Serum Lipid and Protein Fractions
IX. Comparisons of Ninety-six Patients with Vascular Disease and Sixty Normal Controls (with Additional Notes on Blood Donors)

By Irving Leinwand, M.D. and Dan H. Moore, Ph.D.

Ninety-six patients were observed at intervals for as long as three and one-half years in some cases. In most instances multiple determinations were made during this time using the electrophoretic pattern of the proteins before and after cold ether extraction (McFarlane method), to measure lipid transport. Hyperlipemia usually produced an increased in beta globulin. A varying decrease in albumin accompanied any increase in globulin. Phosphate and barbiturate buffers were used in this study. In setting up a control group, it was noted that the sera from professional blood donors contained relatively less albumin and more globulin than the sera of the nondonor group of hospital personnel.

For the past few decades, it has been common knowledge that in certain diseases the incidence of arteriosclerosis is much higher than in other diseases. About five years ago, a study of the lipid and protein fractions in these diseases was undertaken in an effort to determine if possible, some basis for this preferential incidence in these particular diseases.

Material and Methods

The groups of patients consisted of individuals with apparent occlusive vascular disease, in various stages of disability, as well as patients with assumed vascular disease without clinical evidence of occlusion. Included in the first category are 32 patients with arteriosclerosis obliterans, seven patients with myocardial infarction, and 28 patients with thromboangiitis. In the latter category are three patients with angina pectoris, 10 patients with hypertensive vascular disease, four patients with hypothyroidism, and 12 patients with primary essential xanthomatosis. These 96 patients were observed for varying periods of time up to three and one-half years. Sixty normal adults were used as controls.

The blood specimens were obtained in the morning after about 10 to 12 hours of fasting since this appeared to be an optimum time for a constant level of the lipids. Lipid estimations were chiefly concerned with lipid phosphorous and total cholesterol. In some patients the total lipids were determined. Fatty acid determinations were also made in most instances. During the first year of study, the electrophoretic analyses were done at a pH of 7.4 in a phosphate buffer, 0.02 M with respect to the sodium phosphate and 0.15 M with respect to sodium chloride.1,2 Subsequently, a barbiturate buffer was used at a pH of 8.6, 0.1 M with respect to sodium diethylbarbiturate and 0.02 M with respect to the diethylbarbituric acid.3 These two buffers were used in order to permit a comparison of protein patterns with those in previously published reports of other investigators. Phosphate buffer produces a pattern which is easier to measure. The use of barbiturate buffer makes possible the separation of an additional component alpha 1, from the albumin fraction.

Patterns were secured before and after cold ether extraction by the McFarlane method4 since this appeared to leave the protein structure comparatively undisturbed. Two volumes of serum were thoroughly mixed with one volume of ether and quickly frozen in a dry ice-alcohol bath. The samples were then allowed to thaw, whereupon the serum layer was carefully removed from underneath the ether layer with a syringe and long needle. The process was repeated four times on all samples, although most of the changes in the pattern took place after the first extraction.5 As was pointed out by McFarland,6 low temperature treatment of the ether-serum emulsion removes only part of the total lipids. Faber and Chargaff6 have shown that most of the cholesterol is removed at the first extraction but that small quantities of phospholipids may continue to be removed even at the tenth or twelfth extraction. This has been confirmed in our own laboratory.7 By measuring the patterns before and after cold ether

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Circulation, Volume X, July, 1954
Table 1.—Electrophoretic Analysis of Serum from 43 Patients Using Phosphate Buffer at a pH of 7.4. Component Distribution Is Given in Per Cent of Pattern Area with the Standard Deviation, S.D. The Percentage Loss in Pattern Area after Cold Ether Extraction Is Given Underneath the Original Values. These Are Mean Figures of 15 Determinations

<table>
<thead>
<tr>
<th>Disease</th>
<th>Patients</th>
<th>Albumin</th>
<th>Gloablins</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tr>
<td></td>
<td></td>
<td>%</td>
<td>S.D.</td>
<td>%</td>
<td>S.D.</td>
<td>%</td>
<td>S.D.</td>
<td>%</td>
<td>S.D.</td>
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<tr>
<td>Angina</td>
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<td>6.4</td>
<td>9.1</td>
<td>0.5</td>
<td>20.9</td>
<td>0.1</td>
<td>11.2</td>
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<tr>
<td></td>
<td></td>
<td>-4.3</td>
<td>-24.3</td>
<td>-52.9</td>
<td>0.1</td>
<td>11.2</td>
<td>-4.1</td>
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<td></td>
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<tr>
<td>Arteriosclerosis obliterans</td>
<td>15</td>
<td>58.0</td>
<td>5.0</td>
<td>8.7</td>
<td>2.0</td>
<td>17.2</td>
<td>4.3</td>
<td>15.6</td>
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<td>4.8</td>
<td>2.3</td>
<td>17.9</td>
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<td>18.7</td>
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<td>-23.5</td>
<td>-48.0</td>
<td>0.1</td>
<td>11.2</td>
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<tr>
<td>Hypothyroid</td>
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<td>0.9</td>
<td>8.4</td>
<td>2.5</td>
<td>18.7</td>
<td>0.9</td>
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<tr>
<td>Thromboangiitis obliterans</td>
<td>16</td>
<td>59.9</td>
<td>4.6</td>
<td>8.0</td>
<td>2.0</td>
<td>16.4</td>
<td>2.1</td>
<td>15.9</td>
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<td>-46.8</td>
<td>0.1</td>
<td>11.2</td>
<td>-4.1</td>
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<tr>
<td>Primary essential xanthomatosis</td>
<td>6</td>
<td>54.7</td>
<td>3.5</td>
<td>8.6</td>
<td>1.6</td>
<td>20.8</td>
<td>4.1</td>
<td>16.2</td>
<td>4.4</td>
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<td>-15.1</td>
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<td>-52.4</td>
<td>0.1</td>
<td>11.2</td>
<td>-4.1</td>
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<td>Normal</td>
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<td>6.5</td>
<td>0.3</td>
<td>15.6</td>
<td>0.7</td>
<td>16.5</td>
<td>3.3</td>
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<tr>
<td></td>
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<td>0.1</td>
<td>11.2</td>
<td>-4.1</td>
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</table>

Extraction, a comparative study of lipid transport was possible. The possibility that cold ether extraction could modify the electrophoretic mobility of the components was investigated. There was no significant change. Lipids and cholesterol were determined on separate aliquots. When the phosphate buffer was used, the total lipids and fatty acids were determined by the method of Bloor, total cholesterol by a modification of the Sackett method, and the lipid phosphorous after Whitehorn. When barbiturate buffer was used, the fatty acids were determined according to the method of Bauer and Hirsch, the cholesterol determinations according to Bloor, and the lipid phosphorous after Fiske and Subbarow. Occasionally there was not enough serum obtained for all of the determinations. In the group of patients studied with the barbiturate buffer, total lipids were not determined.

Serum was used throughout this study rather than plasma to avoid the fibrinogen peak. Calculations of the areas in the electrophoretic patterns were expressed as percent of the total area.

Results

The figures in the tables are the mean figures of many determinations. It would be impossible to include all of them in any one report. More detailed information will appear in other reports.

Table 1. The analyses in this group of 43 patients studied with a phosphate buffer, showed variable decreases in albumin of minor degree except in primary essential xanthomatosis where there was a marked decrease. The alpha and beta globulins were uniformly elevated to some degree in all of these diseases. The only disease which showed even a slight elevation of the gamma globulin was hypertensive vascular disease. In regard to the lipid associated with these protein fractions, the loss after ether extraction by this method was fairly uniform except for the gamma globulin fraction. This uniformity of the means is, however, quite deceptive, since the individual determinations often showed considerable variation not only with each other in the same disease group, but in the same individual.

Table 2. The analyses in this group of 68
patients studied with barbiturate buffer, showed essentially the same trend. Again variable decreases in albumin were seen which was most marked in primary essential xanthomatosis. The alpha-1 globulin remained fairly constant. The alpha-2 and beta globulins were slightly increased in some groups and more markedly increased in others. The gamma globulin was slightly elevated in hypertensive vascular disease.

After ether extraction the difference in pattern area was calculated and expressed as the per cent change in area loss. This loss was greater in most of these diseases than it was in the normal controls, regardless of the type of buffer used. The percentages of the protein components are not comparable for these two buffers in regard to absolute figures except for the beta globulin. In spite of this the trend of the patterns is the same when compared to the normal controls for each buffer.

The variation of the lipoprotein pattern was previously reported by one of us.\textsuperscript{15} A biologic swing was noted by Jones, Gofman, and their group\textsuperscript{16} which has not been sufficiently studied or emphasized. In our studies, this variation in the same individual was such as almost to nullify the value of any single determination. Multiple determinations in the same individual over some period of time is necessary in order to decrease the errors possible with single specimens of such variable components. This variability is noted in another report from our laboratory,\textsuperscript{17} although it is indicated by the standard deviation given for each group in tables 1 and 2. It is quite possible that this variation is responsible for the recent conflicting reports of ultracentrifugal studies in these diseases. Although most of these studies in this present report were made in the fasting state, there is no difference in patterns obtained in the nonfast-

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Disease} & \textbf{Patients} & \multicolumn{9}{c|}{\textbf{Distribution of Electrophoretic Components}} \\
\hline
 & & \textbf{Albumin} & & & & & & & & \\
 & & \textbf{Globulins} & & & & & & & & \\
 & & \textbf{\%} & \textbf{S.D.} & \textbf{\%} & \textbf{S.D.} & \textbf{\%} & \textbf{S.D.} & \textbf{\%} & \textbf{S.D.} & \textbf{\%} & \textbf{S.D.} \\
\hline
Angina & 2 & 53.7 & 6.6 & 5.1 & 0.5 & 10.7 & 2.3 & 19.9 & 4.2 & 10.2 & 0.4 \\
 & & -20.6 & & -22.0 & & -21.6 & & -68.6 & & -19.7 \\
Arteriosclerosis obliterans & 21 & 56.0 & 3.7 & 4.9 & 1.1 & 11.0 & 2.6 & 16.0 & 2.2 & 12.3 & 1.9 \\
Hypertensive vascular & 11 & 54.2 & 7.2 & 5.7 & 1.1 & 9.7 & 2.7 & 15.8 & 3.0 & 14.1 & 6.6 \\
 & & -16.7 & & -19.6 & & -10.0 & & -58.0 & & -23.1 \\
Hypothyroid & 3 & 54.6 & 4.5 & 5.5 & 1.3 & 10.6 & 1.3 & 16.3 & 0.7 & 13.0 & 4.9 \\
 & & -8.9 & & -19.6 & & -15.0 & & -45.5 & & -10.8 \\
Myocardial infarction & 6 & 54.0 & 7.8 & 5.9 & 2.3 & 12.2 & 4.8 & 17.0 & 1.4 & 10.8 & 2.0 \\
 & & -14.5 & & -18.9 & & -26.4 & & -57.7 & & -20.7 \\
Thromboangiitis obliterans & 13 & 57.3 & 3.9 & 5.3 & 0.9 & 9.8 & 2.4 & 15.7 & 2.2 & 12.1 & 1.0 \\
 & & -12.7 & & -20.6 & & -26.9 & & -57.4 & & -34.3 \\
Primary essential xanthomatosi & 12 & 50.3 & 4.4 & 5.0 & 0.9 & 10.3 & 0.9 & 22.0 & 2.7 & 12.6 & 2.5 \\
 & & -9.6 & & -18.4 & & -12.3 & & -63.2 & & -17.1 \\
Normal & 41 & 61.0 & 4.9 & 4.9 & 0.5 & 8.9 & 0.8 & 14.9 & 2.4 & 12.0 & 2.7 \\
 & & -7.4 & & -5.4 & & -10.2 & & -55.1 & & -16.9 \\
\hline
\end{tabular}
\caption{Electrophoretic Analyses of Serum from 68 Patients Using Barbiturate Buffer at a pH of 8.6. Component Distribution Is Given in Per Cent of Pattern Area with the Standard Deviation (S.D.). The Percentage Loss in Pattern Area after Cold Ether Extraction Is Given Underneath the Original Values. These Are Mean Figures of 88 Determinations.}
\end{table}
ing state as far as we could determine by these methods. Therefore, these variations are probably not directly due to the immediate food intake.

**Arteriosclerosis Obliterans.** Table 3 shows the difference in protein pattern in 32 patients with the identical disease, arteriosclerosis obliterans, divided into two main groups by buffer and the absence or presence of hypercholesteremia. This table demonstrates the higher percentage of beta globulin in those patients with hyperlipemia and/or hypercholesteremia than in those with normal levels regardless of the type of buffer used.

**Thromboangiitis Obliterans.** Table 4 gives the findings in 28 patients with thromboangiitis obliterans divided again into groups by buffer and the presence or absence of hypercholesterolemia. There was practically no difference in the mean beta globulin of these patients regardless of the level of the lipids or the type of buffer used. The patients with hyperlipemia appeared to have a slightly higher alpha-2 globulin and a slightly lower gamma globulin than those with normal levels. The mean determinations of the entire group showed a slight elevation of the beta globulin which was not really significant, so that the electrophoretic pattern as a whole appears much the same as the normal controls.

**Myocardial Infarction.** All of these patients showed an increase in alpha-2 globulin and beta globulin with a decrease in albumin. The highest alpha-2 globulins were seen in three patients who were in shock when the blood specimens were secured. One of these patients died in 12 hours, a second in 24 hours, and the third in several days. The elevation of the alpha-2 globulin in shock has been described in the experimental animal by Moore and by Lewis, Page, and Glasser. The increase in this component in human shock has not to our knowledge been previously reported.

**Angina.** Patients with this complaint all showed hypercholesteremia with a reflected increase in the beta globulin and a decrease in the albumin. None of these patients had evidence of myocardial infarction.

**Hypertensive Vascular Disease.** None of the

### Table 3.—Electrophoretic Analyses of Single Specimens of Serum from 32 Patients with Arteriosclerosis Obliterans. Component Distribution Is Given in Per Cent of Pattern Area

<table>
<thead>
<tr>
<th>Hyperlipemia</th>
<th>Buffer</th>
<th>Distribution of Electrophoretic Components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Albumin</td>
</tr>
<tr>
<td>Present</td>
<td>Phosphate, pH 7.4</td>
<td>57.0</td>
</tr>
<tr>
<td>Absent</td>
<td>pH 7.4</td>
<td>58.0</td>
</tr>
<tr>
<td>Present</td>
<td>Barbiturate, pH 8.6</td>
<td>56.5</td>
</tr>
<tr>
<td>Absent</td>
<td>pH 8.6</td>
<td>58.2</td>
</tr>
</tbody>
</table>

### Table 4.—Electrophoretic Analyses of Single Specimens of Serum from 28 Patients with Thromboangiitis Obliterans. Component Distribution Is Given in Per Cent of Pattern Area

<table>
<thead>
<tr>
<th>Hyperlipemia</th>
<th>Buffer</th>
<th>Distribution of Electrophoretic Components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Albumin</td>
</tr>
<tr>
<td>Present</td>
<td>Phosphate, pH 7.4</td>
<td>60.3</td>
</tr>
<tr>
<td>Absent</td>
<td>pH 7.4</td>
<td>59.5</td>
</tr>
<tr>
<td>Present</td>
<td>Barbiturate, pH 8.6</td>
<td>57.8</td>
</tr>
<tr>
<td>Absent</td>
<td>pH 8.6</td>
<td>54.8</td>
</tr>
</tbody>
</table>

10 patients examined had evidence of myocardial infarction. One female member of this group had had two episodes of cerebral vascular accidents. They had decreases in albumin with slight increases in beta and gamma globulin.

**Hypothyroidism.** These patients showed varying degrees of hypercholesteremia and variable increases in beta globulin. No characteristic pattern was noted. None of these patients had myxedema.

**Primary Essential Xanthomatosis.** Reported in this group are the four types described by Thannhauser, xanthelasma, tendon xanthoma, xanthoma tuberosum, and the forme fruste. Analysis of the serum, which was clear, fulfilled the criteria as described. Eight of these patients were females. All of these patients were from families where there were
histories of death from myocardial infarction of from one to three males in the immediate family. Two pairs of patients were related. Analysis of this group of patients showed a marked decrease in albumin and a marked increase in the beta globulin. Although the mean gamma globulin is within normal limits, at different intervals of time individual determinations frequently revealed a rise in the gamma globulin.

**Normal Controls and Professional Blood Donors.** The normal controls were made up of student nurses, technicians, physicians, and patients without obvious disease seen for routine examination. The professional donors were from two active blood banks in New York City hospitals. These included the usual share of derelicts. The pooled sera was from one of these blood banks and was divided into six samples each containing serum from three or four individuals. Table 5 gives a comparison of several samplings of this blood bank serum and the normal controls.

The analysis of the pooled sera is similar to that reported by Reiner, Fenichel, and Stern for professional blood donors. They reported a difference in professional and nonprofessional blood donors. This difference is also apparent in our study. This difference is even more marked when the nonpooled sera of the other two groups is compared with the sera of our normal nonprofessional group. Protein patterns in many of these individual donors showed marked changes in the albumin-globulin ratio. Thymol turbidity tests and cephalin flocculation tests were often positive. In the group of nine donors, two patterns were within normal range. The other seven were so abnormal as to produce an abnormal mean in spite of two normal patterns. The mean figures of the 10 donors from another blood bank were little better. As one can see from the pooled samples, it is possible with an increase in the number of sera, to approach a more normal pattern. Thus pooled plasma with a sufficient number of normal donors might supply a solution with a normal albumin-globulin ratio but still contain abnormal albumins and globulins. Moreover, it is whole blood that is in common use since it should possess many advantages over plasma. Therefore, the use of blood which does not have a normal protein content is to be deplored. Not only is the attending physician or surgeon misled by a false sense of security, but the patient himself is endangered by inadequate therapy and the possibility of complications from the transfusion itself. Proper screening of donors would eliminate a large part of this risk. Several laboratory procedures could be used to eliminate blood which is found to be undesirable after it has slipped through the screening of the donors. Commercial laboratories using blood donors for various biologic products should also take heed of this problem since this situation is one which could produce some complicated medicolegal problems.

**Discussion**

In the 96 patients with eight types of disease in which atherosclerosis is common, there is one common finding. This is the increase in the beta globulin. This is, however, not true for every individual with these diseases. While an examination of the mean determinations shows a trend toward an average pattern for
each group, it cannot be said that these patterns have any real diagnostic significance as a test for the presence or absence of atherosclerosis. There was no correlation between the severity of the lesions observed clinically, and the degree of increase in the beta globulin.

The groups of patients studied using the phosphate buffer at a pH of 7.4 showed a higher gamma globulin than did the groups studied with the barbiturate buffer at a pH of 8.6. This applied to the normal subjects too. This is probably due to the inclusion of an epsilon boundary in the gamma globulin of the phosphate buffer. Since the albumin plus the alpha1 of the barbiturate buffer should roughly equal the albumin of the phosphate buffer, the correlation of the percentages of these fractions for the two buffers is poor. These are technical considerations which will be discussed at greater length elsewhere. It does however emphasize the importance of the buffer and pH in comparing data on electrophoresis.

In considering these disease groups further, those patients with arteriosclerosis obliterans showed a good correlation between hyperlipemia and an increase in beta globulin. However there was a poor correlation between hyperlipemia and this disease, since 50 per cent of these patients had neither a hyperlipemia or hypercholesteremia.

In thromboangiitis obliterans, the slight increase in beta globulin in the absence of hypercholesteremia is unexpected. Since this disease is basically characterized by an inflammatory lesion, one would have expected an increase in the gamma globulin, since this fraction is said to contain the antibodies. There was, however, no increase in this fraction in the group of patients studied. An increase in beta globulin without an increase in serum lipids is also seen in pulmonary tuberculosis and occasionally in sarcoidosis. The significance of this finding is not clear. Some investigators believe that this increase is an indication of healing. The possibility that the beta globulin contains antibodies must be considered.

In primary essential xanthomatosis, not only is the albumin fraction decreased, but the amount of associated lipid lost after ether extraction is practically the same percentage loss as normal. Therefore, the amount of lipid associated with the albumin fraction is decreased in spite of the great increase in serum lipids. The small amount of lipid associated with the albumin fraction in primary biliary cirrhosis in the presence of greatly increased serum lipids has been noted by Kunkel and Slater.

Conclusions

1. In the disease groups presented, the most significant decrease in the albumin fraction measured by electrophoresis was in primary essential xanthomatosis.

2. The alpha1 globulin remained practically normal.

3. The alpha2 globulin was slightly increased in all of these diseases to about the same degree except in those patients in shock due to myocardial infarction in whom it was markedly increased.

4. The beta globulin was increased to some degree in all of these diseases and was increased most in primary essential xanthomatosis.

5. The gamma globulin was within normal limits except in hypertensive vascular disease where it was slightly increased.

6. In arteriosclerosis obliterans, the beta globulin was usually within normal limits in the absence of hyperlipemia or hypercholesteremia. About 50 per cent of the patients with this disease observed in this study had normal serum lipid levels.

7. In thromboangiitis obliterans, the slight increase in the beta globulin fraction had no relation to the level of the serum lipids.

8. The professional donors had a lower albumin fraction and a higher globulin content than the nonprofessional donors. Many professional donors had a reversal of the albumin-globulin ratio.

Acknowledgments

Technical assistance was ably provided by Betsy Neustadt, B.A., Joan Roberts, B.S., M.S., and Jane Gordon, B.S. Considerable assistance was rendered by the Service Bureau of International Business Machines in analysing the data presented.

Sumario Español

1. En el grupo de enfermedades presentado, el decremento mas significativo en la fracción
de albumina medida por electroforesis fué en xantomatosis esencial primaria.

2. La globulina alpha, permaneció prácticamente normal.

3. La globulina alpha aumentó ligeramente en todas estas enfermedades más o menos el mismo grado excepto en aquellos pacientes en choque debido a infartos de miocardio en los cuales hubo un aumento marcado.

4. La globulina beta aumentó en algún grado en todas estas enfermedades y aumentó en grado mayor en la xantomatosis esencial primaria.

5. La globulina gama se encontró entre los límites normales excepto en la enfermedad hipertensivo vascular donde se encontró ligeramente aumentada.

6. En la arterioesclerosis obliterate, la globulina beta se encontró usualmente entre los límites normales en la ausencia de hiperlipemia o hipercolesterolemia. Aproximadamente 50 por ciento de los pacientes con esta enfermedad observados en este estudio tuvieron niveles de lípido del suero normales.

7. En la tromboangitis obliterate, el pequeño incremento en la fracción de globulinas beta no tuvo relación alguna al nivel de lípidos del suero.

8. Los donantes profesionales tuvieron una fracción de albúmina baja y un contenido mas alto de globulina que los donantes no profesionales. Muchos de los donantes profesionales tuvieron una inversión de la relación de albúmina-globulina.

REFERENCES
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7. Unpublished data.
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