TABLE VI

TITRATION RESULTS OF B_{12r} WITH OXYGEN

Titration no.	Cobalt taken, mmoles	Oxygen required to reach end-point, mmoles 1st	2nd	Ratio Co:O₂ at end-points 1st	2nd	Diffusion current[a] at end-points, amp. 1st	2nd	"n" Electrons at end-point[b] 1st	2nd
1	4.51	1.11	3.26	4.08	1.38	1.10	2.92	0.63	3.74
2	5.25	1.32	..	4.03	..	1.31	..	.64	..
3	4.23	1.07	3.25	3.95	1.30	1.11	2.80	.65	3.82

[a] Diffusion current at the end-point; corrected for dilution. Data for run 2 were taken beyond the end-point. [b] Calculated by the Ilkovic equation; $n = i_d/(605\ CD^{1/2}m^{2/3}t^{1/6})$ in which i_d is the diffusion current at the end-point; C is the millimolar concentration of B_{12r}: (1) in calculations at the first end-point concentration of B_{12r} was used, (2) in calculations at the second end-point concentration that was equivalent to the B_{12a} dimer was used; D is the diffusion coefficient: (1) in the calculations at the first end-point the diffusion coefficient that of B_{12} (2.95×10^{-6} cm.²/sec.), (2) in the calculations at the second end-point the diffusion coefficient that of B_{12a} dimer (2.33×10^{-6} cm.²/sec.) was used; $m^{2/3}t^{1/6}$ is the capillary constant at -0.1 v. toward S.C.E. (18875 mg.$^{2/3}$ sec.$^{1/6}$).

merged with the wave of the dimer at the second end-point. That is, the behavior is identical to that of B_{12a} during this part of the titration.

The titration of B_{12} with standard oxygen solution showed no interaction between the two substances. The diffusion currents throughout the titration were simply those predicted by the addition of the diffusion currents of the two materials measured separately.

AMES, IOWA

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

The Molecular Weights and Dimensions of Some High-density Human Serum Lipoproteins

BY R. NICHOLS HAZELWOOD[1-3]

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Two high-density lipoprotein fractions were isolated by preparative ultracentrifugation. Sedimentation coefficient, apparent partial specific volume and molecular weight were measured centrifugally. The molecular weights at *p*H 6.7 were 1.75×10^5 for the lipoprotein with partial specific volume 0.867 and sedimentation coefficient 4.65 (HDL-3), and 4.0×10^5 for the lipoprotein with partial specific volume 0.905 and sedimentation coefficient 5.45 (HDL-2). Measurements at various solution densities and concentrations indicated that HDL-3 was a fairly homogeneous fraction.

Introduction

In 1949 Gofman, Lindgren and Elliot[4] showed that the boundary anomaly observed by McFarlane[5] and Pedersen[6] in ultracentrifugation of human serum could be interpreted as a pile up of lipoproteins at the albumin boundary. Subsequent work by Gofman and his associates[7] described the isolation of low density lipoproteins[8] by centrifugal techniques. The isolation and characterization of the high density lipoproteins has been reviewed by deLalla and Gofman.[9]

In a recent paper, Lindgren, Freeman, Nichols and Gofman[10] described a model for lipoprotein structures which featured a core of triglyceride, chloresterol and cholesteryl esters surrounded by an outer shell of high-density lipoproteins. It seemed of interest to characterize some of the high-density lipoproteins more precisely than has been done previously. Klainer and Kegeles[11] have described a modification of the Archibald[12] method for determining molecular weights by approach to sedimentation equilibrium. This modification permits determination of molecular weight from centrifugal data obtained over a period of a few hours.

The molecular weight data may be combined with sedimentation coefficients to calculate frictional factors and axial ratios of the macromolecules.[13] The partial specific volume, which is needed for the calculations, could be determined centrifugally by the method of Katz and Schachman.[14]

Experimental

Materials.—Following the nomenclature of deLalla and Gofman,[9] HDL-1, HDL-2 and HDL-3 are high-density

(1) This paper is taken in part from a dissertation submitted to the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy, January, 1957. Presented at the American Chemical Society Meeting, September, 1957, New York City.

(2) National Science Foundation Predoctoral Fellow, 1954–1955. U. S. Public Health Service, National Heart Institute, Predoctoral Fellow, 1955–1956.

(3) Arthur D. Little, Inc., Cambridge 42, Mass.

(4) J. W. Gofman, F. T. Lindgren and H. A. 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) Low-density lipoproteins are those macromolecules which float to the top of a centrifuge tube in the Spinco Model L Preparative Ultracentrifuge at a solution density of d^{26}_4 1.063 when centrifuged for 13 hr. at 40,000 r.p.m. in the 40.3 rotor. By this definition high-density lipoproteins are lipoproteins which sediment under the foregoing conditions.

(9) O. deLalla and J. W. Gofman, in Glick "Methods of Biochemical Analysis," Vol. 1, Interscience Publishers, New York, N. Y., 1954, p. 459.

(10) F. T. Lindgren, N. K. Freeman, A. V. Nichols and J. W. Gofman, *Proc. Roy. Flemish Acad. Sci. Belgium*, in press.

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

(12) W. J. Archibald, *ibid.*, **51**, 1204 (1947).

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

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

lipoproteins with "hydrated density" of 1.055, 1.075 and 1.145, respectively.

HDL-3 was isolated by the following procedure: Human blood serum was mixed in a 1:1 ratio by volume with a solution of $NaNO_3$ in D_2O (d^{20}_4 1.2425). The solutions were centrifuged 24 hr. at 40,000 r.p.m. in a Spinco Model L Preparative Ultracentrifuge using the 40.3 preparative rotor. The top cc. in the lusteroid sample tube, containing all the low density lipoproteins plus HDL-1 and HDL-2, was pipetted off. The lusteroid tube was sliced with a razor at a point *ca.* 1 cm. below the liquid surface, allowing some solution to run out. The remaining 4 to 5 cc. were poured from the tube and saved for isolation of HDL-3.

The "bottom" solution from the first preparative ultracentrifugation was mixed 2:1 by volume with a $NaNO_3$–D_2O solution of d^{20}_4 1.3925. This mixture was centrifuged 24 hr. at 40,000 r.p.m., and the top 1 cc. in each tube, which contained the HDL-3, was pipetted off. These samples contained *ca.* 0.8 g. of HDL-3/100 cc.

HDL-2 was isolated by a somewhat similar procedure. Human blood serum was mixed 1:1 by volume with an aqueous NaCl solution to bring the final mixture to a solution d^{26}_4 1.063. After centrifugation for 13 hr. at 40,000 r.p.m., the floating lipoproteins were pipetted off, the tube sliced with a razor and the bottom 4 ml. adjusted to a d^{20}_4 1.070 ± 0.001 with D_2O-$NaNO_3$. This solution was centrifuged 36 hr. at 40,000 r.p.m. The top 2 ml. was removed, the tube sliced and the bottom 4 ml. adjusted to d^{26}_4 1.125 with $NaNO_3$–D_2O. This was centrifuged 24 hr. at 40,000 r.p.m. to obtain HDL-2. The HDL-2 floats to the top and was obtained in concentrations of 0.5 to 0.7 g. of HDL-2/100 cc.

In the preparation described above, HDL-1 is obtained in low yield in the second centrifugation. However, the repeated recentrifugations initiate denaturation of HDL-1 similar to the irreversible dialysis changes described by Ray, Davisson and Crespi.[15] Hewitt[16] has also noted this denaturation in recentrifugation of lipoproteins.

After isolation of the HDL-2, the solutions were examined for freedom from other lipoprotein components by analytical ultracentrifugation at 52,640 r.p.m. in the Spinco Model E Analytical Ultracentrifuge. At the density of the samples, HDL-2 will float. In some cases, the flotation analysis showed the presence of another floating component which has a higher flotation rate than HDL-2. Shore[17] has observed this fraction in samples isolated from various individuals. In some cases it may contribute as much as 30% of the HDL-2 peak in an analytical determination. This fraction appears to be a lipoprotein of hydrated density (d^{20}_4) *ca.* 1.081.[17] It will be referred to as HDL-1.5 here for convenience, since it floats between HDL-1 and HDL-2 in analytical ultracentrifugation.

HDL-1.5 was removed from HDL-2 samples in one of two ways. Using a phosphate buffer solution of 0.1 ionic strength, the HDL-2 solution was adjusted accurately to d^{20}_4 1.090. The mixture was centrifuged 24 hr. at 40,000 r.p.m. in the 40.3 preparative rotor. The top 1 ml. contained a mixture of HDL-1.5 and HDL-2; due to salt redistribution and convection, HDL-2 was found in the remaining 5 ml. of each tube. It could be removed by slicing the tube as described and pipetting.

The alternative procedure for removing HDL-1.5 is to dialyze the solution against a buffer made up of 0.025 mole of NaH_2PO_4 and 0.025 mole of Na_2HPO_4 per liter H_2O. This buffer has ionic strength 0.1 and *p*H 6.7 at 20°. Dialysis did not denature the HDL-2 (this point will be discussed further below).

After dialyzing the HDL-2 solution until its density fell below 1.01, the mixture was centrifuged 5.5 to 6 hr. at 40,000 r.p.m. in the 40.3 rotor. The HDL-1.5 was packed into the bottom 1 ml. of the preparative tubes. The top 1 ml. was free of all lipoproteins, and after removal, the 2nd through 5th ml. were pipetted out of the tube.

In either procedure, all the HDL-2 recovered was found to be free of contaminating HDL-1.5 by analytical ultracentrifugation. The concentration obtained was in the range 0.45–0.6 g. HDL-2/100 cc. The samples could be further concentrated by preparative ultracentrifugation.

These isolation procedures were carried out on pooled samples of human serum. In most cases, pools representing 3 ml. of serum per individual were used and each pool contained of the order of thirty to fifty individual samples.

Dialysis.—The crude HDL-2 and HDL-3 solutions could be dialyzed into the phosphate buffer without apparent denaturation, although no attempts were made to protect against oxidation.[18] A "Visking" bag was used and rocking dialysis was carried out with daily changes of the phosphate buffer (μ = 0.1, *p*H 6.7).

Samples were dialyzed with fifteen daily changes of buffer (using *ca.* 100 cc. buffer per 1 cc. solution), then resuspended in D_2O–$NaNO_3$ at the original solution density. There were no changes in sedimentation coefficient other than those due to concentration-dependence. From the seventh to the fifteenth day of dialysis, samples in the phosphate buffer showed no change in sedimentation coefficient within the range of experimental error. By ultracentrifugal criteria there was no apparent change in HDL-2 or HDL-3 due to dialysis.[18]

Refractive Index Measurements.—All measurements of re ractive index were made with a Phoenix Precision Differential Refractometer at 20°. Calibration was done with NaCl or sucrose solutions of known composition. Dry weights of lipoprotein solutions were measured by vacuum drying at 70°.[19]

Density Measurements.—Density measurements were made with a bulb and capillary pycnometer of the Lipkin type, at 20.0°. Triplicate measurements were made on each sample. With a 5-ml. pycnometer, the agreement between measured densities was ±0.00006 g./cc. or better in all cases, while with a 1-ml. pycnometer the internal agreement was ±0.0001 g./cc.

Viscosity Measurements.—Viscosities were measured with an Ostwald–Cannon viscometer of 1-ml. capacity. An electrical timer reading to 0.1 second was used to measure the flow times. The viscometer was immersed in a constant temperature bath at 20.0 ± 0.05°. This particular viscometer had an efflux time of *ca.* 220 seconds for distilled water at 20.0°.

Ultracentrifugal Measurements.—A Spinco Model E Analytical Ultracentrifuge was used for the sedimentation analyses. The optical system used a Wolter phase-plate for the Schlieren diaphragm.[20] Double sector centrifuge cells were used, and the bar angle of the phase plate was constant at *ca.* 43° in all runs. A Spinco temperature controller was used to hold the rotor at constant temperature. The optical constants and rotor dimensions had been determined previously.[4,7,9,16]

All analytical ultracentrifuge runs were made at 52,640 r.p.m., using double sector cells. Photographs were taken at 8 minute intervals. The analysis of the photographs follows the "second moment" method,[21] using the enlarging technique described by Bjorklund and Katz.[22]

All molecular weight measurements were made at 12,590 r.p.m., using double sector cells. In runs of less than 1 hr., the temperature rise without using the controller was less than 2°, and the average temperature during the run could be used in the computations. For all runs longer than 1 hr., the temperature controller was used.

It is necessary to locate the true bottom of the centrifuge cell in determining molecular weights. Spurious reflections from the flat cell bottoms prevent accurate locations of the true position of the cell bottom. To remedy this difficulty, 0.1 cc. of Dow Corning high-vacuum silicone oil was added to the cell. The buffered lipoprotein solution is less dense than the silicone oil and does not appear to move into suspension or solution in the silicone oil. In this way, a trans-

(15) B. R. Ray, E. O. Davisson and H. L. Crespi, *J. Phys. Chem.*, **58**, 41 (1954).

(16) J. E. Hewitt, Thesis, University of California, Berkeley, 1955.

(17) B. Shore, "C- and N-Terminal Amino Acids of Human Serum Lipoproteins," University of California Radiation Laboratory Report UCRL 3489, August 6, 1956. Also personal communication.

(18) Experiments carried out by T. E. Davis, T. Pilkington and B. Shore at this Laboratory also showed that dialysis had no apparent effect on HDL-3, using acetate buffer, NaCl solutions or "Tris" buffer.

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

(20) R. Trautman and V. W. Burns, *Biochim. Biophys. Acta*, **14**, 26 (1954).

(21) R. Trautman and V. Schomaker, *J. Chem. Phys.*, **22**, 551 (1954).

(22) R. Bjorklund and S. Katz, THIS JOURNAL, **78**, 2122 (1956).

parent cell bottom is obtained, and it is relatively easy to measure the position of the true base.[23]

Plates were enlarged (5×) and traced. For the Archibald analysis,[12] the ordinates from the base line to the Schlieren pattern were read at each 0.5 cm. of the tracing. The values of dc/dx were computed from the appropriate constants.

For the method of Klainer and Kegeles,[11] the ordinates were read at each 1 mm. and the values of dc/dx computed. In each case, the value of c_0 (corrected for radial dilution)[21] was determined from an analytical ultracentrifugation at 52,640 r.p.m. Several different values of c_0 were used in runs on HDL-2 and HDL-3, since the possibility existed that the activity coefficients were not unity. A run was also made on an HDL-3 solution in phosphate–D_2O buffer. At this higher density, the compressibility effects discussed by Cheng and Schachman[24] might be detected.

Verification of Methods.—To verify the accuracy of the sedimentation equilibrium methods used, runs similar to those described above were made on albumin. Armour Crystalline Bovine Plasma Albumin, Lot #M66909 was dissolved in the 0.1 ionic strength phosphate buffer and dialyzed against phosphate buffer for 28 hr. with one change of buffer. Final concentration was determined from the ultracentrifuge pattern. The specific refractive increment for the albumin was 0.192 (g./ml.), derived from the data of Perlmann and Longsworth.[25] The partial specific volume used was 0.7343 cc./g., the value given by Dayhoff, Perlmann and MacInnes.[26]

Results and Discussion

Bovine Plasma Albumin.—The concentration of the albumin solution was 0.54 g./100 cc. The solution density was 1.0056 g./cc. A run at 12,590 r.p.m. for 48 hr. was made. Photographs taken at 16 minute intervals for the first 128 minutes were analyzed by the method of Klainer and Kegeles.[11] Subsequent photographs made at 13, 20, 38 and 48 hr. were analyzed by the Archibald method.

The molecular weight determined at the cell bottom by the Archibald method was 67,100. At 48, 64, 96 and 128 minutes, respectively, the Kegeles method gave molecular weights of 67,400, 69,500, 69,500 and 71,000. The lower value at the cell bottom is not unexpected. The equation used for computing molecular weight

$$M = \frac{1}{cx}\frac{dc}{dx}\frac{RT}{(1 - \bar{v}\rho)\omega^2} \qquad (I)$$

implies that ρ, the solution density, is constant at all points throughout the cell during the run. Since some albumin sediments during the run, the density will be decreased at the meniscus and increased at the base of the cell with time. Because M is inversely proportional to $(1 - \bar{v}\rho)$, the values at the meniscus should be higher than those at the base.

Values of M for bovine plasma albumin reported in the literature range from 65,000 to 71,300.[11,27] The results reported here are therefore in good enough agreement to conclude that the techniques used are of suitable accuracy.

HDL-3.—A stock solution of HDL-3, dialyzed for ten days against a fifty-fold excess of the phosphate buffer with daily changes, was used to determine the specific refractive increment and the partial specific volume of HDL-3. Dry weights were determined on aliquots of dilutions with buffer of the stock solution. The specific refractive increments were computed from refractive index measurements. For HDL-3 in phosphate buffer at 20° the measured specific refractive increments were 0.151_4 (g./cc.)$^{-1}$ at 435 Å. and 0.166_1 (g./cc.)$^{-1}$ at 5461 Å. These values are lower than those which can be computed from the data for "α-lipoprotein."[19,22] However, "α-lipoprotein" was isolated by low-salt, ethanol fractionation, and the measurements were made at 25°. Because of these differences, intercomparison of the values is unwarranted.

The partial specific volume of HDL-3 was determined pycnometrically from dilutions of stock solutions of HDL-3 in phosphate buffer. The concentrations were measured refractometrically. Using density data obtained with a 5-ml. pycnometer, following the method of Dayhoff, Perlmann and MacInnes,[26] the partial specific volume at infinite dilution for HDL-3 was found to be 0.867 ml./g.

Fourteen samples of HDL-3 in phosphate buffer, ranging in concentration from 0.1 to 0.8 g./100 cc., were used to measure the concentration dependence of the sedimentation coefficient. The samples were taken from these separate pools of HDL-3, each pool representing 36 individuals. Figure 1 is a plot of $1/s$ *versus* c for these samples. Extrapolation of a least-squares line through the points gave an infinite dilution sedimentation coefficient of 4.415. In the expression

$$s^0/(1 + kc) = s \qquad (II)$$

the value of k was computed to be 15.0 (g./cc.)$^{-1}$.

To check this value of k, HDL-3 was dialyzed against 0.025 M Na_2HPO_4 + 0.025 M NaH_2PO_4 in 99.9% D_2O (d^{20}_4 1.110). From sedimentation velocity data for four runs with concentrations ranging from 0.36 to 0.70 g./100 cc., the value of s^0 calculated from equation II with k = 15.0 was 1.097 ± 0.017.

The value of k is higher by an order of magnitude than values reported for other blood macromolecules.[27] It is consistent, however, with the values for other serum lipoproteins.[9,22] An error in the measured value of dn/dc would affect the value of k but not of s^0. Assuming the limits of dn/dc to be from 0.148 (for pure lipid with no associated protein) to 0.190 (for pure protein),[19] the value of k would still be of the order of 10 to 20.

The "hydrated density" given by deLalla and Gofman[9] is 1.145. This corresponds to an apparent partial specific volume of 1/1.145 = 0.873, which is close to the pycnometric value of 0.867. Katz and Schachman[14] have pointed out, however, that a density corresponding to zero sedimentation in a buffer solution may not represent the true reciprocal $\bar{v}$. Rather, the density may be viewed as a measure of the association of water and/or salts with the macromolecule. The density corresponding to a true reciprocal $\bar{v}$ will be found only when the solution density is varied by addition of a substance which behaves like water toward the macro-

(23) This technique was developed by Prof. Howard Schachman and his group at the Virus Laboratory, University of California, Berkeley.

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

(25) G. Perlmann and L. Longsworth, *ibid.*, **70**, 2719 (1948).

(26) M. O. Dayhoff, G. Perlmann and D. A. MacInnes, *ibid.*, **74**, 2515 (1952).

(27) J. T. Edsall, in H. Neurath and K. Bailey, "The Proteins," Vol. 1B, Academic Press, Inc., New York, N. Y., 1953, pp. 549–726.

TABLE I

ULTRACENTRIFUGAL MOLECULAR WEIGHTS—HDL-3

0.025 M NaH_2PO_4 + 0.025 M Na_2HPO_4, μ = 0.1, pH 6.7

Running time (hr.)	Solvent (+PO_4)	T (°C.)	Concn. HDL-3 (g./100 cc.)	d^{20}_4 (soln.) (g./cc.)	Mol. wt. × 10^{-5} Meniscus	Mol. wt. × 10^{-5} Base	Stand. dev. of mol. wt. × 10^{-3} Meniscus	Stand. dev. of mol. wt. × 10^{-3} Base	Ref. to method
96	H_2O	17.6	0.270	1.0046	..	1.78	..	..	12
44	D_2O	20.0	.330	1.1102	1.81	1.69	..	..	12
2					1.82	1.70	6.1	4.1	11
2	H_2O	21.0	.461	1.0049	1.66	1.74	13.7	4.4	11

molecule. Katz and Schachman indicate that D_2O acts like H_2O in such a system.

Since it was not possible to operate in a salt-free system (lipoproteins behave like euglobulins and are insoluble in salt-free systems), it seemed feasible to study the effect of variations in density with mixtures of H_2O and D_2O keeping the ionic strength low. The Katz and Schachman treatment leads to the conclusion that the density of zero sedimentation would approach the true $1/\bar{v}$ as the ionic strength approaches zero. Two sets of HDL-3

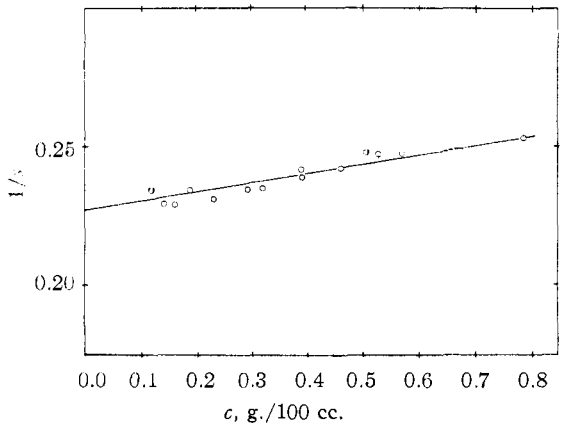

Fig. 1.—$1/s$ *vs.* c for HDL-3 in 0.025 M NaH_2PO_4 + 0.025 M Na_2HPO_4; 52,640 r.p.m., 20.0°.

solutions were prepared, one with ionic strength 0.1 (phosphate buffer), the other with ionic strength 3.0 ($NaNO_3$). With various proportions of D_2O and H_2O, these solvent systems would produce solutions of varying density but constant ionic strength. The sedimentation (or flotation) coefficients were determined at 20° for these solutions and corrected to zero concentration using k = 15.0. From solvent viscosity and s^0 the plots of ηs^0 *versus* density shown in Fig. 2 were made. There is little apparent shift of the density of zero sedimentation with ionic strength. In the $NaNO_3$ system d_0 = 1.148, while in the phosphate system, d_0 = 1.153. These values correspond to apparent $\bar{v}$'s of 0.871 and 0.867 for $NaNO_3$ and phosphate, respectively.

HDL-3 in phosphate buffer had an intrinsic viscosity of 0.033. For prolate ellipsoids, this corresponds to an axial ratio of 3.2 and f/f_0 = 1.126.[27] From viscosity, s^0 and $\bar{v}$, the value of $s_{20,w}$ for HDL-3 was 4.64_7.

The molecular weight of HDL-3 was calculated as 1.74 × 10^5 from $s_{20,w}$ and f/f_0. This molecular weight depends on the choice of a model for molecular shape. A molecular weight determined by the Archibald method does not make any assumptions about molecular shape, and furthermore requires only the partial specific volume. (It can be shown that the Klainer and Kegeles equations apply without knowing the true value of dn/dc.)

Table I summarizes the molecular weight values determined under various conditions by ultracentrifugal transient state methods.[28] The molec-

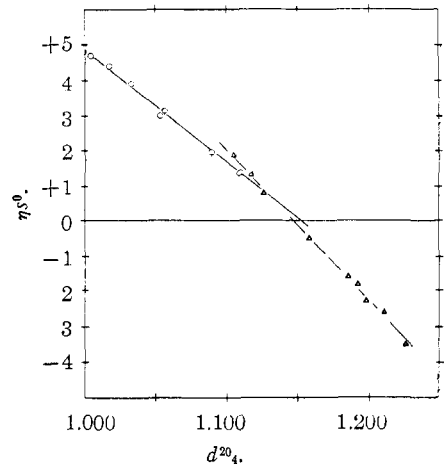

Fig. 2.—ηs^0 *vs.* d^{20}_4 for HCL-3 solutions; 52,640 r.p.m., 20.0°: ———, 0.025 M NaH_2PO_4 + 0.025 M Na_2HPO_4 in mixtures of D_2O and H_2O; - - - -, 3.0 M $NaNO_3$ in mixtures of D_2O and H_2O.

ular weights found by the Archibald method were determined by extrapolation of a molecular weight *versus* time plot. The values determined in D_2O solution indicate that there is little polydispersity of $\bar{v}$. At a density of *ca.* 1.0, a range of 1% in $\bar{v}$ might cause a variation of 1 × 10^4 in molecular weight, while at density *ca.* 1.1, the same range in $\bar{v}$ would cause a variation of 2.5 × 10^4.

It seems reasonable to conclude that the molecular weight of HDL-3 determined by ultracentrifugal transient state methods is 1.75 ± 0.9 × 10^5. The agreement with the value from viscosity and sedimentation coefficient must be regarded as fortuitous.

HDL-2.—Two separate samples of HDL-2 were isolated, with final isolation achieved by preparative ultracentrifugation at d^{20}_4 1.090. The dialysis into 0.1 ionic strength pH 6.7 phosphate buffer produced no evidence of degradative changes, by the criteria described above.

Concentrations were determined using the specific refractive increment found for HDL-3. This assumption probably is not correct, but for purposes of this work the value is sufficiently accurate.

(28) The term "transient state methods" was suggested by an anonymous referee to describe these methods involving approach to sedimentation equilibrium.

As discussed above, only the value of k is affected by errors in dn/dc.

Figure 3 is a plot of $1/s$ *versus* c for HDL-2 in phosphate buffer. At 20.0°, $s^0 = 5.11_2$ and $k = 16.3$ (g./cc.)$^{-1}$. Because of the possible error in concentrations, due to use of the dn/dc value for HDL-3, k may be slightly high. In any event, application of statistical significance tests ("t" test) showed that k_{HDL-2} was not significantly different from k_{HDL-3}. It is sufficient to state that k is of the same order of magnitude for both lipoproteins. The value of s^0_{HDL-2} is significantly different from s^0_{HDL-3}.

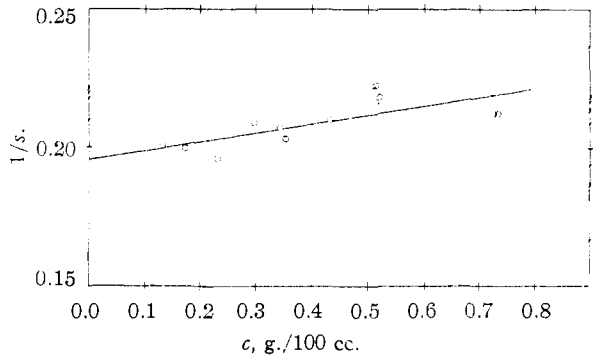

Fig. 3.—$1/s$ *vs.* c for HDL-2 in 0.025 M Na_2HPO_4 + 0.025 M Na_2HPO_4; 52,640 r.p.m., 20.0°.

The "hydrated density" given by deLalla and Gofman[9] is 1.075. This measurement was made with 3 M $NaNO_3$ solutions in H_2O–D_2O mixtures. Since HDL-2 sedimented in 0.05 M phosphate in H_2O–D_2O at d^{20}_4 1.090, the effect described by Katz and Schachman[14] seems significant for HDL-2. Shore[17] found a density of zero sedimentation d^{20}_4 1.093 in NaCl solutions of 1.0–1.5 M in H_2O.

Samples of stock solution of HDL-2 in H_2O-phosphate were diluted 2:1 (v./v.) with mixtures of D_2O and H_2O buffers, at constant phosphate concentration. The density of each sample was measured at 20.0° and the sedimentation coefficient determined. Figure 4 is a plot of ηs^0 *versus* d^{20}_4. All sedimentation coefficients were corrected to infinite dilution with eq. II using $k = 16.3$. Since concentrations were all *ca.* 0.15 g. HDL-2/100 cc., this correction was quite small.

The density of zero sedimentation was 1.105 g./cc. This corresponds to an apparent $\bar{v} = 0.904_8$ (g./cc.)$^{-1}$. In the calculations herein, $\bar{v} = 0.905$ was used. From s^0, $\bar{v}$ and solvent viscosity, $s_{20,w} = 5.44_6$.

Molecular weights were determined at 20.0° at a centrifuge speed of 12,590 r.p.m., at two different concentrations. Analysis of the data was by the method of Klainer and Kegeles.[11] It appeared, that under the experimental conditions, HDL-2 represented some sort of upper limit of application of this technique. At concentrations of the order 0.5 g. HDL-2/100 cc., the Schlieren image ran off the screen at the base of the cell, while at concentrations of the order 0.15 g. HDL-2/100 cc., the Schlieren image at the meniscus was not sufficiently elevated from the base line to permit accurate measurements of Δn. It is not practical to change the bar angle of the Schlieren system to make alternate photographs of base and meniscus during a run. The low-speed attachment for the Spinco ultracentrifuge would permit a reduction in centrigugal force sufficient to give usable data from both meniscus and base in one run. Such equipment was not available in this Laboratory, however.

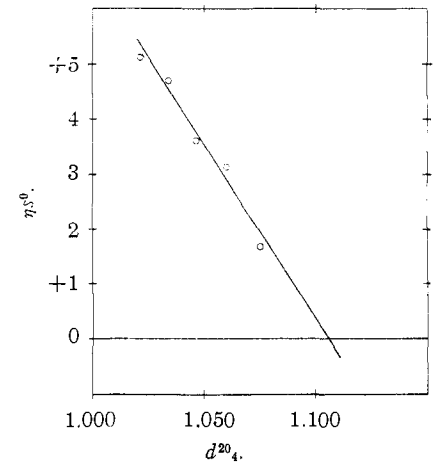

Fig. 4.—ηs^0 *vs.* d^{20}_4 for HDL-2 in 0.025 M NaH_2PO_4 + 0.025 M Na_2HPO_4 with varying proportions of D_2O and H_2O.

At concentration 0.5 g. HDL-2/100 cc., the molecular weight at the meniscus was 4.1×10^5, with a standard deviation of 8.3×10^3. At concentration 0.15 g. HDL-2/100 cc., the molecular weight at the base of the cell was 3.9×10^5 with standard deviation 13.8×10^3. The values are not significantly different by statistical criteria. The best value of molecular weight of HDL-2 is $4.0 \pm 0.3 \times 10^5$.

Table II is a summary of the measured and calculated values for HDL-2 and HDL-3. No data are presented for HDL-1.5, since it was not possible to isolate the material in the course of this work. As discussed by Bjorklund and Katz,[22] intercomparison of results obtained on other lipoproteins isolated by other techniques is not warranted.

TABLE II

MOLECULAR PARAMETERS FOR HDL-2 AND HDL-3

	HDL-2	HDL-3
$s_{20,w}$	5.45	4.65
$\bar{v}$	0.905	0.867
f/f_0	1.187	1.126
b/a	4.1	3.2
Mol. wt.	4.0×10^5	1.75×10^5
Dimensions (assuming prolate ellipsoid), Å.	90 × 365	40 × 130
$[\eta]$	.....	0.033

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