Isolation and Characterization of Lipoprotein B from High-Density Human Serum Lipoproteins

By GERHARD KOSTNER
Institute of Medical Biochemistry, University of Graz, 8010 Graz, Austria
(Received 25 May 1972)

1. Lipoprotein B from female Lp(a)-lipoprotein-negative serum was isolated from the fraction of density 1.073–1.125 by using immunoadsorbent; 2.5mg of freeze-dried material was obtained from 100ml of serum from a fasting patient. 2. The hydrated density of this lipoprotein was found to be 1.084g/cm³. A flotation rate 1.200 of 9.4 and lipid/protein ratio 1.40 were found, similar to that of high-density (d 1.073–1.125) lipoprotein preparations. 3. From immunochemical and electrophoretic studies of the intact and totally delipidized lipoprotein B it was concluded that this lipoprotein represents a separate family within the high-density range of human serum lipoproteins. 4. The possibility that the isolated lipoprotein B is an artifact created by the isolation procedure is discussed.

The fact that lipoprotein density fractions from human serum are composed of multiple lipoprotein families has slowly gained widespread acceptance. Although there seems to exist no density range from which a single lipoprotein family can be isolated solely by ultracentrifugation, there are certain intervals characterized by the predominance of one or the other family. This is the case, for example, with LpB* in d 1.025–1.053 and with LpA in d 1.100–1.21 fractions. On the other hand, the d 1.060–1.100 fraction represents a mixture of lipoproteins with respect to their chemical, immunochemical and physicochemical properties.

The existence of LpB (previously called β, lipoprotein and sometimes equated with LDL) in lipoprotein preparations isolated at a density higher than 1.063 by ultracentrifugation has been reported by several investigators (Ayrault-Jarrier et al., 1963; Seegers et al., 1965; Alaupovic, 1968). In addition, the lipoprotein allotype Lp(a) discovered by Berg (1963), which has been found to be related to a histocompatibility antigen (Berg, 1971) and apparently represents a complex lipoprotein (Ehnholm et al., 1971; Seidel et al., 1971) sharing antigenic determinants with LpB, was isolated almost exclusively

* Abbreviations: LpA, LpB and LpC, lipoprotein family A, B and C respectively, characterized by the protein moiety; Lp(a), allotype of a lipoprotein found by Berg (1963); LDL, low-density lipoproteins, d 1.006–1.063 (unless stated otherwise); HDL₁, lipoproteins isolated at d 1.063 and floating with 1.072 flotation; HDL₂, high-density lipoproteins, d 1.073–1.125; HDL₃, high-density lipoproteins, d 1.125–1.21; LpBHDL, lipoprotein B isolated from HDL fraction; apoAI and apoAII, major apolipoproteins of LpA, corresponding to R-Thr and R-Gln respectively found by Shore & Shore (1968).

from the range d 1.063–1.100. Because of the relatively small amount of LpB present in HDL, few attempts have been successful in isolating and characterizing this LpBHDL. Several groups have recently isolated lipoproteins which reacted with ‘anti-β-lipoprotein’ antisera from HDL₁ or HDL₂ preparations (Utermann & Wiegandt, 1969; Roelcke & Weicker, 1969) and were distinguishable from Lp(a) (Albers et al., 1972). We previously described a successful isolation procedure for LpBHDL (Kostner & Alaupovic, 1971a) and this lipoprotein has been partially characterized (Kostner & Alaupovic, 1972). The present paper presents further results including chemical, immunochemical and physicochemical investigations.

Materials and Methods

Blood samples

Blood samples were collected from apparently healthy female volunteers, who had been informed about the aim of the present investigation. None of the subjects was under medication. Blood was collected after at least 12h of fasting and allowed to clot for 6–10h at 23°C. After being centrifuged in a low-speed centrifuge, the serum was collected and 1mg of NaNO₃ and 0.1mg of disodium EDTA were added/ml. The serum of each individual donor was investigated by lipoprotein electrophoresis in agarose gels (Kostner et al., 1971), and total lipids, phospholipids, total cholesterol and triglycerides were determined as described before (Kostner & Holasek, 1970). Since no antiserum to Lp(a) lipoproteins was available, the possible presence of this lipoprotein was checked by polyacrylamide-gel electrophoresis by the method of
Garoff et al. (1970). Sera of subjects with abnormal lipid concentrations, abnormal lipoprotein patterns in agarose-gel electrophoresis or showing the presence of Lp(a) in polyacrylamide-gel electrophoresis were not used during this study.

Isolation of lipoproteins

The density of 300ml of pooled serum from two or three subjects was adjusted to 1.073 by adding solid NaCl and the serum was centrifuged in the 60Ti rotor of a Beckman L4 preparative ultracentrifuge at 45000rev./min (144000g) for 24h at 15°C. Floating lipoproteins that accumulated in the upper third of the tube were removed by a tube-slicer. The infranatant was brought to the original volume by addition of 0.15M-NaCl (pH7.4 adjusted with 3M-NaOH); the density was again adjusted to 1.073 with solid NaCl, the preparation centrifuged again and sliced under identical conditions. The density of the lower two-thirds of the tubes was then adjusted to 1.125 by addition of solid NaBr and the mixture was spun in the ultracentrifuge for 30h (144000g) at 15°C with the 60Ti rotor. Floating HDL2 was removed by tube-slicing and dialysed against 0.15M-NaCl, pH7.4 (adjusted by addition of 3M-NaOH). HDL3 was isolated (d1.23) from sedimenting proteins (Kostner & Alaupovic, 1972). All density measurements were performed with a digital densitometer from Paar Instruments, Graz, Austria, at a temperature of 15°C.

Immunoadsorption technique

Pure antibodies to LpB were isolated from horse antisera as described by Kostner & Holasek (1969) and an immunoadsorbent was prepared as described by Kostner & Holasek (1970). HDL2 fractions were passed through a column (15cm × 1.2cm) packed with the immunoadsorbent specific to LpB at room temperature and eluted with 0.15M-NaCl (pH7.4) at a flow rate of 10ml/h. The size of the column was sufficient for adsorption of the total amount of LpB present in HDL2 from at least 300ml of serum. After concentration of the eluate to the original volume by vacuum dialysis, no LpB could be detected in the eluate by immunodiffusion. The column was washed with 300ml of 0.15M-NaCl, pH7.4, at 4°C and adsorbed LpB was eluted with 20ml of 0.1M-β-alanine–HCl buffer, pH3.2, followed by 20ml of 0.1M-β-alanine–HCl, pH2.4. The progress of desorption was monitored by photoelectric scanning of the eluate at 280nm. Fractions were dialysed against 0.15M-NaCl, pH7.4, and investigated by immunodiffusion, immunoelectrophoresis, polyacrylamide-gel electrophoresis and in the analytical ultracentrifuge.

For preparation of apoLpBHDL, the combined β-alanine eluates from the immunoadsorbent column was dialysed exhaustively against glass-distilled water and freeze-dried. Removal of lipid from the freeze-dried material was carried out with ethanol–diethyl ether (3:2, v/v), followed by diethyl ether extractations as described by Kostner & Holasek (1972). Polyacrylamide-gel electrophoresis, immunoelectrophoresis, immunodiffusion and lipid analysis were performed as described previously (Kostner & Holasek, 1969, 1970, 1972). Antisera to lipoprotein families, as well as to their constitutive polypeptides, were prepared by immunizing sheep, goats, rabbits and horses with highly purified lipoproteins or separated apoLp polypeptides (Kostner & Alaupovic, 1972; Kostner & Holasek, 1970, 1972; Kostner & Alaupovic, 1971b). Sedimentation and flotation experiments were performed in an analytical ultracentrifuge (Beckman model E) equipped with electronic speed control. Flotation coefficients were calculated from the formula:

\[
(F_c)_{\text{app}} = -\frac{\ln r_2 - \ln r_1}{60\omega^2(t_2 - t_1)}
\]

where \((F_c)_{\text{app}}\) is the apparent flotation coefficient at concentration \(c\), and \(r_1\) and \(r_2\) are the distances of the boundaries from the rotor axis at different time-intervals \((t_1\) and \(t_2\)); \(\omega\) is the angular velocity.

Amino acid analysis

ApoLpBHDL (0.2–0.4mg) was hydrolysed in constant-boiling HCl under vacuum at 110°C for 24, 48 and 72h. Norleucine was used as internal standard and amino acid loss was corrected after extrapolation to zero time. Cysteine was determined as cysteic acid after performic acid oxidation before hydrolysis by the method of Schram et al. (1954). Tryptophan was determined by the method of Gaitonde & Dovery (1970). The analysis was performed in a Bio-Cal BC200 amino acid analyser (Bio-Cal Instruments, München, Germany) with automatic sample injector by using the high-sensitivity cell. The resin Aminex A-G from Bio-Rad Laboratories (Richmond, Calif., U.S.A.) was used in a one-column programme. Amino acid analyses as well as calculation of individual amino acids were performed according to the instruction manual of the Bio-Cal BC200 amino acid analyser.

\[
\bar{v} = \frac{1}{d_2} \left(1 - \frac{d_2 - d_1}{c} \right)
\]

where \(d_2\) is the density of the solvent, \(d\) the density of the solution and \(c\) the concentration in g/cm³. NaCl (0.15M, pH7.4, adjusted with 3M-NaOH) served as solvent and measurements were carried out at 33°C.
EXPLANATION OF PLATE 1(a)

*Immunochemical reaction of HDL₂ (d: 1.073–1.125)* with different antibodies

(a) The immunochemical heterogeneity of HDL₂, isolated from pooled serum of three different subjects, in 1% agarose gel after staining with Amido Black 10B. The centre hole contains HDL₂. Surrounding holes contain antisera to: 1, LpA; 2, LpB; 3, apoLpB; 4, LpC; 5, albumin (Behring) Werke A.G., Marburg, Germany); 6, immunoglobulin G (Behring Werke).

EXPLANATION OF PLATE 1(b) AND (c)

*Gel electrophoresis of LpB<sub>HDL</sub> compared with LDL (d 1.025–1.053)*

(b) Electrophoresis of 1, LpB<sub>HDL</sub> and 2, LDL in 3.5% polyacrylamide gels after staining with Amido Black 10B.

(c) Agarose-gel electrophoresis of 1, LDL, 2, LpB<sub>HDL</sub> and 3, LDL plus LpB<sub>HDL</sub> after staining with Amido Black 10B. For details see the Material and Methods section and the Results section.
EXPLANATION OF PLATE 2(a) AND (b)

Immunochemical examination of intact $LpB_{HDL}$ after elution from immunoadsorbent with 0.1M-$\beta$-alanine buffer, pH 3.2

(a) Immunodiffusion analysis of $LpB_{HDL}$ (centre hole) with antisera to: 1, apoLpB; 2, whole human serum (Behring Werke); 3, ApoAl; 4, apoAII; 5, LpC; 6, albumin. The plate was photographed in diffuse light without staining. (b) Immunelectrophoretic pattern of $LpB_{HDL}$ (top hole) and LDL (bottom hole) with antiserum to LpB. Staining was performed with Sudan Black. For details see the Materials and Methods section.

EXPLANATION OF PLATE 2(c)

Flotation pattern of $LpB_{HDL}$

$LpB_{HDL}$ was dialysed against NaBr solution ($d_1=200$) and examined in the analytical ultracentrifuge at 52000rev./min and 25°C. The concentration was 16mg/ml. A, Photograph at 20min after reaching full speed; B, photograph at 35min after reaching full speed. The acceleration time from zero speed to 52000rev./min was 10min.

G. KOSTNER
EXPLANATION OF PLATE 3

*Immunodiffusion pattern and polyacrylamide-gel electrophoresis of totally delipidized apoLpB*

(a) Two-dimensional immunodiffusion experiment of apoLpB<sub>HDL</sub> (centre hole) with antisera to: 1, apoLpB; 2, whole human serum; 3, apoAI; 4, apoAII; 5, LpC; 6, albumin. Plates were photographed in diffuse light without staining. (b) Electrophoresis in 10% polyacrylamide gels containing 8M-urea. Gel 1, apoHDL<sub>2</sub> solubilized in 0.05M-tris–HCl buffer, pH 8.4, containing 8M-urea; gel 2, apoLpB<sub>HDL</sub> solubilized in 0.05M-tris–HCl buffer, pH 8.4, containing 0.9% (w/v) sodium dodecyl sulphate. Staining was performed with Coomassie Blue. For details see the Materials and Methods section.

G. KOSTNER
out at 25.0°C. Densities were determined with a digital densitometer (Paar Instruments, Graz, Austria) as described by Kratky et al. (1969). Concentrations of lipoprotein solutions were determined gravimetrically after evaporation of the solvent in a desiccator.

Results

Plate 1(a) shows the immunochemical reaction of HDL₂ with different antisera. In HDL₂ and d>1.23 fractions from at least 30 different male and female subjects, no reaction with anti-LpB could be obtained at any antigen/antibody ratio tested. It was concluded that no LpB-containing lipoproteins are present in human serum with hydrated density higher than 1.125.

Fig. 1 shows the elution pattern of LpB from the immunoadsorbent column with 0.1 M-β-alanine at pH 3.2 and 2.4 respectively. The total amount of freeze-dried LpB isolated from 100 ml of serum from fasting patients was 2-3 mg. Electrophoretic and ultracentrifugal studies of intact LpBHDL were performed only on fraction I since part of fraction II was precipitated during dialysis and showed signs of 'denaturation'. For immunodiffusion studies and preparation of apoLpBHDL, fractions I and II were mixed, since in previous experiments no difference in the amino acid composition between these two fractions could be detected.

In Plate 1(b,c) the electrophoretic patterns of LpBHDL in polyacrylamide gel and 1% agarose gel are shown and compared with that of LpB from LDL (d1.025-1.055). A somewhat faster migration of LpBHDL was noticed but, after these two fractions were mixed, only a single band was detectable.

Plate 2(a,b) shows the immunochemical examination of LpBHDL by immunodiffusion and immunoelectrophoresis. The only antiserum giving a positive immune reaction was anti-LpB.

Plate 2(c) shows the flotation pattern of LpBHDL in the analytical ultracentrifuge at d1.200. In a NaBr solution (d1.200) the flotation coefficient of a LpBHDL solution (8.5 mg/ml) at 25°C was calculated to be -9.45 S. A molecular polydispersity may be deduced from the broadening of the peak after prolonged running time. The partial specific volume from two separate preparations was calculated to be 0.924 and 0.922 g/cm³ and the corresponding values for the hydrated densities were 1.082 and 1.084 g/cm³ respectively. The molecular weight from one preparation was calculated as described by Archibald (1947) and an apparent value for molecular weight of 1.3×10⁶ was found in a solution containing 4.5 mg of LpBHDL/ml.

After total removal of lipid, apoLpBHDL was completely soluble in 0.05 M-tris-HCl buffer, pH 8.4, containing 0.9% sodium dodecyl sulphate or sodium decyl sulphate, or in 2 M-acetic acid. The solution of LpBHDL in detergents showed an immunochemical reaction of identity only for antisera to LpB or apo-LpB and gave none with antisera to the two major apoLpA peptides apoAI or apoAII (Kostner & Alaupovic, 1971b) or antisera to the three major LpC polypeptides (Kostner & Holasek, 1972). In polyacrylamide-gel electrophoresis (Plate 3) most of the material migrated through the concentrating gel and came to a dead stop at the beginning of the separating gel, at monomer concentrations of 3.5, 7 or 10% acrylamide. Some diffuse broad bands stainable with Coomassie Blue were observed in the separating gel but none of those bands exhibited a migration rate

![Fig. 1. Elution pattern of LpBHDL from immunoadsorbent with 0.1 M-β-alanine at pH 3.2 and pH 2.4.](image)

Table 1. Chemical composition of LpB isolated from serum HDL₂ by immunoadsorption compared with that of Lp(a) lipoprotein isolated by Ehnholm et al. (1971)

<table>
<thead>
<tr>
<th>Component</th>
<th>LpB (mg/ml)</th>
<th>Lp(a) (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein+carbohydrates</td>
<td>41.5±1.2</td>
<td>35</td>
</tr>
<tr>
<td>Glycerides</td>
<td>7.1±0.9</td>
<td>2</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>6.9±0.4</td>
<td>9</td>
</tr>
<tr>
<td>Cholesterol esters</td>
<td>23.7±0.8</td>
<td>38</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>13.2±0.9</td>
<td>9</td>
</tr>
<tr>
<td>Lysoosphatidylcholine</td>
<td>4.4±0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>2.1±0.3</td>
<td>5</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>0.7±0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

For details see the Materials and Methods section.
Table 2. *Amino acid composition of LpB from HDL₂ compared with that of Lp(a) isolated by Ehnholm et al.* (1971)

A pooled sample from eight different individuals was used. Values for serine, threonine and tyrosine were obtained by linear extrapolation of recoveries from 24–48h and 72h hydrolysis. For details see the Materials and Methods section.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>LpB&lt;sub&gt;HDL&lt;/sub&gt; (mol/1000mol of amino acids)</th>
<th>Lp(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>86</td>
<td>98.9</td>
</tr>
<tr>
<td>Thr</td>
<td>75</td>
<td>68.0</td>
</tr>
<tr>
<td>Ser</td>
<td>90</td>
<td>78.2</td>
</tr>
<tr>
<td>Glu</td>
<td>110</td>
<td>117.8</td>
</tr>
<tr>
<td>Pro</td>
<td>68</td>
<td>54.1</td>
</tr>
<tr>
<td>Gly</td>
<td>64</td>
<td>75.1</td>
</tr>
<tr>
<td>Ala</td>
<td>60</td>
<td>82.6</td>
</tr>
<tr>
<td>Val</td>
<td>82</td>
<td>60.1</td>
</tr>
<tr>
<td>Met</td>
<td>11</td>
<td>15.8</td>
</tr>
<tr>
<td>Ile</td>
<td>46</td>
<td>43.5</td>
</tr>
<tr>
<td>Leu</td>
<td>81</td>
<td>84.9</td>
</tr>
<tr>
<td>Tyr</td>
<td>35</td>
<td>34.8</td>
</tr>
<tr>
<td>Phe</td>
<td>38</td>
<td>35.2</td>
</tr>
<tr>
<td>Lys</td>
<td>63</td>
<td>62.8</td>
</tr>
<tr>
<td>His</td>
<td>20</td>
<td>21.3</td>
</tr>
<tr>
<td>Arg</td>
<td>27</td>
<td>40.3</td>
</tr>
<tr>
<td>Cys</td>
<td>7</td>
<td>17.8</td>
</tr>
<tr>
<td>Trp</td>
<td>Trace</td>
<td>8.7</td>
</tr>
</tbody>
</table>

like that of the two major apoLpA or any of the apoLpC bands.

Tables 1 and 2 show the protein/lipid ratio, the distribution of individual lipids and the amino acid composition of apoLpB<sub>HDL</sub> as compared with that of Lp(a) lipoprotein from Ehnholm et al. (1971). Values for amino acids in Table 2 were calculated from a special batch of apoLpB<sub>HDL</sub>, which was pooled from eight different individuals. On summing the weights of individual amino acids after 24h hydrolysis, 86% of the original weight of apoLpB<sub>HDL</sub> could be recovered.

**Discussion**

It has recently become evident that the lipoproteins which react with antibodies to LpB and which can be found in lipoprotein fractions isolated at a density greater than 1.063 may no longer be dismissed by attributing them to 'contaminations' or 'improper isolation procedure'. This has been verified by the isolation and characterization of Lp(a), a lipoprotein which apparently represents a complex of LpB with other known or unknown lipoproteins or apolipoproteins (Ehnholm et al., 1971; Seidel et al., 1971; Utermann & Wiegandt, 1969; Utermann et al., 1972). LpB has also been found in HDL₃ preparations of Lp(a)-negative subjects (Alaupovic, 1968; Roelcke & Weicker, 1969), but because the small amount present was masked by the predominant LpA and LpC (Kostner & Alaupovic, 1971a), its isolation and characterization were unsuccessful. Results from the present study demonstrate that, by using the procedure outlined above, it is possible to isolate a LpB fraction from human serum HDL₂ with 70.923 and hydrated density 1.083. Considering the flotation rate (F₁₂₀₀0 g·h⁻¹) and the lipid/protein ratio (1.40), the LpB<sub>HDL</sub> exhibits most of the characteristics found for HDL₃ preparations.

A LpB<sub>HDL</sub> showing the above-mentioned physicochemical characteristics should not be interpreted as a contaminant LpB from HDL as a consequence of the isolation procedure (low pH for desorption from immunoabsorbent), since a density of 1.073 was used for removal of LDL and an additional wash of the infranatant at the same density was carried out. Ultracentrifugation at 34.5 × 10⁶ g·h⁻¹ and the removal of the upper third from the tubes containing LDL by tube-slicing should also prevent contamination of the HDL fraction with LDL. In addition, it has been observed (Scaru, 1971), and could be confirmed by our own investigations, that HDL loses some lipids (mainly neutral lipids) during isolation by ultracentrifugation, which shifts it to a fraction of higher hydrated density. This phenomenon could not be observed with freshly prepared LDL (G. Kostner, unpublished work). When LDL preparations were centrifuged again at a density of 1.073 under the conditions outlined in the present paper, no material reacting with anti-LpB could be found in the sediment.

Chemical, immunochemical, physicochemical and electrophoretic properties of LpB<sub>HDL</sub> differed significantly from those of Lp(a) preparations (Tables 1 and 2). It therefore does not seem likely that LpB<sub>HDL</sub> prepared during the present work was contaminated with Lp(a) lipoproteins. It has been stated that apoLp(a) lipoproteins react with antiserum to albumin (Utermann et al., 1972) and to LpC (Seidel et al., 1971); apoLpB<sub>HDL</sub> gave no reaction with those antisera. The preparation of LpB<sub>HDL</sub> described in the present paper cannot be compared directly with the LDL₃ isolated by Albers et al. (1972), since in their studies another density for isolation was used. In addition pooled sera were used, which probably contained Lp(a) lipoproteins, and it therefore cannot be excluded that part of the LDL₃ found represented split fragments from Lp(a) lipoproteins, since it has been discovered that Lp(a) disintegrates on storage or chemical treatment (Ehnholm et al., 1971; Utermann et al., 1972).

It has been suggested previously (Alaupovic et al.,
1972) that lipoprotein families with a density above a certain value exist primarily as individual entities and below that range mainly as associated complexes. This could be verified by isolating different lipoprotein families from HDL (Kostner & Alaupovic, 1971a, 1972) and by isolating LpA from chyle LDL preparations (Kostner, 1972) on the one hand and by isolation of chylomicrons as complexes of lipoprotein families (Kostner & Holasek, 1972) on the other. The present report is another example of the correctness of the 'lipoprotein family concept' proposed by Alaupovic et al. (1972). Intact LpB_HDL reacts only with antibodies to LpB and gives no reaction with any other antiserum to LpA, LpC or to their constitutive polypeptides. After removal of lipid, the apoprotein moiety also showed the same immunochromatographic behaviour.

In a recent study, Scanu & Edelstein (1971) demonstrated and partially determined quantitatively the nature and amount of apolipoproteins soluble in organic solvents. When we dissolved the lipid moiety of LpB_HDL in chloroform–methanol (2:1, v/v) and extracted with 0.15M-NaCl, pH 7.4, according to Folch et al. (1957), no protein could be detected either in the aqueous phase or in the interphase. We could therefore exclude the possibility that any protein was lost during removal of lipid. Although no physicochemical studies were performed on material eluted from immunoabsorbent by ß-alanine at pH 7.4, it seems unlikely that there is any difference between material from peak I and peak II in Fig. 1. In one experiment, samples from peaks I and II were examined separately and no difference in the immunochromatographic behaviour or in the amino acid composition was detectable. Rather it is assumed that, owing to the different avidity of antibodies bound to the adsorbent, part of the LpB can be eluted only at lower pH values. Results of studies presented in the present paper clearly indicate that a single lipoprotein family exhibiting characteristics of LpB can be isolated at a density range 1.073–1.125 by immunoadsorption.

I express my appreciation for valuable criticism by Dr. P. Alaupovic and for the interest shown by Dr. A. Holasek during this investigation. The technical assistance of Miss E. Schön is gratefully acknowledged. Part of this work was carried out at the Oklahoma Medical Research Foundation, Oklahoma City, Okla., U.S.A. and was supported by grants HE-7055 and HE-6221 from the U.S. Public Health Service and by the Oklahoma Heart Association. I am grateful to the Fonds zur Förderung der Wissenschaftlichen Forschung, Wien, Austria, for providing a Spinco model E analytical ultracentrifuge.

References

Berg, K. (1971) Science 172, 1136
Kostner, G. & Holasek, A. (1972) Biochemistry 11, 1217
Seegers, W., Hirschhorn, K., Burnett, L. B., Robson, E. & Harris, N. (1965) Science 149, 303
Shore, B. & Shore, V. B. (1968) Biochemistry 7, 2773