# Serum Lipoprotein Distribution, Flotation Rates and Protein Analysis

F. T. LINDGREN, N. K. FREEMAN, A. M. EWING and L. C. JENSEN,
Donner Laboratory, Lawrence Radiation Laboratory, University of California, Berkeley, California

## Abstract

Flotation rates of the major $S_f$ 0–12 low-density lipoprotein component in human serum may be calculated from ultracentrifuge data utilizing two computer programs. One program calculates a classical moving boundary uncorrected flotation rate by a best fit straight line for the points ($\ln x_i$, $\omega^2 t_i$). The other program permits correction for concentration dependence and correction to standard reference conditions. Preliminary application of these methods indicates significantly greater flotation rates in normal human females than in males for the 35–49 year age group.

The significance of interrelationships between the serum lipoprotein spectra, the serum lipids and the serum proteins is considered, resulting in the development of a revised method of measuring serum proteins by precision refractometry. The refractometric measurement is corrected in accordance with (any of various) lipid measurements in order to account for the contribution of lipoproteins to the total refractive increment. Such a technique, giving potentially a very accurate protein measurement, has application in screening studies involving abnormalities of both serum lipoprotein and serum protein metabolism.

## Introduction

ALTHOUGH SERUM LIPOPROTEINS have been analyzed ultracentrifugally for well over a decade (1), the recent availability of high-speed computers of large memory permits more extensive lipoprotein analyisis. Such a method for computer analysis of the low- and high-density lipoprotein spectra is presented in detail elsewhere (2). However, this technique can be used in combination with additional computer programs for both the calculation of flotation rates and for serum protein analysis. These serum parameters may be of importance in evaluating more fully the metabolic role of the serum lipoproteins and the serum lipids they transport.

## Experimental

All preparative and analytical ultracentrifugal analyses were made according to the procedure described by Ewing et al. (2). Measurements from the schlieren curve for use in calculating flotation rates were made on a fivefold enlargement of the original film.

Observed flotation rates are dependent on lipoprotein concentration, on temperature, and on density of the solution. As temperature and density are carefully controlled, the major correction is due to concentration. We are able to approximate these corrections for the peak flotation rate using a special computer program (2). The correction for concentration dependence is made for each lipoprotein class by the relationship $F = F_0(1-KC)$, using a value of $K = 0.89 \times 10^{-4}$ $(mg/100\ ml)^{-1}$. Additional corrections to the standard conditions of temperature and density ($\rho_s$ and $\eta_s$ are the density and viscosity of 1.745 molal NaCl at 26C) are made yielding a corrected $S_f^\circ$ [1] value from the following relationship: $S_f^\circ = F_o(\rho_s - \sigma)\eta/(\rho - \sigma)\eta_s$, where $\rho$ and $\eta$ are the actual density and viscosity of the solution in analytic run and $\sigma$ is the hydrated density of lipoprotein class under consideration. Although this program is primarily concerned with the computation of concentrations for the various flotation rate classes of lipoproteins using data from schlieren films, it will also accept as input data a measure of peak position from the base-of-cell as observed in the 30′ up-to-speed (UTS) schlieren frame. Using this value, the program computes the uncorrected flotation rate of the peak, an $S_f^\circ$ rate corrected for the effect of concentration and density, and the ratio of corrected to uncorrected rate.

Provision also is made in the program for correcting run temperatures which differ from the standard 26C; however, there are difficulties in evaluating temperature, particularly during the early period of the run. Therefore, temperature correction at this time is based on mean rotor temperature as measured before and immediately after the analytic run. With analysis at 52,640 rpm, the actual rotor temperature during the run is approximately 0.7C lower than this value, primarily the result of adiabatic cooling of the rotor (3) during acceleration. Because of the uncertainty of real cell temperature, corrections for these cooling effects have not been made in our present data. Since the density of the run is normally quite close to the standard density of 1.0630 g/ml, the usual effect of the density correction is relatively small. The slowing effect of concentration on the observed flotation rate, however, may be as much as one Svedberg. Thus, the corrected $S_f^\circ$ rate of the peak is substantially greater than the observed flotation rate.

Although the program also corrects observed concentrations for the Johnston-Ogston (4) effect, no attempt has been made in the current version to incorporate this correction in the corrected peak rate. Since this correction distorts the observed schlieren pattern, it can, potentially, change the peak position.

### Moving Boundary Flotation Rate

Since the above method is based on a single measurement of peak position and is very sensitive to the correct selection of base-of-cell position, we have also developed a program to analyze peak flotation rates by the classical moving boundary method (5,6). This programs accepts data from several schlieren frames. In this case, peak position is measured not from the base-of-cell but from the knife edge (standard refer-

[1] $S_f$ rate is defined as Svedbergs of flotation, measured at 26C in a medium of 1.745 molal NaCl ($\rho/26 = 1.0630$ g/ml). Flotation rates corrected for the effects associated with concentration dependence are indicated by the symbol $S_f^\circ$. F rate denotes a flotation rate measured at any other density.

ence position). Using these measured distances $x_i$ and the times of the associated frames $t_i$, the program computes the best fit (least squares) straight line for the points ($\omega^2 t_i$, ln $x_i$). As shown in Figure 1, the slope of this line is the uncorrected flotation rate of the peak. Actually, such points would be fit better by a slightly curved line. In general, if best-fit slopes for early and late frames from the same run are separately computed, the early frames show a slightly higher flotation rate. Thus, the flotation rate computed from the 8-, 14-, 22-, and 30-min UTS frames is about 0.2 Svedbergs faster than that computed using the 30-, 48-, and 64-min frames. Such time dependent factors as increasing radial concentration and slight differences in density within the cell might be expected to cause slowing of about this magnitude. In addition, the behavior of a continuous distribution of inhomogeneous macromolecules, such as the low-density lipoproteins, is incompletely defined.

This method also permits the program to solve for the base-of-cell position. Regardless of the frames used, and allowing for some uncertainty about the equivalent UTS (up-to-speed) time, the calculated base-of-cell position can be displaced from the observed base-of-cell by as much as 0.2 mm in the direction of the knife edge. A part of this displacement may be attributed to cell tilt (7), estimated to be the order of 0.1 mm. In addition, the presence of an inconspicuously small amount of sedimenting lipoprotein or protein can give rise to a false base-of-cell that moves out slightly from the true base-of-cell as a function of time. As a consequence of this discrepancy, the uncorrected flotation rates computed by the best-fit moving boundary method may be significantly faster than those computed from the single measurement from base-of-cell in the 30′ UTS frame. To approximate corrected best-fit flotation rates we multiply the uncorrected best-fit rate by the corrected-to uncorrected ratio computed by the first program.

A typical calculation utilizes data from 3 to 6 schlieren frames. As shown in Figure 2, the program also computes the deviation of each point from the best fit straight line and then recalculates the slope by successively omitting the most deviant value until only two points remain. Such presentation of the data allows frequent detection of both reading errors and subtle cell leaks.

## Results and Discussion

We have used these computer programs to analyze schlieren data from small normal nonfasting male and female populations. Since not all serum samples

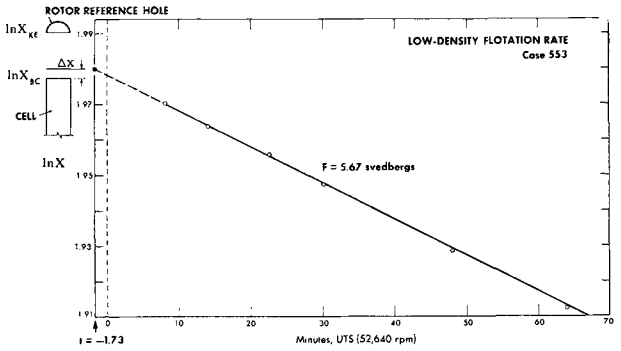


FIG. 1. Uncorrected flotation rate calculated from the 8-, 14-, 22-, 30-, 48-, and 64-min photographs after reaching full speed (52,640 rpm). The acceleration time of 5.2 min is approximately equivalent to 1.73 min at full speed.

FILM 16573D, FACTOR = 11.64

| FRAME | DX(CM) | T*W**2 | LN(X) | D LN(X) |
|---|---|---|---|---|
| 5 | 1.84 | 0.001459 | 1.96864 | −0.00037 |
| 6 | 2.33 | 0.002552 | 1.96274 | −0.00022 |
| 7 | 2.93 | 0.004011 | 1.95548 | 0.00057 ← |
| 8 | 3.62 | 0.005470 | 1.94705 | 0.00021 |
| 9 | 5.10 | 0.008751 | 1.92874 | 0.00003 |
| 10 | 6.40 | 0.011668 | 1.91238 | −0.00022 |

BEST FIT FLOTATION RATE = −5.525

| BASE OF CELL/ | UTS | DX(CM) | ABS R |
|---|---|---|---|
| | 1.733 | 0.99 | 7.2341 |
| | 1.833 | 0.98 | 7.2349 |

CALC. #1

| FRAME | DX(CM) | T*W**2 | LN(X) | D LN(X) |
|---|---|---|---|---|
| 5 | 1.84 | 0.001459 | 1.96864 | −0.00018 |
| 6 | 2.33 | 0.002552 | 1.96274 | −0.00005 |
| 8 | 3.62 | 0.005470 | 1.94705 | 0.00033 |
| 9 | 5.10 | 0.008751 | 1.92874 | 0.00010 |
| 10 | 6.40 | 0.011668 | 1.91238 | −0.00019 |

BEST FIT FLOTATION RATE = −5.509

| BASE OF CELL/ | UTS | DX(CM) | ABS R |
|---|---|---|---|
| | 1.733 | 1.01 | 7.2326 |
| | 1.833 | 1.00 | 7.2333 |

CALC. #2

FIG. 2. Data format for computer flotation rate calculations showing complete input data and best-fit flotation rate and base-of-cell output data.

can be run at the same time after drawing, we have checked the stability of the low-density peak flotation rate by different runs from the same sample over a period of 43 days. The corrected best-fit data shown in Table I show no observable deterioration of the sample in so far as flotation rate of the major $S_f$ 0–12 peak is concerned. In two normal male and female populations, each consisting of 16 individuals between the ages of 35 and 49, we have computed mean corrected best-fit flotation rates. Table II presents the means, standard deviations and standard errors for these small male and female populations. The difference in $S_f^\circ$ values, 6.32 Svedbergs for the males and 7.34 for the females, is significant at the 1% level (by the t-test). Since $S_f$ rates in this region of the lipoprotein spectra are more sensitive to slight differences in lipoprotein hydrated density than to differences in molecular weight, this difference in rate may suggest a difference in chemical composition. We estimate that, in this region, a 3% increase in glyceride or a decrease of 2% in protein (by weight) would cause an increase in $S_f$ rate of about one Sved-

TABLE I

Stability of Flotation Rates, Serum Sample No. 573

| Run | Lipoprotein fraction | Age (days) | $S_f^\circ$ value (Svedbergs) | Remarks |
|---|---|---|---|---|
| 1 | $S_f$ 0–$10^5$ | 7 | 6.42 | |
| 2 | $S_f$ 0–$10^5$ | 7 | 6.43 | |
| 3 | $S_f$ 0–$10^5$ | 8 | 6.20 | Student lab |
| 4 | $S_f$ 0–$10^5$ | 8 | 6.19 | Student lab |
| 5 | $S_f$ 0–20[a] | 11 | 6.32 | Two prep runs (minimize solvent back flow) |
| 6 | $S_f$ 0–20[a] | 12 | 6.28 | Two prep runs (minimize solvent back flow) |
| 7 | $S_f$ 0–$10^5$ | 12 | 6.23 | Prep rerun (after remixing 1st prep run) |
| 8 | $S_f$ 0–$10^5$ | 13 | 6.15 | Prep rerun and analytic rerun |
| 9 | $S_f$ 0–$10^5$ | 13 | 6.18 | Prep rerun (after remixing 1st prep run) |
| 10 | $S_f$ 0–$10^5$ | 26 | 6.32 | |
| 11 | $S_f$ 0–$10^5$ | 26 | 6.55 | |
| 12 | $S_f$ 0–$10^5$ | 28 | 6.25 | Separates $S_f$ 0–20, $S_f$ 20–$10^5$, then remix |
| 13 | $S_f$ 0–20[a] | 28 | 6.13 | Two prep runs (minimize solvent back flow) |
| 14 | $S_f$ 0–$10^5$ | 35 | 6.30 | Pool two fractions (stored 11 days) |
| 15 | $S_f$ 0–$10^5$ | 43 | 6.50 | |
| 16 | $S_f$ 0–$10^5$ | 43 | 6.41 | |

Mean $S_f^\circ$ rate (16 samples) = 6.30 ± 0.12 Svedbergs.

[a] No VLD ($S_f$ 20–$10^5$) lipoproteins present.

TABLE II
Flotation Rates of Sf 0–12 Component, 16 Cases, Ages 35–49 Years

| | Mean | SD | SE |
|---|---|---|---|
| Normal males | 6.32 | 0.92 | 0.24 |
| Normal females | 7.34 | 0.75 | 0.19 |

berg. Previous studies by Oncley et al. (8) have yielded corresponding experimental differences in $S_f$ rate and protein content within subfractions of the $S_f$ 0–12 lipoprotein class. Since the lipid compositions of the male and female $S_f$ 0–20 class lipoproteins are closely similar (2), it seems more likely that these $S_f^\circ$ rate differences may be the result of differences in protein content.

Our values for flotation rates are somewhat lower than those reported by Mills and Wilkinson (9), who in their study of English males obtained peak $S_f$ rates of 7–10 Svedbergs. However, their method of correction for concentration dependence and their constant for the flotation versus concentration correction differed from ours.

## Lipoprotein Distribution and Serum Protein Analysis

At this point we would like to extend our perspective beyond the lipoproteins and to consider potential relationships between the other principal serum macromolecules, i.e., the serum proteins. These relationships would be certainly of interest in the full discussion of the metabolic role of lipids. A logical question arises as to how one can accurately measure serum protein or those protein macromolecules exclusive of the lipoproteins. In the past, total serum protein usually has been measured by a variety of techniques, including both chemical and physical methods. The former include measurement of total protein nitrogen by modifications (10) of the original Kjeldahl procedure or by such colorimetric reactions as the Folin reagent developed by Lowry et al. (11), or the biuret reagent described by Gornall et al. (12). These chemical methods have certain limitations; for example, each class of protein may have slightly different nitrogen or tyrosine and tryptophane content. Further, the analytical methods themselves are difficult to carry out with accuracy and reproducibility. On the other hand, physical methods for quantitative serum protein analysis including electrophoretic (13) and ultracentrifugal (14) techniques usually lack either precision and/or simplicity.

## Serum Refractometry

For over 60 years, the serum proteins have been determined by refractometry (15). This measurement, however, includes both the serum small molecule background as well as the total content of serum lipoprotein macromolecules. Because the small molecule background is nearly constant, and a valid correction can be applied, this method actually measures quite accurately the total serum macromolecules. The measurement of serum proteins by refractometry has recently been reviewed extensively by Naumann (16), yet the influence of serum lipoproteins on the accuracy of this method for total serum protein measurement has not been fully discussed. For instance, serum lipoprotein content contributes considerable variability to the serum macromolecular measurement. Also, lipoprotein specific refractive increments (17–19) are different for each lipoprotein class and are substantially lower than those of the uncomplexed proteins (20).

Since lipoproteins are measured in salt solutions of various densities and refractive indices, there is a slight complication in evaluating the refractive index contribution of the total lipoprotein spectra as measured by total serum refractometry. What is required is knowledge of the change in the specific refractive increments of each lipoprotein class with change in the refractive index of the reference medium. For macromolecules of small particle-size, the relationship is approximately given by the familiar formula (21, 22): $dn/dc = 3n_1(m^2 - 1)/2D(m^2 + 2)$ where D is the density of the anhydrous macromolecule, $n_1$, the refractive index of the solvent, and m is the ratio of the refractive index of the solute to the solvent. Figure 3 shows the almost linear form of this relationship in which the various curves are slightly displaced (or rotated) to fit through available experimental specific refractive increment values and the calculated or estimated refractive index of the anhydrous macromolecule. Thus, from these relationships, it is possible to convert the measured refractive increment of each lipoprotein class to the appropriate value it would have in a serum background environment.

We have made the above calculations for our normal male and female lipoprotein data. Very low-density and low-density lipoprotein values were measured by precision refractometry (19) at 5893 Å and high-density data evaluated from ultracentrifugal analysis (2) at 5460 Å. Thus, for very low-density lipoproteins ($S_f$ 20–$10^5$), a specific refractive increment of 0.00158 Δ n/g/100 ml (19) as measured in 0.199 molal NaCl is used. For low-density lipoproteins ($S_f$ 0–20), a specific refractive increment of 0.00154 (18) as measured in 1.745 molal NaCl is used with correction to an anticipated value of 0.00166 Δ n/g/100 ml in 0.199 molal NaCl. High-density lipoproteins were measured in 0.199 molal NaCl plus 2.771 molal NaBr using a specific refractive increment of 0.00149 Δ n/g/100 ml. Similarly, corrections were made using a value of 0.00173 Δ n/g/100 ml for high-density lipoproteins in 0.199 molal NaCl. Because of the uncertainty of the high-density specific refractive increment, no correction was made for dispersion.

Extrapolation to 5893 Å of the Perlmann and Longsworth (20) specific refractive increment data for the serum proteins (at 5770 Å, and 5460 Å and 4358 Å) was made using a best-fit Cauchy plot. From this it was estimated that the refractive increment of the serum proteins (such as serum albumin and

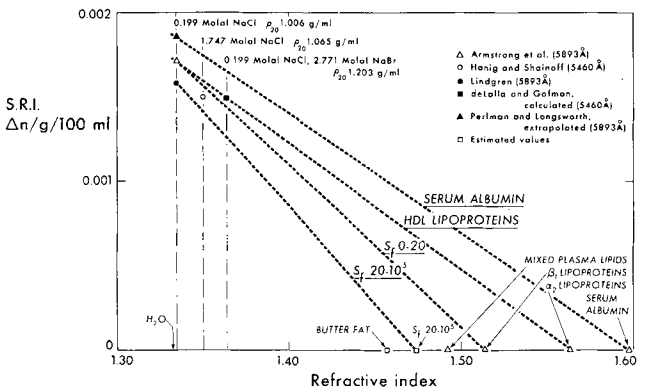


FIG. 3. Relationship between specific refractive increment of serum proteins and lipoprotein classes and the refractive index of the reference media.

globulin) would be approximately 1% lower at 5893 Å. Accordingly, we have used a specific refractive increment of 0.00185 Δ n/g/100 ml for calculations of total serum protein content.

The refractive index contribution of the serum small molecule background was evaluated from the 2nd ml infranatant of an unaltered serum preparative run with corrections for sedimentation of the small molecule solutes (19). Subtracting the corrected lipoprotein and serum background refractive increments from the total serum refractive increment (5893 Å) measured by precision refractometry (23) yields the total serum protein macromolecules, whose densities are greater than 1.20 g/ml, excluding essentially all the known lipoproteins. Such a measurement, of course, includes complex proteins such as mucoproteins and any lipoproteins that might exist in the density region of 1.20–1.33 g/ml. The amounts of both are normally very small.

## Results and Discussion

The mean values and standard deviations of serum protein concentrations determined from our refractometric data, together with the calculations by other formulas are given in Table III. For the purposes of clarity and comparison with other serum protein data, we have defined three quantities: these are total serum macromolecules, total serum protein (excluding all the known lipoproteins) and total real protein (which includes the protein moieties of the serum lipoproteins). Thus, these values illustrate the some 900 mg% contribution of the lipoproteins to the total serum macromolecules, approximately 300 mg% of which is lipoprotein protein. Within our small population, no significant differences were observed between males and females in either total serum protein or total real protein. We have broken our data into these components because of the procedure for calibration of the refractometric method (16). The usual calibration has been to compare refractive increment above the water reference against, either total real serum protein evaluated by conventional chemical methods, or a standard protein solution, such as serum albumin. The former procedure ignores the contribution of nonprotein moieties of the serum macromolecules, particularly the lipoproteins of density less than 1.20 g/ml. On the other hand, the latter, neglecting differences in specific refractive increment, would approximate the serum content of total macromolecules. Table III also presents the usual calculations, showing the wide discrepancies observed between the standard regression formulae (24–26).

Figure 4 shows all the components measured by serum refractometry, together with the mean refractive increment values and their standard deviations for our small populations. It is apparent that neither the total serum proteins (excluding the known lipoproteins) nor total real protein can be measured accurately by serum refractometry unless the total content of serum lipoproteins is considered. Although

TABLE III

Serum Protein Concentrations
16 Males, 16 Females, 35–49 Years

| Method | Males | Females |
|---|---|---|
| Total serum macromolecules | 8,158 ± 462 | 8,133 ± 465 |
| Total real protein | 7,525 ± 415 | 7,553 ± 482 |
| Total serum protein | 7,263 ± 420 | 7,237 ± 485 |
| Sunderman[a] (1944) | 6,882 ± 430 | 6,797 ± 447 |
| Drinkman-McKeon[a] (1962) | 6,795 ± 287 | 6,738 ± 298 |
| Bausch and Lomb[a] (1963) | 7,831 ± 442 | 7,744 ± 459 |

[a] Usual Δ n calculations.

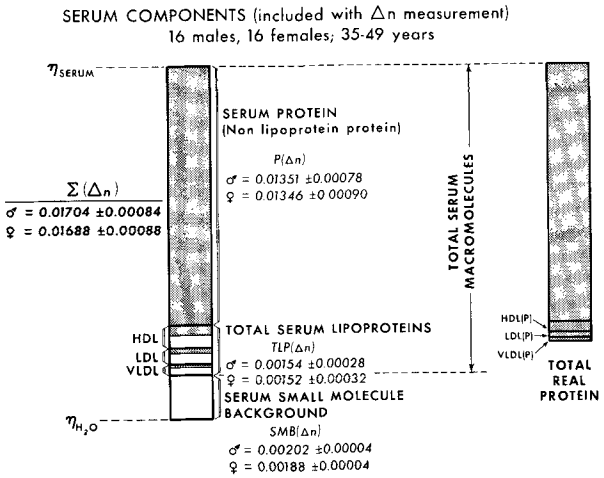


FIG. 4. Serum components included with Δ n measurement giving mean values ± standard deviations for 16 males, 16 females, 35–49 years.

the usual differences in serum lipoprotein content between the males and females were observed (27,2), it is interesting to note the significantly ($P > 0.01$) elevated serum small molecule background in the male group. This is of additional interest in that the standard deviations of these values are only slightly higher than the relative accuracy of the precision refractometry itself.

The individual calculation for total serum protein (excluding the lipoproteins) are plotted in Figure 5, along with the regression formulas of Sunderman (24), Drinkman-McKeon (25) and Bausch and Lomb (26). Gross discrepancies for total serum protein amounting to the order of 1,000 mg%, as calculated by these regression formulas, are readily apparent. It is therefore understandable why in the past this refractive index method for serum protein determination has been in a state of controversy, and has not received widespread acceptance as a reliable analytic procedure. However, considering the accuracy of precision refractometry of serum as well as the accuracy of the calculated contributions of lipoproteins to the total serum refractive increment, this improved version of serum refractometry should have great potential accuracy. It further would have the stability inherent in a physical rather than a chemical measurement. Here our anticipated accuracy, based on

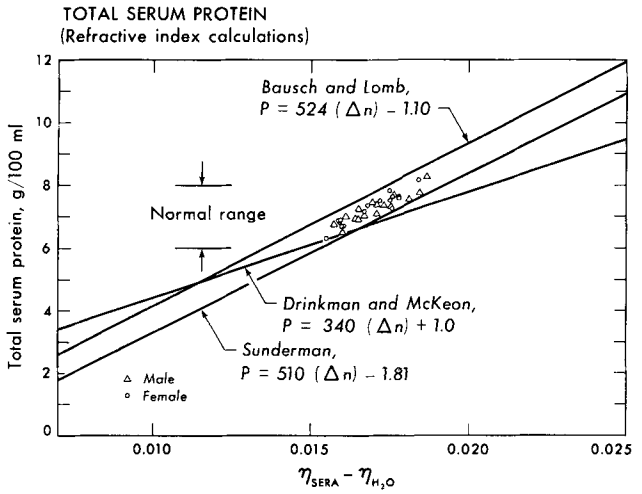


FIG. 5. Individual total serum protein values for our male and female populations showing the three commonly used regression formulae.

the limiting factor of reproducibility of lipoprotein analysis (a standard error of measurement of approximately ±5%), should be the order of 50–100 mg% for total serum proteins (exclusive of the lipoproteins). Such factors as small differences in specific refractive increment among various classes of serum proteins (17,20), which would be the order of 1%, also must be considered. However, such a revised measurement of total serum protein with a potential error in the neighborhood of 1–2% represents substantial improvement over the refractometric method whose several regressive formulas differed by as much as 15% within the normal range of serum values!

If it were necessary to isolate and measure lipoproteins either by refractometry or the ultracentrifuge to make all these corrections, (which also has been done with a small computer program) such a method of defining total serum macromolecules, total real protein and nonlipoprotein protein would perhaps have limited application. However, lipoproteins and lipoprotein Δ n may be estimated adequately for this correction from serum lipid measurements. In general, either total serum lipid, or a combination of serum triglyceride and cholesteryl ester values may be used to calculate total lipoprotein Δ n. However, for the females, inclusion of serum phospholipid tends to improve the calculation slightly. Table IV presents regression formulas for total lipoprotein Δ n as calculated from total serum lipid and from combinations of serum triglyceride, serum cholesteryl ester and serum phospholipid. The correlation coefficients refer to the relationship between the total lipoprotein Δ n obtained from actual lipoprotein measurement and the derived Δ n value calculated from total lipid data. It is to be understood that these values are slightly higher than the correlation coefficients to be expected when the regression formula is used to predict Δ n in another population. This is because the coefficients were calculated by a least squares method to optimize the relationship of the derived Δ n variable to the calculated Δ n value (within one population). Random variation of values would therefore tend to lower the correlation when the same regression coefficients are used for prediction in another population. It is evident, however, that lipoprotein Δ n can be estimated with considerable accuracy from these serum lipid parameters. Perhaps the most promising application of this method may be its value as a screening test for possible lipid or protein abnormalities. This would be especially true for small animal studies because of the very limited amounts of serum required for analysis. Thus, serum refractometry can be done on one drop of serum and the serum cholesteryl ester and triglyceride can be done simultaneously by the high resolution infrared spectrometry technique of Freeman (28) on as little as 0.050 ml of serum. Further, the infrared lipid analysis has been partly automated (29) and is sufficiently simple and reproducible that complete automation appears to be possible.

In order to utilize serum refractometry to measure accurately the nonlipid-containing serum macromolecules, it is necessary to measure or estimate the serum lipoprotein spectra. This revised technique may become important because of possible interrelationships between serum protein and lipid metabolism. In the present preliminary study of normal males and females, however, only low order nonsignificant correlations were observed. Yet, in many of the known hyper- and hypoproteinemias (16), where both lipid and protein abnormalities may exist, there indeed may be fruitful application of precision serum refractometry in combination with measurement or estimation of the serum lipoproteins.

TABLE IV

Regression Formulas for Total Lipoprotein Δ n [a]

| | |
|---|---|
| **Normal males** | |
| Δ n (TLP)=1.704K (TGL)+0.00015 | r=0.96 |
| Δ n (TLP)=1.248K (STG)+3.605K (SCE)+0.00011 | r=0.98 |
| Δ n (TLP)=0.860K (STG)+1.803K (SCE)+ 3.454K (SPL)−0.00018 | r=0.99 |
| **Normal females** | |
| Δ n (TLP)=1.867K (TGL)+0.00017 | r=0.95 |
| Δ n (TLP)=2.558K (STG)+3.776K (SCE)+0.00015 | r=0.90 |
| Δ n (TLP)=0.335K (STG)+2.269K (SCE)+ 3.690K (SPL)−0.00020 | r=0.95 |

[a] Lipid abbreviations are: TLP (total lipoprotein), TGL (total gravimetric lipid), STG (serum triglyceride), SCE (serum cholesteryl ester) and SPL (serum phospholipid). Lipid values are in mg/100 ml. $K = 10^{-6}$ (mg/100 ml)$^{-1}$.

ACKNOWLEDGMENTS

This work was supported by Research Grants 5-RO1-HE-01882-11 and 5-R01-HE-02029-11 from the National Heart Institute, USPHS, and the U.S. Atomic Energy Commission.

REFERENCES

1. deLalla, O., and J. W. Gofman, in "Methods of Biochemical Analysis," Vol. 1, edited by D. Glick, Interscience Publishers, New York, 1954, p. 459.
2. Ewing, A. M., N. K. Freeman and F. T. Lindgren, in "Advances of Lipid Research," Vol. 3, edited by R. Paoletti and D. Kritchevsky, New York, Academic Press, Inc., 1965, Chap. 2, p. 25.
3. Waugh, D. F., and D. A. Yphantis, Rev. Sci. Instr. *23*, 609 (1952).
4. Johnston, J. P., and A. G. Ogston, Trans. Faraday Soc. *42*, 789 (1946).
5. Svedberg, T., and K. O. Pederson, "The Ultracentrifuge," Oxford University Press, London and New York, 1940.
6. Shachman, H. I., "Ultracentrifugation in Biochemistry," Academic Press, New York, 1959.
7. Gropper, L., Anal. Biochem. *6*, 170 (1963).
8. Oncley, J. L., K. W. Walton and D. G. Cornwell, J. Am. Chem. Soc. *79*, 4666 (1957).
9. Mills, G. L., and P. A. Wilkinson, Clin. Chim. Acta *8*, 701 (1963).
10. Zipf, R. E., B. J. Katchman and F. W. Sunderman, in "Serum Proteins and the Dysproteinemias," (ed. F. W. Sunderman and F. W. Sunderman, Jr., J. B. Lippincott Co., Philadelphia, Pa., 1964, p. 40.
11. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall, J. Biol. Chem. *193*, 265 (1951).
12. Gornal, A. G., C. J. Bardawill and M. M. David, J. Biol. Chem. *177*, 751 (1949).
13. Williams, F. G., and M. C. Nixon, in "Serum Proteins and the Dysoproteinemias," ed. F. W. Sunderman and F. W. Sunderman, Jr., J. B. Lippincott Co., Philadelphia, Pa., 1964, p. 110.
14. deLalla, O. F., University of California Lawrence Radiation Laboratory Report, UCRL-8550, November (1958).
15. Reiss, E., Inaug. Diss., Strassburg (1902).
16. Naumann, H. N., in "Serum Proteins and the Dysproteinemias," ed. F. W. Sunderman and F. W. Sunderman, Jr., J. B. Lippincott Co., Philadelphia, Pa., 1964, p. 86.
17. Armstrong, S. H., M. J. E. Budka, K. C. Morrison and M. Hasson, J. Am. Chem. Soc. *69*, 1747 (1947).
18. Hanig, M., and J. R. Shainoff, J. Biol. Chem. *219*, 479 (1956).
19. Lindgren, F. T., A. V. Nichols, N. K. Freeman, R. D. Wills, L. Wing and J. E. Gullberg, J. Lipid Res. *5*, 68 (1964).
20. Perlmann, G. E., and L. G. Longsworth, J. Am. Chem. Soc. *70*, 2719 (1948).
21. Zimm, H., and W. B. Dandliker, J. Phys. Chem. *58*, 644 (1954).
22. Heller, W., and T. L. Pugh, J. Coll. Sci. *12*, 294 (1957).
23. Bauer, N., K. Fajans and S. Z. Lewin, in "Physical Methods of Organic Chemistry," 3rd ed., Vol. I, ed. A. Weissberger, Interscience Publishers, New York, 1960, part II, p. 1239.
24. Sunderman, F. W., J. Biol. Chem. *153*, 139 (1944).
25. Drickman, A., and F. A. McKeon, Am. J. Clin. Path., *38*, 392 (1962).
26. Bausch and Lomb, Abee-3L Refractometer Reference Manual; Serum Protein Meter Reference Manual, Rochester 2, New York (1963).
27. deLalla, O. F., and J. W. Gofman, in "Blood and Other Body Fluids," ed. D. S. Dittmer, Washington, D.C., Federation of American Societies for Experimental Biology, 1961, p. 64.
28. Freeman, N. K., J. Lipid Res. 5, 236 (1964).
29. Freeman, N. K., E. Lampo and A. A. Windsor, University of California Lawrence Radiation Laboratory Report (in press).