Protein-Lipid Relationships in Normal Dog Plasma

Application of Cohn Method 10

By ELLA M. RUSS AND JULIE RAYMUNT

Although Cohn's microfractionation method number 10 was specifically devised to meet the conditions of protein concentration and distribution in human plasma, it is shown in this contribution that it may be applied to studies of canine plasma. Essentially all of the lipids of plasma are recovered in Fractions IV + V + VI and I + III. Only negligible amounts appear in Fraction II. Electrophoretic studies proved that the lipoproteins of Fraction IV + V + VI have the mobility of alpha globulins and those in Fraction I + III have the mobility of beta globulins.

WITH the use of Cohn's microfractionation method number 10, almost all of the lipids of normal human plasma may be quantitatively recovered from two distinct fractions. In one, they are bound to the alpha globulins as alpha lipoproteins, and in the other to beta globulins as beta lipoproteins. The method has been found applicable to the separation of lipoproteins in a wide variety of normal and pathologic plasmas.

That the greatest relative amount of lipid in dog plasma, in contrast to human plasma, is bound to alpha globulins while only a small part is bound to beta globulins was first demonstrated by Zeldis, Ailing, McCoord, and Kulka. By electrophoretic analyses of dog plasma before and after an elaborate alcohol-ether extraction, they showed marked changes in the configuration and number of alpha globulin peaks, with little or no change in the remaining electrophoretic components.

The present communication is a report of the effectiveness of the Cohn method in separating the lipid-bearing globulins of dog plasma. It includes some preliminary observations on their lipid composition.

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Procedures

Studies were made on plasma from six apparently normal dogs three to four years of age. Blood was drawn from the jugular vein into ACD solution, and the plasma was fractionated by the technique identical to that employed in previously reported studies of human plasma. Three protein fractions were obtained under the same precise conditions necessary for the isolation of Fractions IV + V + VI, II, and I + III from human plasma. Since, however, dog plasma is known to contain electrophoretic components never found in human plasma, the Cohn nomenclature for protein fractions was avoided and the designation of Fractions A, B and C + D was substituted.

Protein analyses were made by the standard Kjeldahl and biuret procedures and, whenever necessary, by the micro methods of Jacox or Lowry and coworkers. Cholesterol and phospholipid determinations were performed as described elsewhere with an adaptation of the phospholipid method to a micro scale whenever it was essential.

Moving boundary electrophoretic analyses of dog plasma and its fractions were carried out with the aid of an Aminco-Stern apparatus using veronal-citrate buffer pH 8.0, ionic strength 0.1 at 2°C. The samples were prepared for electrophoresis in the same manner as described for human plasma.

For rapid identification of lipoproteins or other protein components, paper electrophoresis was employed. The fractions were prepared as for moving boundary analyses, but dialysis was not essential. This ionophoretic system was a modification and combination of several methods.

PROCEEDINGS

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hours. The experiments were carried out in a constant vapor chamber at 5 C. using veronal-citrate buffer pH 8.6, ionic strength 0.05. The lipoproteins were specifically identified with Sudan black B, while all protein components were stained with bromphenol blue.\textsuperscript{14, 16}

Starch electrophoresis was carried out at 5 C. using veronal-citrate buffer pH 8.6, ionic strength 0.1, employing the system described by Kunkel and Slater.\textsuperscript{16} A potential of 300 V was applied to a starch block 10 cm. by 45 cm. and 1 cm. thick for approximately twenty hours, or until complete resolution had taken place. Prior to electrophoresis, Fraction A of dog plasma was concentrated with zinc reagent and redissolved with citrate-sodium chloride reagent to a concentration of 4.0 gm. per cent as described previously for human Fraction IV + V + VI.\textsuperscript{12} Of this solution, 4.0 ml. was applied to the starch block. Following complete electrophoretic separation of the protein components, the block was cut into 1.0 cm. segments. The protein was eluted with 0.5 per cent sodium chloride. Cholesterol, phospholipid, and protein analyses were made on each segment employing, when necessary, the micro procedures described elsewhere in this communication.

RESULTS

The type of separation characteristically obtained from the Cohn fractionation of dog plasma may be seen from the moving boundary electrophoretic patterns shown in figure 1.

The percentage area distribution of the electrophoretic components in plasma and Fractions A, B, and C+D from three dog plasmas are listed in table 1.

Albumin, which on an average represents approximately 35 to 40 per cent of the total protein, is recovered almost entirely in Fraction A; only very small amounts are found in Fraction C+D. Two extra peaks together representing approximately 10 per cent of the plasma total area are designated as alpha-3 and alpha-4 on the basis of their mobilities and positions relative to the alpha-2 and beta-1 peaks. In this scheme of designation, the beta-1 component migrates somewhat more slowly than does the beta-1 of human plasma. The alpha-1 globulins appear to be concentrated entirely in Fraction A while the alpha-2, alpha-3, and alpha-4 components are scattered in various amounts between Fractions A and C+D. Resolution of the alpha-1, beta-1, and beta-2 components in Fraction A varies considerably in the dogs. In one, there is a merging of the alpha-4 and beta-1 peaks, and, in another, of the beta-1 and beta-2 peaks, resulting in one electrophoretic component.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{moving_boundary_electrophoretic_patterns.png}
\caption{Moving boundary electrophoretic patterns of dog plasma and three fractions.}
\end{figure}
which migrates between the alpha-3 and beta-2 areas. Beta-1 globulins, aside from the small amount found in Fraction A, are localized chiefly in Fraction C+D where they represent approximately 27 per cent of the fraction. The large concentrations of beta-2 globulins and fibrinogen ranging from 9 to 15 per cent of the total proteins in unfractionated plasma are recovered in both Fractions B and C+D. Approximately 60 per cent of the plasma gamma globulins are recovered in Fraction B; the remainder is found in Fraction C+D.

Sudan black B lipid stains of the paper electrophoretic patterns of dog plasma and its fractions indicate that most of the lipids of dog plasma migrate with proteins from the middle of the albumin to the alpha-3 area, while very small amounts appear to be bound to the beta globulins. The patterns also indicate that the alpha lipoproteins are concentrated entirely in Fraction A and appear to be effectively separated from the beta lipoproteins which are localized entirely in Fraction C+D. This lipoprotein distribution in dog plasma is a marked contrast to that in the human, where approximately three-fourths of the lipids are bound to beta globulins.

In table 2, the mean concentration and distribution of cholesterol, phospholipids, and protein, including the cholesterol-phospholipid ratios from the plasma and fractions of six dogs, may be compared to those obtained from human plasma. Although the concentration of cholesterol in the dog plasmas varies greatly, it is in general lower than in humans. The concentration of the phospholipids in the two species is essentially the same. Thus the cholesterol-phospholipid ratio is considerably lower in canine plasma, in which the figure of 0.53 may be compared with the human value of 0.84.

In Fraction A of dogs, the concentrations of both the cholesterol and phospholipids are approximately twice as great as in the corresponding human Fraction IV+V+VI, with no essential difference in the cholesterol-phospholipid ratios. The concentration of cholesterol in Fraction C+D is less than one-tenth, and the phospholipid is less than one-fifth of that in the human Fraction I+III. The cholesterol-phospholipid ratio which averages only 0.61 in Fraction C+D in comparison to 1.25 for humans would indicate the possibility of a species variation in the constitution of the lipoproteins or perhaps unusual mixtures of lipoproteins.

The decreased albumin concentration characteristic of dog plasma (table 1) accounts in part for the diminished total plasma protein concentration. The greatest species difference appears in Fraction A, where the protein concentration is markedly reduced. This may be attributed to the diminution of albumin in the unfractionated plasma, as 96 per cent of the total albumin is recovered in Fraction A. Fractions B and C+D show no essential difference.

Values for the relative distribution of lipids
The Average Concentration and Comparative Distribution of Cholesterol, Phospholipid, and Protein in the Plasma of Dogs and Man, Including the Cholesterol:Phospholipid Ratios

<table>
<thead>
<tr>
<th></th>
<th>Concentration in Dog Plasma</th>
<th>Distribution in Dog Plasma</th>
<th>Human Plasma</th>
<th>Distribution in Human Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>± S.D.</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated plasma</td>
<td>134.0</td>
<td>83-179</td>
<td>189</td>
<td>± 35</td>
</tr>
<tr>
<td>Fraction A</td>
<td>114.0</td>
<td>64-165</td>
<td>61</td>
<td>± 13</td>
</tr>
<tr>
<td>Fraction B</td>
<td>1.8</td>
<td>0-3</td>
<td>3</td>
<td>± 1</td>
</tr>
<tr>
<td>Fraction C + D</td>
<td>10.2</td>
<td>13-22</td>
<td>123</td>
<td>± 32</td>
</tr>
<tr>
<td>Phospholipid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated plasma</td>
<td>257.0</td>
<td>158-310</td>
<td>225</td>
<td>± 28</td>
</tr>
<tr>
<td>Fraction A</td>
<td>234.0</td>
<td>131-294</td>
<td>117</td>
<td>± 20</td>
</tr>
<tr>
<td>Fraction C + D</td>
<td>23.7</td>
<td>19-28</td>
<td>98</td>
<td>± 23</td>
</tr>
<tr>
<td>Cholesterol:Phospholipid Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated plasma</td>
<td>0.53</td>
<td>0.46-0.58</td>
<td>0.84</td>
<td>± 0.11</td>
</tr>
<tr>
<td>Fraction A</td>
<td>0.50</td>
<td>0.44-0.55</td>
<td>0.52</td>
<td>± 0.06</td>
</tr>
<tr>
<td>Fraction C + D</td>
<td>0.61</td>
<td>0.46-0.50</td>
<td>1.25</td>
<td>± 0.16</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated plasma (Kjeldahl)</td>
<td>5.72</td>
<td>5.09-9.99</td>
<td>6.68</td>
<td>± 0.25</td>
</tr>
<tr>
<td>Fraction A (Biuret)</td>
<td>3.34</td>
<td>3.02-3.60</td>
<td>5.04</td>
<td>± 0.21</td>
</tr>
<tr>
<td>Fraction B</td>
<td>0.83</td>
<td>0.66-1.08</td>
<td>0.87</td>
<td>± 0.15</td>
</tr>
<tr>
<td>Fraction C + D</td>
<td>1.68</td>
<td>1.59-1.94</td>
<td>1.37</td>
<td>± 0.19</td>
</tr>
</tbody>
</table>

In Fractions A and C+D emphasize the striking differences between the species; 85.4 per cent of the canine plasma cholesterol is recovered in Fraction A as compared to 33.2 per cent for humans. The species difference in phospholipid distribution is almost as great.

Recoveries for cholesterol and phospholipids were 94 per cent and 100 per cent, respectively; the protein recovery averaged 102 per cent.

The analytical results of an electrophoretic protein separation of Fraction A employing a starch medium are shown in figure 2. This analysis was made on a pool of Fraction A from three dogs. The concentrations of protein, cholesterol, and phospholipids are plotted for each 1.0 cm. segment of the block according to the legend described on the graph. The protein curve indicates the same type of electrophoretic distribution of the components in Fraction A as was shown by the moving boundary patterns in figure 1 and also by the paper electrophoretic patterns. The curves of the cholesterol and phospholipid concentrations indicate that these lipids are concentrated chiefly between the alpha-1 and alpha-2 areas, with a spread from the start of the albumin through the alpha-3 area.

In this experiment, the protein recovery was 96 per cent, while the cholesterol and phospholipids each showed an 80 per cent recovery. In three similar experiments employing starch electrophoresis of dog Fraction A the protein recoveries ranged from 91 to 101 per cent, while that for the cholesterol was 65 to 80 per cent, and the phospholipids 65-75 per cent. The rather poor recovery for the lipids may be.
partially explained by the inevitable adherence of some of the protein to the waxed paper which is used to envelop the starch block during electrophoresis. Also, the large amounts of lipid-bearing proteins characteristically found in dog Fraction A may be extremely difficult to elute completely from the starch granules.

Analysis of Fraction A prior to electrophoresis showed a cholesterol-phospholipid ratio of 0.57. Since the recoveries for both cholesterol and phospholipids were of the same order, cholesterol-phospholipid ratios were calculated for each segment. These showed a range of 0.40 to 0.65 except for the two segments 6 and 7. This discrepancy was not apparent in observations on Fraction A of other dogs. It is believed that in this experiment it is attributable to faulty analysis of phospholipids in these two segments.

SUMMARY

Cohn method number 10, although originally developed for human plasma, may be applied to canine plasma for an effective separation of many protein components.

In dog as in man the plasma lipids are combined chiefly with alpha and beta globulins. In the dog and unlike man, however, the lipids are bound almost entirely to alpha globulins.

As judged from the cholesterol-phospholipid ratios, the alpha lipoproteins of dogs are similar to those of man. The beta lipoproteins have, however, a much lower cholesterol-phospholipid ratio than those in human beings. This may indicate that in the dog there are qualitative differences or unusual mixtures of the beta lipoproteins.

REFERENCES


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