

The common practice has been to plot the sedimentation rate against the concentration of the solution which prevails at the start of the centrifugation. During the course of the centrifugation, however, the concentration decreases continuously. This decrease, due to the sector shape of the cell and the increase in centrifugal force with increasing distance from the axis of rotation, was shown by Svedberg and Rinde[5] to follow the equation

$$c = c_1x_1^2/x^2 \qquad (2)$$

where c and c_1 are the colloid concentrations at the boundary distances, x and x_1, respectively, from the axis of rotation. The combined effects expressed by equations (1) and (2) result in a continuous increase in the sedimentation rate during each run. This increase was assumed by Sanigar, Krejci and Kraemer,[6] and was demonstrated experimentally by Lauffer.[7] The rate measured in the usual way is, therefore, a composite value, equaling the instantaneous rate at some time *after* the start of the centrifugation. The concentration at *that* particular time should be used for obtaining the correct relation between sedimentation rate and concentration; or, the rate at *any* given time, and the concentration which prevails at the same time, may be used. Kegeles and Gutter[8] related the concentrations at the mid-points between the first and last photographs of the runs to the over-all sedimentation rates. As will be shown, this is a very close approximation to the correct relation.

The correct relation between sedimentation rate and concentration may be found by substituting equations (1) and (2) into Svedberg's equation[9] defining sedimentation rate

$$S = k(dx/dt)/\omega^2x \qquad (3)$$

where dx/dt is the velocity of the sedimenting boundary at the distance x from the axis of rotation, ω is the angular velocity of the rotor and k represents a constant which comprises the different corrections for reducing the observed rate to its value at the standard conditions of sedimentation in water at 20°. The combined equations may be written

$$S_0dt = (k/\omega^2x)(1 + Ac_1x_1^2/x^2)dx \qquad (4)$$

Taking $t = 0$ at x_1, and $t = t$ at x_2, and integrating, one obtains

$$S_0 = (k/\omega^2t)[\ln(x_2/x_1) + (Ac_1/2)(1 - x_1^2/x_2^2)] \qquad (5)$$

S_0 and A may be evaluated by the usual methods of solving simultaneous equations. Measurements from two runs at different initial concentrations would generally be best, but measurements from two intervals within a single run may be used if they are precise enough. The values of c_1 may be found in each case with the aid of equation (2). The greater the difference between the two values of c_1 used in equation (5), the more accurate will be the determination of A.

The specific sedimentation slope of rabbit myosin,[10] determined in the above manner, is about 1% lower than that determined by the method of Kegeles and Gutter. Since myosin has an unusually high change of sedimentation rate with concentration, most other substances should show even better agreement. The use of the method of Kegeles and Gutter is, therefore, fully justified for most work; further, because it is simpler, it is generally preferable to the more complicated procedure described above. When sedimentations are carried out over the full range of the cell, it is found that the concentrations at the mid-points are about 85% of the starting concentration.[11] This approximation may be used in calculating A by Kegeles and Gutter's method from ultracentrifuge studies in which more detailed information is not given. Of course, the value of S_0 obtained by either of these methods should be the same as that found by simply plotting $1/S$ against c.

Relation between Specific Sedimentation Slope and Intrinsic Viscosity.—Both theoretical[2,3,4,12] and

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TABLE I

SPECIFIC SEDIMENTATION SLOPES, INTRINSIC VISCOSITIES AND MOLECULAR WEIGHTS OF VARIOUS PROTEINS

Protein	A	$\frac{A-5}{0.75}$	$[\eta]$	Molecular weights calculated from equation (14) Using A	Using $\frac{A-5}{0.75}$	Using $[\eta]$
Carbon monoxide hemoglobin	6.4[8]	1.9	6.4[8]	68,000	43,000	68,000
Bovine plasma albumin	6.6[8]	2.1	5.8[8]	64,000	42,000	61,000
β-Lactoglobulin	7.6[11]	3.5	6.0[15]	41,000	30,500	37,000
Southern bean mosaic virus	8.9[16]	5.2	6.2[16]	7.6×10^6	6.2×10^6	6.6×10^6
Pyrophosphatase	11.9[17]	9.2	4.1[17]	89,000	81,000	60,000
Egg albumin	13.8[8]	11.7	5.3[8]	65,000	61,000	45,000
Lee influenza virus	15.8[18]	14.5	9.5[18]	6.3×10^8	6.2×10^8	5.3×10^8
Fibrinogen	23.5[19]	24.7	43.4[19]	220,000	220,000	270,000
Tobacco mosaic virus	48.6[7,a]	52.8	44.6[7]	34×10^6[a]	36×10^6	33×10^6
Myosin	193[10]	250	230[10]	400,000	440,000	420,000

[a] Calculated from two intervals in the single run of ref. 9.

experimental[7,8,10] investigations have demonstrated correlations between specific sedimentation slope and intrinsic viscosity, and these correlations have been subjected to criticism.[6,8,13,14] These relations have only been expressed implicitly, but the following explicit relations may be obtained from them. From Schachman and Kauzmann's derivations,[3] which are based on the hydrodynamic calculations of Burgers,[2] it can be deduced that

$$A \backsimeq 0.75[\eta] + 5 \qquad (6)$$

where $[\eta]$ is the intrinsic viscosity. Kermack, McKendrick and Ponder[12] indicate that for spherical and disc-shaped particles

$$A \approx [\eta] + 5.5 \qquad (7)$$

where $[\eta]$ is given a theoretical value of 1.6 for spheres and somewhat less for discs. From the thermodynamic calculations of Powell and Eyring,[4] it may be deduced that

$$A \approx [\eta] \qquad (8)$$

Lauffer[7] has presented experimental data supporting equation (8), but his data are not uniform enough to exclude (6).

In the second, third and fourth columns of Table I are shown values of A, $(A - 5)/0.75$, and $[\eta]$, respectively, for a number of proteins, which are comparatively rigid and compact, for which data are available in the literature. For these calculations, c is taken as the volume fraction. It is seen that Powell and Eyring's relation holds better for values of A less than 8, while Burgers' is generally better for values of A greater than 8. Although the agreement is not as close in either case as may be desired, it is clear that despite the preliminary nature of the theories involved, the specific sedimentation slope gives at least a first approximation of the viscosity.

It is not intended, of course, that the ultracentrifuge be generally used in place of a viscometer. The relations shown above have been brought out because of their theoretical interest, and their practical value in special cases like the following: (1) A sedimentation rate *versus* concentration curve is often obtained to find the extrapolated sedimentation constant or for other purposes, so that the data are often already available. (2) With highly precise data, like that reported by Lauffer, it is possible to estimate the viscosity and, as shown below, the molecular weight, from a single sedimentation experiment.

Calculation of Molecular Weight.—Methods of calculating molecular weight which are based on sedimentation data require also data on the partial specific volume and on either the diffusion rate or the intrinsic viscosity.

The approximate agreement between the specific sedimentation slope and the viscosity makes it possible, however, to estimate molecular weights from sedimentation rate and partial specific volume data alone. This is done by eliminating the viscosity factor from Lauffer's expression[20] for calculating molecular weights from intrinsic viscosity, sedimentation rate, and partial specific volume. Lauffer uses Simha's equation[21]

$$[\eta] = \frac{(b/a)^2}{15[\ln(2b/a) - 3/2]} + \frac{3(b/a)^2}{15[\ln(2b/a) - 1/2]} + \frac{14}{15} \qquad (9)$$

to get the axial ratio of elongated particles, a/b, from the intrinsic viscosity; and the equation of Perrin[22] and Herzog, Illig and Kudar[23]

$$\frac{f}{f_0} = \frac{(a/b)^{2/3}}{[1 - (a/b)^2]^{1/2}} \ln\left(\frac{1 + [1 - (a/b)^2]^{1/2}}{a/b}\right) \qquad (10)$$

to get the frictional factor, f/f_0, from the axial ratio. He then calculates the molecular weight from the equation

$$M^{2/3} = 6(f/f_0)S_0\eta^0_{20}\pi N(3V_{20}/4\pi N)^{1/3}/(1 - V_{20}\rho^0_{20}) \qquad (11)$$

where M is the molecular weight, η^0_{20} is the viscosity, in poises, of water at 20°, N is the Avogadro number, V is the partial specific volume of the sedimenting material at 20°, and ρ^0_{20} is the density of water at 20°.

We have combined equations (8) and (9) by graphical methods, and found that the intrinsic viscosity and frictional factor are related by the simple equation

$$[\eta] = 2.38\,(f/f_0)^4 \qquad (12)$$

It will be noted that for the special case of spherical,

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unhydrated particles, for which $f/f_0 = 1$, equation (12) is in good agreement with Einstein's theoretical value for these particles[24]

$$[\eta] = 2.5 \qquad (13)$$

Furthermore, equation (12) is supported in the more general case by the data shown in Table II.

TABLE II

FRICTIONAL FACTORS AND INTRINSIC VISCOSITIES OF VARIOUS PROTEINS

Protein	1 f/f^{0a}	2 $[\eta]$ calcd.^{b}	3 $[\eta]$ obsd.^{a}
Pepsin	1.08	3.2	5.2
Hemoglobin	1.16	4.3	5.3
Egg albumin	1.17	4.5	5.7
Helix pomatia hemocyanin	1.24	5.6	6.4
Serum albumin	1.25	5.8	6.5
Lactoglobulin	1.26	6.0	6.0
Homarus hemocyanin	1.27	6.2	6.4
Amandin	1.28	6.4	7.0
Octopus hemoglobin	1.38	8.7	9.0
Serum globulin	1.41	9.5	9.0
Thyroglobulin	1.43	10.0	9.9
Gliadin	1.60	15.5	14.6
Helix hemocyanin	1.89	30.4	18.0

^{a} From a table compiled by Mehl, Oncley and Simha.[15] The values of f/f^0 were calculated from sedimentation and diffusion data by the method of Svedberg and Pedersen.[9] ^{b} Calculated from values in column 1 by use of equation (12).

Equations (11) and (12) may now be combined to give

$$M^{2/3} = 4.82[\eta]^{1/3} S_0 \eta_{20}^0 \pi N(3V_{20}/4\pi N)^{1/3}/(1 - V_{20}\rho_{20}^0) \qquad (14)$$

$[\eta]$ may finally be replaced by its equivalent as given in equation (6) or (8). Molecular weights calculated with either substitution, and also with experimentally determined $[\eta]$, are shown in the last three columns of Table I. The last mentioned method is, of course, the equivalent of Lauffer's method.[20] It is seen that using equation (6) for values of A greater than 8, and equation (8) for values of A under 8, reasonably good approximations of molecular weights are obtained based only on sedimentation rate and partial specific volume data.

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PHILADELPHIA, PENNA.

Utilization of Guanine by *Tetrahymena geleii*[1]

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Tetrahymena geleii has been shown to have an absolute requirement for guanine, although adenine will satisfy a portion of this requirement.[2] It has been assumed, therefore, that all purines of the organism are derived from these exogenous sources. Flavin and Graff showed combined nucleic acid purines to be derived from preformed purines in strain H by administration of labeled guanine[3] and adenine,[4] followed by isolation from the organisms of purines with approximately the same specific activity. Although these workers did not isolate the purines from the acid-soluble nucleotides, the copper precipitate from this fraction was reported to have low activity. This finding suggested the possibility of a synthesis of acid-soluble purines from non-purine precursors, as found by Abrams[5] in the case of a purine-requiring yeast. This was found not to be true for *Tetrahymena* in the studies described below.

Experimental

Guanine-8-C^{14} was prepared by a modification of the Traube[6] method. Three hundred mg. (0.0044 mole) of sodium formate[7] containing 0.6 millicurie of carbon-14 was mixed with 0.94 ml. (0.022 mole) of 90% formic acid. 475 mg. (0.0022 mole) of 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride[8] was added, and the mixture refluxed for 24 hours (yields of approximately 75% were obtained after 12 hours). A trap containing sodium hydroxide pellets was placed at the top of the condenser. At the conclusion of the reaction, the excess radioactive formate was recovered by distillation.

The crude guanine was dissolved in N hydrochloric acid, partially decolorized with charcoal, and reprecipitated twice as the free base at *p*H 6, to give 315 mg. (95%) of partially purified product. The guanine was recrystallized twice as the sulfate and twice as the hydrochloride. The product gave a negative phosphotungstic acid test[9] before and after heating in dilute acid, indicating the absence of unreacted triaminohydroxypyrimidine and the intermediate formamidopyrimidine. The white guanine-8-C^{14} hydrochloride (225 mg., 54%, with more recoverable from supernatants) gave the ultraviolet absorption spectrum of pure guanine. Paper strip chromatograms in four solvent systems and ion-exchange chromatography[10] showed only one component, which also contained all the radioactivity.

Culture of Organisms.—*Tetrahymena geleii* W. was grown (4 days, 25°, in the dark) in sterile culture in one liter of medium A[11] modified as follows: Tween 80 (10 mg./ml.) was substituted for Tween 85, 12.4 mg. of guanine-8-C^{14}-HCl (10 μg. guanine/ml.) and 40 μg. uracil/ml. were used in place of the nucleic acid derivatives listed, concentrations of thioctic acid (= protogen) and the other eight vitamins were doubled. The culture was aerated by placing it in a 3.5-gallon Pyrex bottle which was rotated on its side at 10 r.p.m. by two motor-driven rollers. An air inlet and outlet, protected by sterile cotton plugs, were mounted in a swivel joint in the rubber stopper; air was supplied by an aquarium pump. Respiratory carbon dioxide was collected in 2 N sodium hydroxide and precipitated with barium chloride.

Isolations.—The procedures were similar to those previously described,[10] aliquots of various fractions being removed for radioactivity assay. After the medium had been removed from the organisms by settling in the cold, they were washed three times with 1% sucrose in the same manner, and once with water by centrifugation, wet weight *ca.* 10 g. Acid-soluble nucleotides were extracted with three 100-ml. portions of 5% trichloroacetic acid (TCA) in the Waring blendor at 0–5°, and centrifuged cold. This

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