Effect of Chylomicrons on the Fibrinolytic Activity of Normal Human Plasma in Vitro

By THOMAS C. MERIGAN, M.D., JOHN W. FARQUHAR, M.D., JAMES H. WILLIAMS, M.D., AND MAURICE SOKOLOW, M.D.

The reported inhibition of fibrinolytic activity of blood plasma after ingestion of fat has been confirmed. It is shown that the chylomicrons present in such plasma are responsible for this phenomenon.

THE PLASMA of normal fasted human subjects possesses fibrinolytic activity which can be quantitated by measuring the in vitro lysis of fibrin clots, plasma clots, or whole blood clots.1-3 This fibrinolytic activity may be important both in maintaining the normal fluidity of blood4 and in preventing the deposition and organization of fibrin on the endothelial lining of blood vessels. In 1956 Greig reported that fibrinolysis of human plasma is inhibited by prior ingestion of varying amounts of mixed animals fats;5 later he concluded that the inhibition resulted from the appearance of both chylomicrons and beta-lipoproteins in the plasma.6

The purposes of this study were to confirm the effect of lipemia on fibrinolysis, to evaluate the effect of ingestion of butterfat on this system, and to determine if the lowest density lipoproteins that appear in the blood post-prandially (chylomicrons) are responsible for inhibiting fibrinolysis. Since the change in plasma lipids after ingestion of fat consists primarily in the appearance of chylomicrons,8 it seemed appropriate to study first the effect of this fraction on the fibrinolytic process. This was done by contrasting the fibrinolytic activity of a chylomicron-rich and a chylomicron-poor fraction of plasma. The plasma was derived from blood of normal subjects drawn both after ingestion of butterfat and in the fasting state.

METHODS

The study was divided as follows: Part A. Six experiments were performed on plasma drawn from 5 subjects four hours after they had ingested a meal containing 1.5 Gm. of butterfat (as cream)/Kg. of body weight. These subjects had otherwise fasted for 12 hours prior to venesection. Part B. Eight similar experiments were performed on plasma drawn from fasting subjects 12 hours after their last meal.

All steps involving blood or plasma, except the blood sampling, were carried out at a temperature of approximately 5 C. (All venesections were performed between 7:30 and 8:30 a.m.) Free-flowing venous blood was drawn from each subject into a sterile siliconized precooled syringe. The blood was immediately placed in an ice-cooled Erlenmeyer flask for 2 to 3 minutes. It was then delivered into lusteroid tubes, 13.5 ml. in capacity, and spun for 5 minutes at 27,500 r.p.m. (50,000 X gravity) in the no. 40 rotor of a Spinco Model L refrigerated ultracentrifuge. This step separated the plasma into a clear bottom fraction and a turbid top fraction containing chylomicrons. Immediately after centrifugation the bottom fraction (fraction II) was removed from the tubes. The residual top fraction was also removed, and its turbid material homogenously dispersed throughout it (fraction I). A series of 40 siliconized tubes (15 by 100 mm.) containing 2.4 ml. of 0.04 M veronal buffer (pH 7.4) and 0.1 per cent CaCl₂ were prepared. One tenth ml. of fraction I was added to 20 of the tubes, and 0.1 ml. of fraction II to the remaining 20. The contents of each tube were mixed with individual roughened glass stirring rods. The tubes (with rods in place) were transferred to a 38 C. water bath, where clotting occurred promptly (within 20 minutes). After a 40 minute period of incubation, duplicate
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1/2 INCUBATION TIME
TOTAL INCUBATION TIME

FIG. 1. Average percentage of clot lysis of plasma of 6 subjects four hours after ingestion of 1.5 Gm. butterfat per Kg. body weight.

or triplicate measurements of fibrinolytic activity were made at timed intervals.

Fibrinolytic activity was measured by the method of Fearnley and Lackner, residual clot protein was determined by the colorimetric method of Bidwell. In this method the degree of clot lysis is measured at serial time intervals until visible clot fragmentation occurs. The percentage of the original clot lysed was expressed as change in optical density following the incubation period divided by optical density of the digestant of the initial (40 minute) sample.

Lipid determinations were performed as follows: Total cholesterol was measured by the method of Abell et al., phospholipid by the method of Stewart and Hendry, and total lipids by the method of Bragdon. Plasma triglycerides were calculated by the method of Bragdon; in these calculations a free cholesterol/total cholesterol ratio of 0.27 was assumed, and lipid phosphorus was converted to phospholipid by the use of the factor 25.

RESULTS

Plasma derived from the subjects showed varied fibrinolytic activity. In order to compare the data obtained, the fibrinolysis times were expressed as total incubation time (averaging 15 hours) and one half total incubation time (averaging 7½ hours). To test the reliability of the procedure we determined the coefficient of variation between the duplicate measurements of fibrinolysis of all samples. These figures were ± 9 per cent for part A and ± 2.7 per cent for part B of the study.

Comparison of Fibrinolytic Activity of Plasma Fractions Following Ingestion of Cream (Summarized in Figure 1). The fibrinolytic activity of fraction I (containing "chylomicrons") was consistently less than that of fraction II (optically clear) in each of the cream-feeding experiments. At the final incubation time, fraction I averaged 17 per cent in clot lysis and fraction II averaged 44 per cent in clot lysis; therefore the fibrinolytic activity of fraction I was 61 per cent less than that of fraction II (table 1). The average difference in lysis in the two fractions at all points in time was statistically significant (p < 0.001). The correlated means formula was used to calculate the significance of the differences between lysis of the two fractions.

The results of plasma lipid determinations in the butterfat study are given in table 2. Fraction I contained slightly more phospholipid and cholesterol than did fraction II. The triglyceride content of the two fractions, however, differed strikingly, amounting to 270 mg./100 ml. in fraction I and 162 mg./100 ml. in fraction II, a difference of 108 mg./100 ml. The difference between the total lipid of fraction I and fraction II was 82 per cent triglyceride; this is consistent with the per cent triglyceride composition of human chylomicrons, as reported by Bragdon et al. and by Laurell, and confirmed our supposition that the lipoproteins removed by ultracentrifugation were chylomicrons.

Comparison of Fibrinolytic Activity of Plasma Fractions in Fasting Subjects (Summarized in Figure 2). In the 8 experiments

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasted 12 hours</th>
<th>Post-prandial 4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. All</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>B. Clear F I</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>C. Turbid F I</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>LI - F I difference</td>
<td>27</td>
<td>0.001</td>
</tr>
</tbody>
</table>

(1.5 Gm. Butterfat/Kg. B.W.)

Table 1. — Summary of Average Percentage Plasma Clot Lysis of Normal Subjects (Measurements after Fasting and After Cream Ingestion)
EFFECT OF CHYLOMICRONS ON HUMAN PLASMA

TABLE 2.—Summary of Average Plasma Lipid Differences in Fraction I and II of Plasma of Normal Subjects after Fasting and Cream Ingestion

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>Fraction</th>
<th>Total lipid (mg./100 ml.)</th>
<th>Total cholesterol (mg./100 ml.)</th>
<th>Phospholipid (mg./100 ml.)</th>
<th>Calculated triglyceride (mg./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted 12 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. All</td>
<td>8</td>
<td>I</td>
<td>631</td>
<td>211</td>
<td>262</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>609</td>
<td>211</td>
<td>252</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Difference</td>
<td>22</td>
<td>0</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>B. Clear fraction I</td>
<td>4</td>
<td>I</td>
<td>593</td>
<td>208</td>
<td>253</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>590</td>
<td>210</td>
<td>244</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Difference</td>
<td>3</td>
<td>-2</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>C. Turbid fraction I</td>
<td>4</td>
<td>I</td>
<td>669</td>
<td>213</td>
<td>271</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>628</td>
<td>211</td>
<td>260</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Difference</td>
<td>41</td>
<td>2</td>
<td>11</td>
<td>33</td>
</tr>
<tr>
<td>Postprandial 4 hours</td>
<td>6</td>
<td>I</td>
<td>858</td>
<td>209</td>
<td>301</td>
<td>270</td>
</tr>
<tr>
<td>1.5 Gm. butterfat per Kg. body weight</td>
<td></td>
<td>II</td>
<td>727</td>
<td>208</td>
<td>286</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Difference</td>
<td>131</td>
<td>1</td>
<td>15</td>
<td>108</td>
</tr>
</tbody>
</table>

Fig. 2. Average percentage of clot lysis of plasma (8 determinations) of 6 fasted subjects.

in this series the average degree of lysis at the end of the incubation period was 6 percent less in fraction I than in fraction II (table 1). This difference was not statistically significant (p < 0.2). Although the rate of fibrinolysis of fraction I and II did not differ significantly, in four experiments fraction I was slightly turbid and showed a modest decrease in fibrinolytic activity (table 1). The turbidity was believed to result from the small amounts of low density lipoproteins normally found in plasma from fasting subjects, or possibly from the presence of chylomicrons derived from previously ingested fat. Lipid determinations in these four experiments showed that the average triglyceride content was slightly higher in fraction I than in fraction II (33 mg./100 ml.) (table 2). In the remaining four experiments both fraction I and II were visibly clear. Little difference was found in the rate and amount of fibrinolysis (fig. 2) and in the lipid concentration of these fractions (table 2).

DISCUSSION

The plasma of our subjects was separated into two fractions, one from which chylomicrons had been removed by centrifugation (fraction II) and one in which the chylomicrons of the total sample had been resuspended (fraction I). Results of experiments on these fractions confirmed the in vitro fibrinolytic activity of plasma from normal human subjects. In addition, although our observations were limited to plasma drawn four hours after the subject had ingested fairly large amounts of butterfat (average, 110 Gm.), we concluded that the inhibition of fibrinolysis after ingestion of animal fat reported by Greig is caused by an increased concentration of plasma chylomicrons. This conclusion is based on our observations that (1) the fibrinolytic activity of fraction I in subjects who ingested butterfat was consistently less than that of fraction II, (2) the fibrinolytic activity of fraction I and fraction II of plasma from the fasting subjects did not differ, and (3) the composition of the inhibitory lipid in fraction I was
similar to that reported for chylomicrons isolated either by precipitation with toluidine blue\textsuperscript{15} or by ultracentrifugation.\textsuperscript{14} Our conclusions appear to differ from those of Greig and Runde,\textsuperscript{7} who stated that the inhibitory lipid of postprandial plasma is beta-lipoprotein. This apparent contradiction results in part from the fact that we do not consider chylomicrons to be a subgroup of beta-lipoproteins since they migrate with alpha-2 globulins in electrophoretic methods.\textsuperscript{10} The use of ultracentrifugal techniques for separation of chylomicrons allowed us to define with certainty the nature of the lipid fraction which affects fibrinolytic activity. The lipid solvent methods utilized by Greig and Runde did not allow such definition, since their electrophoretic data indicated that both beta-lipoprotein and chylomicrons were removed during extraction of the plasma.

The question arises as to the significance of the inhibition of lysis we observed, particularly since a much greater change in lysis occurs normally on a diurnal basis and after exercise or administration of epinephrine.\textsuperscript{2-3} We cannot be sure that the in vitro system we have measured reflects a process that operates in vivo, particularly since the activity is increased in greater dilutions of plasma.\textsuperscript{4} However, the fact that the intravenous administration of plasmin (fibrinolysin) increases the rate of disappearance of intravascular clots in experimental animals\textsuperscript{17} supports the hypothesis that clot dissolution in vivo depends on the fibrinolytic mechanism. Also, plasmin has been found to adsorb on plasma clots in vitro.\textsuperscript{18} Accordingly, an enzyme which is present in small quantities in plasma, by adsorption, may reach sufficient concentration in the region of the clot to account for its dissolution by lysis.

**Summary**

The in vitro fibrinolytic activity of the plasma of 5 normal subjects was found to be consistently inhibited (average inhibition, 61 per cent) when measured four hours after they had ingested a test meal containing 1.5 Gm. of butterfat/Kg. of body weight. Our experiments indicate that a single species of plasma lipoproteins (chylomicrons) is responsible for the observed inhibition. Studies now in progress will attempt to define the properties of human chylomicrons responsible for this inhibition.

**Acknowledgment**

We wish to thank Mrs. J. Moore and Miss R. Zinow for their technical assistance and Dr. Richard J. Havel for his advice and encouragement.

**SUMMARIO IN INTERLINGUA**

Esseva constatate que le activitate fibrinolytic in vitro de specimen du plasma ab 5 subjectos normal esseva inhibite regularmente (con un inhibition medie de 61 pro cento) quando illo esseva mesurate 4 horas post que le subjectos habeva ingerite un repasto experimental continente 1.5 g de grassia butyric per kg de peso corporee. Nostre experimentos indica que un unic specie de lipoproteinas del plasma (chylomicrons) es responsabile pro le observate inhibition. Studios nunc in progresso ha le obiectivo de definir le proprietates de chylomicrons human que es responsabile pro le inhibition.

**References**


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