TABLE II

METAPERIODATE OXIDATION DATA

	Moles IO_4^- consumed per mole of compound							
	Within 3 minutes	1 hr.	4 hr.	8 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Uridine	0.92	1.00	1.00	..	1.05	..	..	..
Xylopyranosylthymine	..	0.15	..	0.73	1.58	2.10	2.20	..
Xylofuranosylthymine	.02	0.08	0.14	..	0.58	0.79	1.01	1.05
"Ribofuranosylthymine" A	.90	..	1.03	..	1.03	..	..	..
Ribofuranosylthymine B	..	1.05	..	1.05	1.08	..	..	..
Ribofuranosylthymine C	.80	0.96	..	..	1.01	..	..	..
Glucopyranosylthymine	..	.29	..	..	2.02	2.06	..	..
Thymidine	..	.00	..	..	..	..	..	0.04

Metaperiodate Oxidation Studies.—Concentrations of nucleosides ranging between 0.001 to 0.002 mM/ml. were treated with excess metaperiodate and aliquots titrated iodometrically according to the usual procedures.[36,37] The acidity produced was determined according to Jackson and Hudson.[38] The results are listed in Table II. The extent of the oxidation within three minutes is noteworthy, and would seem to correlate with the presence of *cis*-hydroxyls.

(36) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 994 (1937).
(37) B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 592 (1944).
(38) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **61**, 1530 (1939).

Acknowledgments.—The authors are indebted to Dr. J. O. Lampen for samples and for enzymatic assay of our compounds, to Dr. D. W. Visser for the sample of "ribosythymine" A, and to Schwarz Laboratories, Inc., for a supply of crude guanosine. The authors wish to thank Dr. Aaron Bendich for helpful discussions and Iris Wempen and John Vitols for valuable assistance.

NEW YORK 21, NEW YORK

[CONTRIBUTION FROM THE DONNER LABORATORY OF MEDICAL PHYSICS AND THE VIRUS LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY]

The Molecular Weights and Dimensions of Some Human Serum Lipoproteins

BY RUSSELL BJORKLUND[1,2] AND SIDNEY KATZ[3]

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Three fractions of low density human serum lipoproteins have been examined by means of light scattering and velocity ultracentrifugation. The molecular weights at *p*H 6.7 were 2.77×10^6, 2.80×10^6 and 3.08×10^6 for fractions of flotation constants 5.9, 6.4 and 8.1 svedbergs respectively. One of the fractions was also studied over the *p*H range 3–9.6 and showed little or no change in molecular weight. The densities of the salt solutions in which two of the fractions have zero sedimentation velocity were determined and the molecular volumes derived therefrom correlated with the molecular weight and dissymmetry data from light scattering. The available data are most satisfactorily fitted by assuming ellipsoidal molecules of small axial ratios. A spherical model provides a less satisfactory fit while rod-like or coil-like molecules are ruled out.

Introduction

In 1949 Gofman, Lindgren and Elliot[4] showed that the boundary anomaly which McFarlane[5] and Pedersen[6] had observed in the ultracentrifugation of human serum could be interpreted as a piling up of lipoproteins at the albumin boundary. Later, Gofman and his associates[7] gave methods for isolating fractions of low density lipoproteins (density less than d^{26}_4 1.063) by centrifugal techniques and used ultracentrifugal data to estimate the molecular weights of these fractions.

In view of the significant statistical correlations between the concentrations of certain low density serum lipoproteins and the clinical manifestations of coronary disease,[8,9] we thought it worthwhile to characterize some of these fractions more precisely than has been done previously. To this end we have applied the techniques of light scattering to determine the molecular weights and dissymmetries of the scattering envelopes of three fractions of human serum lipoproteins and compared these data with ultracentrifugal data on the same fractions.

Materials.—The lipoprotein fractions were isolated by a method described by Lindgren, Elliot and Gofman.[7] Plasma from blood which had been discarded by a blood bank because of positive serology was centrifuged in 9-ml. tubes in a Spinco preparative centrifuge for 24 hr. at 80,000*g*. A layer of material which contained most of the lipoproteins with flotation coefficients[10] between S_f 4 and S_f 10 was pipetted out. Enough solution from the bottoms of the tubes was added to this to give a resulting solution whose concen-

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(2) Supported in part by the United States Atomic Energy Commission.
(3) Supported in part by the Office of Naval Research, Contract NR 121-175.
(4) J. W. Gofman, F. T. Lindgren and H. Elliot, *J. Biol. Chem.*, **179**, 973 (1949).
(5) A. S. McFarlane, *Biochem. J.*, **29**, 660 (1935).
(6) K. O. Pedersen, *J. Phys. Colloid Chem.*, **51**, 156 (1947).
(7) F. T. Lindgren, H. A. Elliot and J. W. Gofman, *ibid.*, **55**, 80 (1951).
(8) J. W. Gofman, H. B. Jones, F. T. Lindgren, T. P. Lyon, H. A. Elliot and B. Strisower, *Circulation*, **2**, 161 (1950).
(9) H. B. Jones, J. W. Gofman, F. T. Lindgren, T. P. Lyon, D. Graham, B. Strisower and A. V. Nichols, *Am. J. Med.*, **11**, 358 (1951).
(10) Following Gofman, we use the term flotation coefficient to denote the negative of the sedimentation coefficient in Svedberg units of species undergoing centripetal migration in a sodium chloride solution of density 1.0630 g./ml. at 26°. The symbols S_f and S_f° will be used to denote the observed flotation coefficient and the flotation coefficient corrected to infinite dilution, respectively. Centrifuge data for all other media will be expressed as sedimentation coefficients.

tration of albumins and globulins was about 5%. Sufficient sodium chloride to make the density of the solution d^{20}_4 1.039 in the absence of protein was then added. Four ml. of this solution was pipetted into a 6-ml. centrifuge tube and 2 ml. of a 4.95% sodium chloride solution was carefully layered on top. This was then centrifuged for 18 hr. at 110,000g. This second centrifugation fractionates the lipoproteins into narrower density groups. The top, second and third milliliters were pooled separately.

Light scattering measurements of the isolated lipoproteins were made in buffers of the following compositions and *p*H's: 0.0794 *M* citric acid, 0.0410 *M* disodium phosphate, 0.0889 *M* sodium chloride, *p*H 3.0; 0.0315 *M* citric acid, 0.0669 *M* disodium phosphate, *p*H 4.9; 0.0500 *M* disodium phosphate, 0.0500 *M* monosodium phosphate, *p*H 6.7; 0.512 *M* sodium carbonate, 0.512 *M* sodium bicarbonate, *p*H 9.6. The ionic strength of all but the *p*H 9.6 buffer was 0.16; that of the latter was 1.4.

Centrifugal Analysis.—Samples of the three fractions were adjusted to d^{26}_4 1.0630 by the addition of sodium chloride and centrifuged at 52,640 r.p.m. in a Spinco Model E analytical ultracentrifuge using a Wolter phaseplate[11] as a schlieren diaphragm. Enlarged tracings (5 ×) of the schlieren peaks at 16 minute intervals of the run were made using a photographic enlarger. All centrifuge runs were made using double-sector cells containing solution in one sector and solvent in the other. Ordinates measured from the solvent baseline to the schlieren peak were determined for every 0.25 cm. of the tracing. Using these data and the optical constants of the centrifuges the following quantity was calculated for each frame

$$\langle x^2 \rangle = \frac{\int x^2 y \, dx}{\int y \, dx} \qquad (1)$$

where x is the distance from the center of rotation of the position in the cell corresponding to the abscissa on the tracing, y is the ordinate defined above, and the integration extends over the peak. The integrals were evaluated numerically using Simpson's rule. If the integral in the numerator of the right side of eq. 1 is divided by the square of the initial radius of the schlieren boundary and is then multiplied by appropriate optical constants of the centrifuge one obtains the refractive index increment of the solution relative to the solvent.[12] The concentrations of the fractions used in this study were determined by dividing the refractive increment so determined by the specific refractive increment of the lipoprotein. For a single sedimentable component the quantity

$$s = \frac{10^{13}}{2\omega^2} \cdot \frac{d \ln \langle x^2 \rangle}{dt} \qquad (2)$$

is the sedimentation coefficient of the molecules in the plateau region.[12] For a multicomponent system this same quantity is the weight average sedimentation coefficient of the molecules in the plateau region if all of the components have the same specific refractive increments.[13] Sedimentation coefficients were determined by substituting slopes of plots of $\ln \langle x^2 \rangle$ *vs.* t into eq. 2. These slopes were almost straight indicating that compressibility effects[14] were either small or were masked by other factors that cause sedimentation coefficients to change during a run.

A rough estimate of the heterogeneity of the fractions was obtained by calculating the rate of spreading of the half-width of the schlieren peaks in Svedberg units using the relation

$$\Delta s = \frac{2 \cdot 10^{13}}{\omega^2 (x_1 + x_2)} \cdot \frac{d(x_2 - x_1)}{dt} \qquad (3)$$

where x_1 and x_2 are the radii of the two points of the boundary whose ordinates are half of the maximum ordinate. A value of approximately 1.6 Svedberg units was obtained. Using a reasonable estimate of the diffusion constant for these molecules (about 2×10^{-7} cm.^{2}/sec.) it is easily shown that most of the spreading must arise from polydispersity of flotation coefficients rather than diffusion.

The densities of zero sedimentation of two of the fractions were determined in the following way. Two samples of a fraction were adjusted to two densities close to the density of zero sedimentation such that at one density the fraction floated and at the other density it sedimented. This adjustment was accomplished by the addition of sodium chloride and water in such proportions as to reach the desired densities and yet have the same concentration of lipoprotein at both densities. Both samples were centrifuged at the same time in the same rotor. The density of zero sedimentation was determined by linear interpolation of the values of ηs for the two densities; η is the viscosity of the solvent relative to that of water at the same temperature, and s is the measured sedimentation coefficient. By following this procedure, errors caused by the dependence of the sedimentation coefficients upon temperature and lipoprotein concentration were minimized. The relative viscosities were interpolated from values given in the International Critical Tables. The measured sedimentation and flotation coefficients are listed in Table I. The flotation coefficients at infinite dilution were calculated using the relation

$$S_f^\circ = S_f(1 - kc)^{-1} \qquad (4)$$

where c is the concentration of lipoprotein. The value of k used[15] was 10 ml./g., obtained from measurements over the concentration range 0.003–0.009 g./ml. The densities of the solutions were determined to ±0.0002 g./ml. with a 1-ml. pycnometer.

TABLE I

DENSITY DEPENDENCE OF SEDIMENTATION COEFFICIENTS FOR THE THREE LIPOPROTEIN FRACTIONS

	Top, ml.	Second, ml.	Third, ml.[c]	Soln. density d^{26}_4	
Concn., g./ml.	0.0118_6	0.0109	0.0070_3		
S_f	7.18	5.73	5.53	1.0630	
S_f^0	8.1	6.4	5.9	1.0630	
s	2.08			1.0186(1.0183)[a]	
s	−3.48			1.0431(1.0436)[a]	
s		2.38		1.0202(1.0199)[a]	
s		−2.29		1.0456(1.0464)[a]	
s (calcd.)	0.0			1.0274(1.0274)[b]	
s (calcd.)		0.0		1.0328(1.0330)[b]	

[a] Time average value of d^{26}_4 at the position of the boundary (corrected for redistribution of salt but not for the compressibility of the solution). [b] ηs *vs.* d^{26}_4 intercept using the time average d^{26}_4 values. [c] The density dependence of the sedimentation coefficient of this fraction was not measured.

Light Scattering Measurements. Apparatus and Calibration.—The light scattering photometer, designed by one of us (S. K.) makes use of an optical system similar to that used by Brice, *et al.*[16] Signal detection and power supplies are the same as those used previously.[17] The 436 mμ line of an AH-3 mercury vapor lamp was isolated by using a Bausch and Lomb No. 33-79-43 multi-layer interference filter together with a Corning No. 3060 glass color filter. This combination provides a narrower band width and nearly twice the intensity of the combination used by Brice. A quarter-wave plate precedes the photomultiplier, as suggested by Mommaerts.[18]

The instrument was calibrated by using du Pont "Ludox" as described previously.[19] It proved to be impossible to clean the concentrated solution (*ca.* 30%) by filtration either through ultrafine fritted glass or Millipore filters because these clogged before passing any solution. It was found that about 40 ml. of a 3% solution of Ludox (made by diluting the 30% solution with distilled water) could be made to pass through a Millipore type HA filter before clogging the pores, enabling us to accumulate sufficient quantities of cleaned 3% solution. Further dilutions of the 3% solution were made with distilled water which had also been cleaned by Millipore filtration.

(11) For a discussion of the theory and use of a Wolter phaseplate in schlieren optical systems, *cf.* R. Trautman and V. W. Burns, *Biochim. Biophys. Acta*, **14**, 26 (1954).

(12) R. Trautman and V. Schumaker, *J. Chem. Phys.*, **22**, 551 (1954).

(13) V. Schumaker, Thesis, Univ. Calif., Berkeley, 1954.

(14) P. Y. Cheng and H. K. Schachman, THIS JOURNAL, **77**, 1498 (1955).

(15) T. Davis, personal communication.

(16) B. A. Brice, M. Halwer and R. Speiser, *J. Optical Soc. Am.*, **40**, 768 (1950).

(17) S. Katz, THIS JOURNAL, **74**, 2238 (1952).

(18) W. F. H. M. Mommaerts, *J. Colloid Sci.*, **7**, 71 (1952).

(19) S. H. Maron and R. L. H. Lou, *J. Polymer Sci.*, **14**, 29 (1954).

A Beckman model DU spectrophotometer equipped with 10-cm. cells was used for the turbidity measurements. Dissymmetry, depolarization and Δ_{90} were measured in the photometer in a semioctagonal dissymmetry cell. The quantity, Δ_{90}, is defined by

$$\Delta_{90} = (G_{90}f_0/G_0f_{90})_2 - (G_{90}f_0/G_0f_{90})_1 \qquad (5)$$

where G_θ is the galvanometer reading at angle θ and f_θ is the transmission of the neutral filters used in measuring G_θ. The subscripts 1 and 2 refer to solvent and solution, respectively. At angles other than zero the photomultiplier views scattered light, while at zero it measures a quantity proportional to the incident intensity. Figure 1 shows the results of these measurements. The negligible dissymmetry of 1.002 and small limiting depolarization of 0.0035 confirm the findings of other workers and again justify the use of Ludox solutions for calibration of light scattering photometers. In agreement with Maron and Lou[19] we also find that a plot of log (Δ_{90}/τ) *vs.* τ is a straight line permitting the necessary extrapolation to zero. Our technique differed from theirs in that we measured G_0 through the cell, but it can be shown that measuring G_0 with the cell removed from the beam would not affect the linearity of the plot.

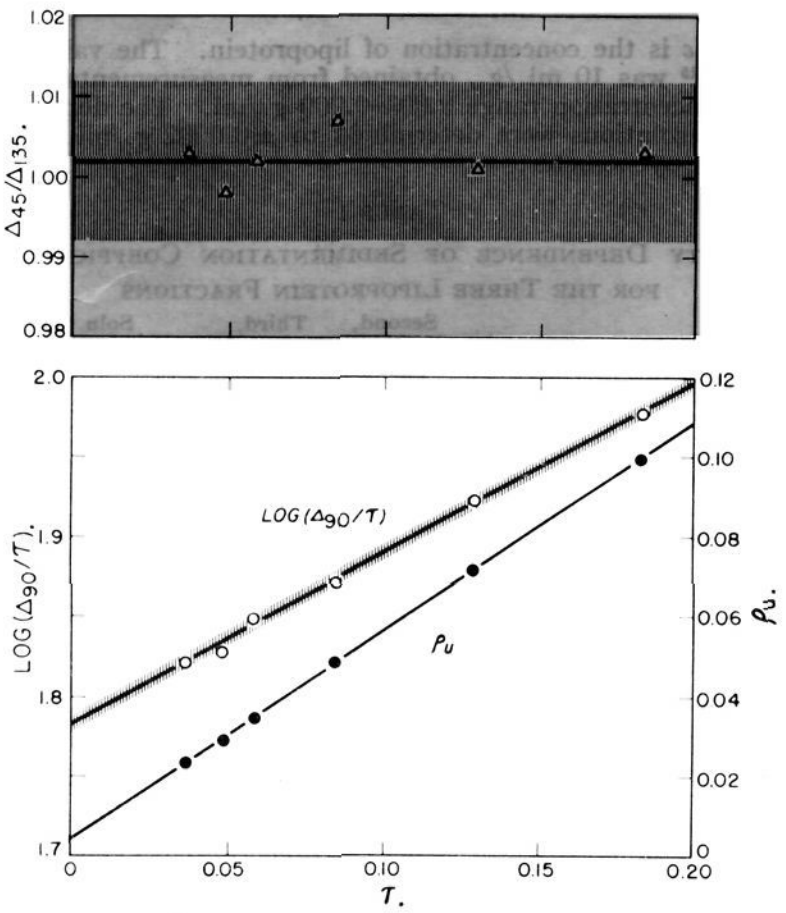

Fig. 1.—Dissymmetries, depolarizations and log (Δ_{90}/τ) as a function of τ for the Ludox solutions used to calibrate the light scattering photometer. The shaded area in the lower half of the figure indicates a variation of $\pm 1\%$ in the quantity Δ_{90}/τ.

Apertures 4 mm. in diameter were used before and after the 10-cm. spectrophotometer cell to determine the correction to the turbidity as measured in the Beckman arising from the lack of angular resolution. This correction was small amounting to a decrease of only 0.9% of the measured turbidity.

Using the limiting value of Δ_{90}/τ and the relation

$$\tau = \frac{16\pi}{3} R_{90,\,u} \qquad (6)$$

which is valid for small isotopic particles, measured values of Δ_{90} were converted to the corresponding reduced intensities. The symbol $R_{90,\,u}$ denotes the reduced intensity for the scattering of unpolarized incident light. Because the refractive indices of the buffers differed from that of water the scattering data for the protein solutions were corrected for refraction of light in passing from the solution into air.

Cleaning Methods for Light Scattering Measurements. All glassware was cleaned in dichromate–sulfuric acid cleaning solution, rinsed with distilled water and finally rinsed in freshly distilled, dust-free acetone. Buffers were filtered through type HA Millipore filters before use. The isolated lipoprotein at the isolation concentration was pipetted into a clean erlenmeyer flask and an appropriate amount of filtered buffer added with gently stirring. The protein solutions and buffer were then centrifuged 2 hr. at 20,000 *g.* in a Servall SS-1 centrifuge. The buffer was pipetted into a clean dissymmetry cell and the protein solution into a clean Polythene bottle. The scattering of the buffer was always measured first; aliquots of the cleaned protein solution were then transferred from the Polythene container to the cell to measure the scattering of the protein solution.

Specific Refractive Increments.—Values of dn/dc, the specific refractive increments of the lipoprotein, were calculated from Armstrong's[20] value of 0.171 ml./g. at 25° for β_1-lipoprotein. Since this value refers to a wave length of 578 mμ and was measured at a salt concentration different from those used in this study, it was necessary to correct it to what it would be under our experimental conditions. The calculations were made as follows. Values of the refractive indices of the various buffers at the wave lengths λ 436 mμ and λ 546 mμ were obtained by measuring their refractive index increments, Δn, relative to water in a differential refractometer at 25°. A two-term Cauchy dispersion formula then gave the refractive indices of the buffers at λ 578 mμ. Values of dn/dc at λ 578 mμ could then be computed from Heller's formula[21] using the density of zero sedimentation rate as the "density" of the lipoprotein.

To convert the values of dn/dc at 578 mμ to their value at 436 mμ, a lipoprotein fraction was isolated by a procedure similar to that used in isolation of the S_f° 8 class. The refractive index increments of this fraction, at about 1% concentration relative to the subnatant salt solution from which it was centrifugally isolated, were measured at λ 436 mμ and at λ 546 mμ. Substitution of these values into a two-term Cauchy dispersion formula gave the information necessary to correct the specific refractive increments from λ 578 mμ to λ 436 mμ. The results at λ 436 mμ were dn/dc = 0.175, 0.177, 0.177 and 0.162 for the buffers of refractive indices 1.3437, *p*H 3; 1.3421, *p*H 4.9; 1.3419, *p*H 6.7; and 1.3575, *p*H 9.6, respectively.

Results and Discussion

Figure 2 gives the values of $Kc/R_{90,\,u}$ as a function of concentration for the three fractions all at *p*H 6.7. K is equal to $2\pi^2(dn/dc)^2n_0^2/N\lambda_0^4$ where n_0 is the refractive index of the solvent, N is Avogadro's number and λ_0 is the wave length of the light *in vacuo*. Concurrent measurements of depolarization, ρ_u and dissymmetry, $Z \equiv \Delta_{45}/\Delta_{135}$, gave the results shown in Table II. The superscript

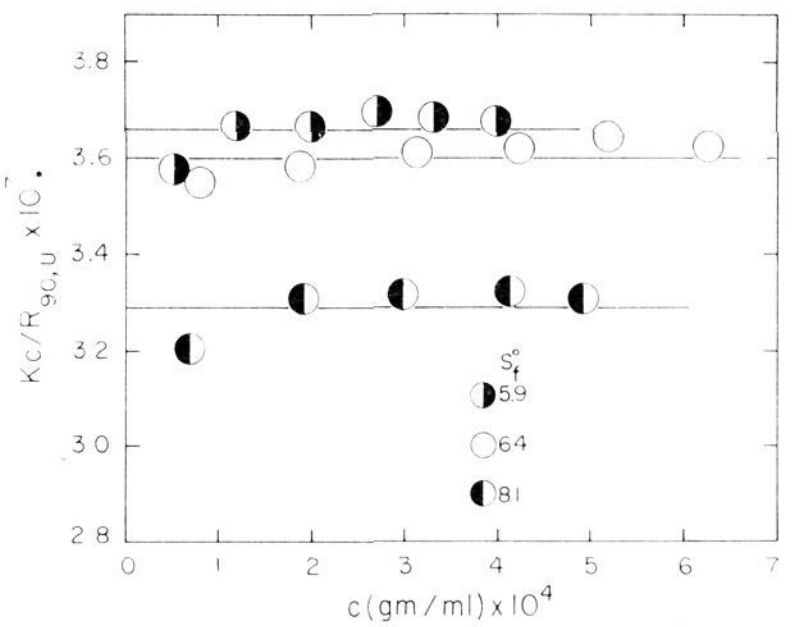

Fig. 2.—Plots of $Kc/R_{90\,u} \times 10^7$ *vs.* concentration for the three fractions all at *p*H 6.7.

(20) S. H. Armstrong, Jr., M. J. E. Budka, K. C. Morrison and M. Hasson, THIS JOURNAL, **69**, 1747 (1947).

(21) W. Heller, *Phys. Rev.*, **68**, 5 (1945).

TABLE II

MOLECULAR PARAMETERS OF THE LIPOPROTEIN FRACTIONS

S_f^0	pH	$\langle \frac{Kc}{R_{90,u}} \rangle \times 10^7$	$\rho^0_u \times 10^3$	$\langle Z \rangle$	s	$P(90)$	M.W. $\times 10^{-6}$	D	S_f^0 spheres[a]	S_f^0 ellipsoids[a]	Axial ratio	2b	2a
5.9	6.7	3.66	5.0	1.034	0.003	0.977	2.77						
6.4	6.7	3.61	4.6	1.030	.002	.980	2.80	205	6.84	6.49	2.16	159	342
8.1	6.7	3.29	5.1	1.035	.002	.976	3.08	212	8.64	8.06	2.42	158	382
8.1[b]	3.0	3.26	4.7	1.042	.002	.972	3.12	213	8.73	8.15			
8.1[b]	4.9	3.32	4.9	1.053	.004	.964	3.10	212	8.68	8.09			
8.1[b]	9.6	3.36	5.2	1.029	.004	.980	3.00	210	8.50	7.93			

[a] These values are corrected for redistribution of salt but not for compressibility of the solution. The former correction is very small, however, amounting to less than 0.2%. [b] This is the fraction which had an S_f^0 8.1 at *p*H 6.7. No ultracentrifugal measurements were made at other *p*H's.

zero on ρ_u^0 denotes the value obtained by extrapolating plots of ρ_u (corrected for the depolarization of the solvent) *vs.* concentration to zero concentration. The plots were linear in all cases. The dissymmetry values reported are the average values, because no concentration dependence of Z was observed. The following column of the table, which gives values of s, the standard deviation from $\bar{Z}$ illustrates this point. Although the dissymmetry values are small the high precision indicated by the small values of s justifies regarding them as significant.

The scattering of one of the fractions, S_f^0 8.1, was measured over the *p*H range 3.0 to 9.6 with the results shown in Fig. 3 and Table II. With the possible exception of the results at *p*H 9.6, this fraction appears to be stable with respect to molecular weight over the range of *p*H covered.

The molecular weights given in Table II were calculated from the equation

$$\text{M.W.} = \frac{(6-7\rho_u^0)/(6 + 6\rho_u^0)}{\langle Kc/R_{90,u} \rangle P(90)} \qquad (7)$$

where $\langle Kc/R_{90,u} \rangle$ is the average value of $Kc/R_{90,u}$. Average values were used in the calculations, because inspection of the scattering data (including that for 45 and 135°) showed that $Kc/R_{\theta,u}$ had no statistically significant tendency to increase with increasing c when the same combinations of neutral density filters were used. The values of $P(90)$ were taken from a plot of $P(90)$ *vs.* Z computed from the particle scattering factor for spherical particles. In this range of Z, $P(90)$ is very nearly independent of the model used and therefore the molecular weights are independent of the assumption of spherical shape.

With molecular weights, flotation coefficients and dissymmetries available it was of obvious interest to examine the internal consistency of these quantities by calculating flotation coefficients from the measured molecular weights, dissymmetries and the "densities" of the hydrodynamic units. The last mentioned quantity was taken to be d_0, the density of zero sedimentation. For the purposes of these calculations we assumed no preferential adsorption of either buffer or water.[22] The densities and the molecular weights were used to calculate the molecular volumes of each species. The diameters, D, of spheres having these volumes are shown in column 9 of Table II. Flotation coefficients for spheres with these densities and volumes are shown in column 10. Using the same molecular volumes we calculated the dimensions of prolate ellipsoids of revolution having the measured dissymmetries using a relation derived by Debye.[23] In Table II, b is the length of the semi-minor axis and a is the length of the semi-major axis determined this way. With these dimensions, flotation coefficients can be calculated using the relation

$$S_f^0 = -\frac{M(1 - d/d_0)}{(Nf/f_0)f_0} \qquad (8)$$

where f/f_0 is the frictional ratio for prolate ellipsoids of revolution calculated using Perrin's formula and f_0 is the frictional factor for spheres. The density of the solution, d, is 1.0630 g./ml.

The results are shown in columns 10–14 of Table II. Evidently, granting the assumption of no preferential adsorption, only the ellipsoidal shape is consistent with the measured flotation rates, densities, dissymmetries and molecular weights. Since the dissymmetries are much too low for rod-like or coil-like molecules of these molecular weights these models are also ruled out.

To illustrate that the molecular weights are virtually independent of the assumption of spherical shape used to calculate $P(90)$, it can be stated that

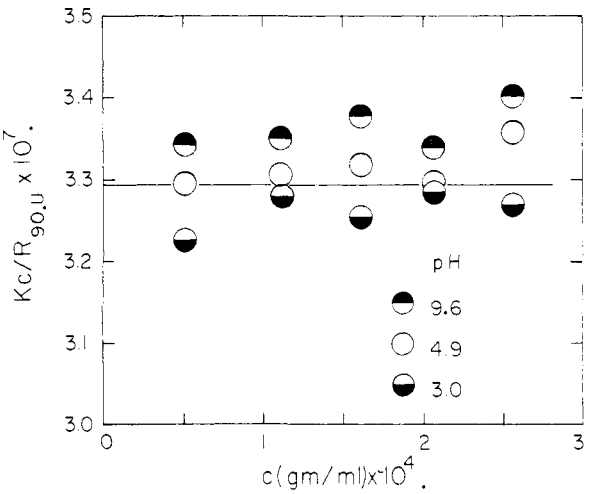

Fig. 3—Plots of $Kc/R_{90,u} \times 10^7$ *vs.* concentration for the S^0_f 8.1 fraction at *p*H 3.0, 4.9 and 9.6. The horizontal line is the average value of $Kc/R_{90,u} \times 10^7$ for this fraction at *p*H 6.7.

(22) S. Katz and H. K. Schachman, *Biochim. Biophys. Acta*, **18**, 28 (1955).

(23) Cited in "Advances in Protein Chemistry," Vol. 6, Academic Press, New York, N. Y., 1951, p. 324.

this factor when calculated for the ellipsoidal models given in Table II differs by less than 0.02% from the $P(90)$ for spheres having the same dissymmetry.

It is difficult to compare our results with some of the earlier studies of human serum lipoproteins. On the basis of ultracentrifugal and diffusion measurements Pedersen[6] obtained the value 2.6×10^6 for what he assumed to be the "hydrated" molecular weight of "X-protein." His estimate assumes that the partial specific volume of the sedimenting unit[22] (which may consist of protein plus associated water and salt) is independent of the salt concentration. This assumption is particularly critical in calculating a molecular weight since the partial specific volume was determined to be 0.97 and an error of only 0.01 in this figure would give an error of about 30% in the molecular weight calculated from the usual Svedberg equation. The light scattering method is, of course, free of this difficulty. In addition more recent ultracentrifugal studies[4,7] have shown that Pedersen's "X-protein" probably consisted of molecules with flotation constants ranging at least from S_f^0 4 to S_f^0 10. Without more information one can only say that there is no necessary disagreement between our results and Pedersen's. Oncley, Scatchard and Brown[24] have obtained an anhydrous molecular weight of 1.3×10^6 for β_1-lipoprotein, a lipoprotein whose ultracentrifugal characteristics are similar to our $S_f^0 \sim 6$ fraction. However, the degree of ultracentrifugal homogeneity of this preparation has not yet been reported, and as the preparation of β_1-lipoprotein involved ethanol fractionation and dialysis (the latter having been shown to effect irreversible changes in serum lipoproteins)[25] a comparison of our results and those of Oncley, Scatchard and Brown appears unwarranted.

Lindgren[7] has calculated minimum molecular weights for some lipoprotein fractions using ultracentrifugal data and assuming the molecules to be spherical. This method of calculation applied to our S_f^0 8.1 and S_f^0 6.4 fractions gave molecular weights which were 10% smaller than those calculated from the light scattering data.

Acknowledgments.—It is a pleasure to thank Professor John W. Gofman for his interest in and encouragement of this work. We also wish to thank Professor Howard K. Schachman and Dr. Verne Schumaker for helpful criticism of the manuscript.

(24) J. L. Oncley, G. Scatchard and A. Brown, *J. Phys. Colloid Chem.*, **51**, 184 (1947).

(25) B. R. Ray, E. O. Davisson and H. L. Crespi, *ibid.*, **58**, 841 (1954).

BERKELEY, CAL.

[CONTRIBUTION FROM THE MEDICINAL CHEMICAL RESEARCH SECTION, RESEARCH DIVISION, AMERICAN CYANAMID COMPANY]

The Use of Phosphorous Acid Chlorides in Peptide Synthesis[1]

BY RICHARD W. YOUNG, KATHRYN H. WOOD, R. JANICE JOYCE AND GEORGE W. ANDERSON

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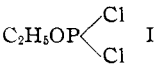
The preparation of peptide derivatives using ethyl dichlorophosphite and ethylene chlorophosphite under varying conditions is reported. The best conditions involve the addition of the chlorophosphite to a suspension or solution of the N-acylamino acid or peptide and amino acid or peptide ester in diethyl phosphite containing triethylamine. The novel use of a trialkyl phosphite as an acid-acceptor in these peptide-forming reactions is described. The methods have been investigated for extent of racemization.

In previous papers of this series the use of diethyl chlorophosphite,[2,3] *o*-phenylene chlorophosphite[2,3] and tetraethyl pyrophosphite[4] for the synthesis of peptides was reported. Since these publications appeared, several reports on the use of these reagents for the synthesis of peptides of asparagine and glutamine,[5] arginine[6] and of oxytocin[7] have been published.

In this paper we wish to report the use of some other chlorophosphites and a study of various conditions under which the reagents may be employed for peptide synthesis.

Continuing the investigation of simple reactive halophosphites, ethyl dichlorophosphite (I)[8] was prepared. This reagent may be prepared in large



I

quantities from ethanol and phosphorus trichloride without using a tertiary base. Both halogens in I are reactive, the compound being both more stable (thermally) and reactive than diethyl chlorophosphite. The reagent may be employed exactly as are the monochlorophosphites; *i.e.*, I may react first with the acylated amino acid to form the "mixed anhydride" II which then may be used to acylate the amino ester (equation 1), or the amino ester may first be converted to the "phosphite amide" III which then may be treated with the

(1) Fourth paper in a series on phosphorus derivatives. Portions of this paper were presented at the September, 1951, Meeting of the American Chemical Society. In order to maintain consistent nomenclature with previous papers in this series, the currently accepted names for phosphorus compounds (*Chem. Eng. News*, **30**, 4515 (1952)) are not being employed.

(2) G. W. Anderson, J. Blodinger, R. W. Young and A. D. Welcher, THIS JOURNAL, **74**, 5304 (1952).

(3) G. W. Anderson and R. W. Young, *ibid.*, **74**, 5307 (1952).

(4) G. W. Anderson, J. Blodinger and A. D. Welcher, *ibid.*, **74**, 5309 (1952).

(5) H. K. Miller and H. Waelsch, *Arch. Biochem. Biophys.*, **35**, 176 (1952); S. J. Leach and H. Lindley, *Aus. J. Chem.*, **7**, 173 (1954); A. Miller, A. Niedle and H. Waelsch, *Arch. Biochem. Biophys.*, **56**, 11 (1955).

(6) G. W. Anderson, THIS JOURNAL, **75**, 6081 (1953).

(7) V. duVigneaud, C. Ressler, J. M. Swan, C. W. Roberts and P. G. Katsoyannis, *ibid.*, **76**, 3115 (1954).

(8) N. Menschutkin, *Ann.*, **139**, 343 (1866).