Studies of Blood Coagulation and Fibrinolysis in Patients with Idiopathic Hyperlipemia and Primary Hypercholesterolemia before and after a Fatty Meal

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In view of the current interest in the possible relationship between elevated serum lipid levels, increased coagulability of the blood (or decreased blood fibrinolysis) and ischemic heart disease, the clotting and fibrinolytic activity of the blood were studied before and after a fatty meal in 9 patients with idiopathic hyperlipemia, 10 patients with primary hypercholesterolemia, and 10 normal subjects. Six patients had evidence of coronary heart disease. The methods of study included procedures designed to assess the over-all coagulability of the blood as well as the measurement of specific clotting factors. Blood fibrinolytic activity was measured by both direct and indirect methods. Serum lipid studies were performed simultaneously.

During recent years, increasing attention has been paid to the role of the serum lipids in the development of atherosclerosis with particular reference to coronary atherosclerosis. There has also been much discussion concerning the possible significance of the state of coagulability of the blood in the pathogenesis of coronary atherosclerosis since the suggestion by Duguid that atherosclerotic plaques may be derived from mural fibrin thrombi. Indeed, it has even been postulated that intravascular coagulation is a constant process and that small fibrin strands are being continuously deposited on the vascular endothelium only to be removed by the naturally occurring process of fibrinolysis. According to this concept, any shift in such a delicate equilibrium toward either an increase in blood clotting activity or a decrease in blood fibrinolytic activity would allow excessive deposition of fibrin even to the extent of causing complete vascular occlusion. Some workers have contended that hyperlipemia may disturb this balance, leading not only to acceleration of blood clotting but also to retardation of blood fibrinolysis. Most of the previous reports in this field, however, have been based upon evaluation of the clotting and fibrinolytic activity of the blood either after the ingestion of a fatty meal in normal subjects or after the addition of fat to plasma in vitro. So far only a single report is available regarding blood coagulation activity in patients with abnormal lipid metabolism in whom hyperlipemia or hypercholesterolemia, or both, exist as part of an inherent disease process. In these patients a high blood lipid level is present even in the fasting state.

It was therefore decided to study the clotting and fibrinolytic activity of the blood in a selected group of patients with idiopathic hyperlipemia and primary hypercholesterolemia (including those with familial xanthomatosis) before and after a fatty meal. Moreover, investigation of these patients seemed of additional interest, since many of them develop widespread atherosclerosis and early coronary disease. Observations on this unique group of patients might be of value in defining the relationship between elevated serum lipid levels (i.e., hyperlipemia or hypercholesterolemia), increased coagulability of the blood (or decreased blood fibrinolysis), and ischemic heart disease.

Material and Methods

The subjects were divided into 3 groups. The first group consisted of 9 patients with idiopathic

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hyperlipemia, a disorder of fat metabolism, usually familial, characterized by lactescent serum and high concentrations of triglycerides in the fasting state, with or without high serum cholesterol and phospholipid levels. There were 7 males and 2 females in this group, ranging in age from 25 to 55 years. Cases 1, 3, and 9 had extensive xanthomatous lesions. Cases 1, 3, and 5 had clinical and electrocardiographic evidence of ischemic heart disease, amounting in case 1 to acute coronary heart disease without major infarction.

The second group was composed of 10 patients with primary hypercholesteremia, a disorder by clear serum with high concentrations of cholesterol in the fasting state. There is usually a concomitant elevation of the serum phospholipid levels. None of the patients in this series showed evidence of diabetes mellitus, nephrosis, or myocardia. There were 5 males and 5 females in the hypercholesteremic group, ranging in age from 27 to 61 years. Cases 10, 11, 13, 17, and 18 had extensive xanthomatous lesions. Cases 10, 17, and 18 had clinical and electrocardiographic evidence of ischemic heart disease amounting in cases 10 and 17 to acute coronary heart disease without major infarction.

Ten normal control subjects comprised the third group (8 males and 2 females, ages 19 to 39). The subjects in this group were chosen for the following reasons: (1) relative youth, (2) clear fasting serum with normal triglyceride and cholesterol levels, (3) absence of xanthomatous lesions, (4) no evidence of ischemic heart disease.

The subjects were instructed to fast after 10:00 p.m. and blood was drawn at 9:00 a.m. on the following morning. They were then given a meal consisting of 2 eggs, 3 strips of bacon, 2 pats of butter, and 4 ounces of 20 per cent cream along with toast, cereal, and coffee; this meal contained 85 Gm. of fat. All subjects were supervised during the breakfast to ensure that the entire meal was consumed. Postprandial blood specimens were obtained 4 hours after ingestion of the meal during the maximal lipemic effects. Blood was withdrawn by clean venipuncture with a 19-gage siliconized needle with use of the 2-syringe technic. Blood obtained in the first syringe was used for the determination of the serum lipids. The subsequent blood was drawn into a chilled 30-ml siliconized syringe and a stop watch was started as soon as the blood entered the syringe. Subsequently 1.5 ml of blood was withdrawn through the same needle into a calibrated siliconized tuberculin syringe for thrombelastography, a separate stop watch being started as soon as blood entered the syringe. While a technician was emptying the blood from the large silicone syringe into the appropriate test tubes, one of the workers immediately placed 0.35 ml of the blood from the tuberculin syringe into each of the 3 cuvettes of the thrombelastograph. The blood from the large silicone syringe was distributed as follows: (a) 1.0 ml into each of 6 siliconized test tubes for the whole blood clotting time, (b) 9.0 ml into a chilled plastic tube containing 1.0 ml of 2.5 per cent sodium citrate for spinning in the refrigerated centrifuge for 30 minutes at 4,000 r.p.m. to obtain platelet-poor plasma, (c) 4.5 ml into each of 3 plain glass tubes containing 0.5 ml of 0.1 M sodium oxalate for centrifuging at 1,500 r.p.m. for 10 minutes to obtain platelet-rich plasma.

**Coagulation Methods**

The *prothrombin concentration of plasma* was determined by the modified method of Owren as described by Alexander. A saline extract of acetone-dried human brain was used as the source of thromboplastin. Factor V (labile factor) *activity* was measured by determining the corrective effect on the prothrombin time of aged plasma (The substrate had normal values for prothrombin, factor VII, and fibrinogen.) Factor VII (S.P.C.A.) *activity* was measured by a modification of the Owren procedure. The *plasma fibrinogen level* was determined by the method of Ratnoff and Menzie as modified by Holburn. The normal fasting levels were between 200 and 400 mg. per 100 ml. of plasma. The *coagulation time of recalified plasma* was performed on platelet-rich plasma and platelet-poor plasma: 0.1 ml of 0.9 per cent saline and 0.1 ml of 0.02 M calcium chloride were added to 0.1 ml of the respective plasmas and the clotting times were estimated in duplicate. The normal fasting values for the platelet-rich plasmas were between 75-105 seconds and for the platelet-poor plasmas were greater than 2 minutes. The error between duplicate samples was ± 15 seconds (platelet-rich plasma) and ± 30 seconds (platelet-poor plasma). The *whole blood clotting time in silicone glassware* (Lee and White method as modified by Merskey) was recorded as the mean of the clotting time in 6 tubes (13 × 100 mm.). The normal fasting values for this test were 20 to 40 minutes. The *prothrombin consumption* of plasma and serum was determined as mentioned above. Serum for this test was obtained by recalifying 0.5 ml of platelet-poor plasma with 0.1 ml of 0.1 M calcium chloride. At the end of 1 hour 0.1 ml of 3.8 per cent sodium citrate was added and the serum was separated. The normal fasting values for this test were 30 to 75 per cent. The *heparin tolerance test* was performed as described by Soulier and Le Bolloch. The end-point in the fourth tube was used for comparison of the clotting ability of the fasting and postprandial
plasmas in the presence of heparin. The normal fasting clotting time in the fourth tube ranged between 10 and 14.5 minutes. Thrombelastography. A Hellige thrombelastogram was employed, determinations being performed on whole blood according to the method of Harter. The 3 components of the thrombelastogram that reflect the coagulability of the blood are 1. "R" (reaction time) indicating the period from the entry of blood into the syringe to the initial formation of fibrin in the cuvette. The normal fasting values were between 9.0 and 11.5 minutes. 2. "K" indicating the interval from the end of "R" to the end-point of the clot formation. The normal fasting values were 5.0 to 6.75 minutes. 3. "MA" (maximal amplitude) measuring the elasticity of the clot. The normal fasting values were between 46 and 50 mm. Shortened "R" and "K" values and lengthened "MA" values indicate increased coagulability of the blood. The thromboplastin generation test as originally modified by Hicks and Pitney was further modified as follows: 1. Platelet-poor plasma was used. 2. Inosithin was diluted in saline in a concentration of 0.5 mg./ml. to determine the potency of this thromboplastin material. 3. The test was repeated by further diluting the previously prepared Inosithin to 1 in 150 of veronal-buffer-isotonic saline mixture at pH 7.4. Clotting times were determined at 1-minute intervals for 15 minutes. With normal fasting plasma, clotting times of 8 to 10 seconds were obtained after 10 minutes of incubation. The Inosithin was diluted in order to increase the sensitivity of the test. 4. The thromboplastin generation test was also determined without the addition of any foreign thromboplastin material in order to detect thromboplastin activity in the plasma were such present. Clotting times were determined at 5-minute intervals for 30 minutes. Fasting clotting times ranging between 20 and 50 seconds were obtained after 15 to 25 minutes of incubation. This procedure was performed at 37 C. The 1-stage prothrombin time (Quick's method). A saline extract of acetone dried human brain was used as the source of thromboplastin. The normal fasting values were between 12 and 14 seconds. The Stypven time (Russell viper venom test). Russell viper venom (Stypven) was diluted 1 in 10,000 with 0.9 per cent saline; 0.1 ml. of platelet-poor plasma diluted 1:2 with 0.9 per cent saline was incubated at 37 C. with 0.1 ml. of the previously prepared Stypven, and after 1 minute 0.1 ml. of 0.02 M calcium chloride was added, and the clotting time was determined in duplicate. All determinations were performed immediately after the plasma was obtained. The normal fasting values were between 20 and 30 seconds.

Fibrinolysis Methods

Two methods were employed. Method 1 measured the fibrinolytic activity of blood as described by Fearnley et al. Method 2 measured the profibrinolysin activity of plasma. A modification of the method described by Loomis was used: 0.1 ml. of bovine thrombin (Upjohn) containing 50 units per ml. and 0.5 ml. of citrated platelet-poor plasma were added in rapid succession to 0.1 ml. of Varidase (Lederle Laboratories) containing 2,000 units of streptokinase per ml. and the resulting mixture was incubated at 37 C. Ten seconds after the reagents had been thoroughly mixed, at which time a clot had already formed, an open-ended capillary tube was inserted perpendicularly into the bottom of the clot. The tube was left undisturbed in a glass water bath at 37 C. Once fibrinolysis had begun, the liquefied plasma ascended into the capillary tube. The end-point was taken at the instant that the liquid level in the capillary tube reached the upper surface of the clot. Normal fasting values ranged between 60 and 80 seconds.

The 2 methods employed in the estimation of fibrinolytic activity of the fasting and postprandial plasma differed not only in their technic but also in that they measured separate phases of the fibrinolytic process. The lysis time as measured by the Fearnley technic (method 1) essentially reflects the concentration of free or active fibrinolysin in freshly shed blood. Method 2 does not measure fibrinolysis directly but rather expresses the concentration of the precursor of fibrinolysin present in the plasma which, in the presence of streptokinase, becomes converted to fibrinolysin. This activated lysin is independent of the free fibrinolysin normally present in the plasma inasmuch as the latter type is neutralized, upon standing, by the plasma antifibrinolysin. It is evident, therefore, with method 1 a longer fibrinolysis time following the fatty meal is indicative of inhibition of fibrinolysis, whereas a shorter fibrinolysis time signifies that fibrinolysis has become accelerated postprandially. The interpretation of results is more difficult when method 2 is used and the following scheme is presented for clarification of the problem.

```
   streptokinase
   ↓ proactivators
   activators
   ↓ profibrinolysin (plasminogen)
   fibrinolysin (plasmin)
   fibrin clot
   products of fibrinolysis
```
Table 1.—Fasting and Postprandial Values of Silicone Clotting Time, Thrombelastography, Heparin Tolerance, and Prothrombin Consumption

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*MA* values were always between 46-50 mm.

F = fasting.

PP = postprandial.

Thus with this method prolongation of postprandial lysis could imply one of the following: 1. A decrease in the level of pro-activators or pro-fibrinolysins. 2. Inhibition of activation of pro-activators or inhibition of activation of fibrinolysins. 3. Inhibition of lysis of the fibrin clot. 4. An increase in plasma fibrinogen concentration. The error between duplicate samples was considered to be ± 20 minutes with method 1 and ± 5 seconds with method 2. The degree of significant alteration in the postprandial fibriolysis time could not be established with certainty using method 1, since the individual variability in the fasting state was very marked (i.e., between 3 and 13 hours). With method 2, the significant alteration in the fibriolysis time following the fatty meal was taken to be ± 15 seconds.

**Chemical Methods**

The serum cholesterol and cholesterol esters were determined by the method of Schoenheimer and Sperry. The lipid phosphorus was obtained by extracting the serum with Bloor's reagent and petroleum ether. The lipid phosphorus was converted to inorganic phosphorus by digestion and the inorganic phosphate so obtained was measured by the procedure of Fiske and SubbaRow. A factor of 25 was used to convert the lipid phosphorus to phospholipids. The total serum fatty acids were determined by the method of Stoddard and Drury and the total serum lipids by the gravimetric method as described by Peters and Van Slyke. The serum triglycerides were not determined directly but were calculated by means of the following formula. Triglycerides...
mg. per cent = fatty acids mg. per cent – 0.7
(cholesterol esters mg. per cent + phospholipids
mg. per cent). The serum beta lipoproteins were
determined by the nephelometric method of Bern-
field et al. The serum turbidity was determined
by the nephelometric method of Bern-
fild et al.31

RESULTS

Blood Coagulation and Serum Lipid Data

The results of the coagulation studies in the
3 groups of subjects were divided into 2
main categories.

Category 1 was composed of the results of
those tests that measure specific coagulation
factors. These consisted of the prothrombin
concentration of plasma, factor V (labile fac-
tor) activity, factor VII (S.P.C.A.) activity,
and the plasma fibrinogen level. The values
obtained for these factors were within normal
limits in the fasting state and remained essen-
tially unchanged after the fatty meal in the
3 groups of subjects.

Category 2 comprised the results of those
procedures that measure the over-all coagu-
lability of the blood (or plasma) with or with-
out the addition of thromboplastic material.
These consisted of the coagulation time of re-
calcified platelet rich and platelet poor plas-
ma, the silicone clotting time of whole blood,
the prothrombin consumption test, the hepa-
rin tolerance test, thrombelastography, the
thromboplastin generation test, the 1-stage
prothrombin time, and the Stypven time.

The results of the first 5 tests inclusive,
performed without the addition of foreign
thromboplastic material, were within normal
limits in the fasting state and remained essen-
tially unchanged after the fatty meal in the
3 groups of subjects (table 1).

The thromboplastin generation test gave
essentially inconclusive results. In the fasting
state, however, there appeared to be a tending
cy for the hyperlipemic and hypercholes-
teremic groups to develop a more potent
thromboplastin. Thus 6 of the 9 patients with
hyperlipemia and 6 of the 10 patients with
hypercholesteremia showed clotting times be-
tween 20 and 35 seconds, whereas only 3 of
the 10 normal subjects fell within this range.
Postprandially there was even less difference
between the normal subjects and the other 2
groups of patients.

The last 2 tests involve the use of thrombo-
plastic material. The 1-stage prothrombin
time, in which tissue thromboplastin was em-
ployed, constantly gave normal results in the
3 groups of subjects both before and after the
fatty meal. On the other hand, the Styp-
ven time, entailing the use of Russel viper
venom as the thromboplastic agent, was the
one coagulation test that almost always
showed significant changes (i.e., acceleration)
in the presence of hyperlipemia either in the
fasting state (i.e., patients with idiopathic
hyperlipemia) or following the fatty meal in
all groups of subjects. For this reason the
results of this test are considered in detail
below.

As seen from table 2, the patients with
idiopathic hyperlipemia were unique in that
their fasting Stypven times were consider-
ably shorter than those of the other 2 groups.
On the other hand, the fasting Stypven times
of the patients with primary hypercholesterol-
emia fell within the normal range, even though
they were slightly shorter than those of the
control subjects. The degree of postprandial
shortening of the Stypven time did not exceed
4 seconds in the hyperlipemic and hypercho-
lesteremic groups, except for 1 patient in the
former group (case 4) and 2 patients in the
latter group (cases 12 and 18). In contrast,
7 of the 10 control subjects showed more than
4 seconds’ shortening after the fatty meal.
In an attempt to establish the postprandial
variability of the Stypven response, tests were
repeated on 3 additional days in 8 of the 10
normal subjects. Although the response of
some of these subjects varied to a certain
degree from day to day, in 70 per cent of in-
stances there was still more than 4 seconds’
postprandial shortening, a finding identical
with the initial observations in this group.
Thus it is clear that the change in the Styp-
ven time following a fatty meal was more pro-
nounced in the control subjects than in the
patients with hypercholesteremia or hyperli-
Table 2.—Fasting and Postprandial Values of Stypven and Fibrinolysis Times in Relation to the Serum Lipid Changes

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (yr.)</th>
<th>Sex</th>
<th>Fastig</th>
<th>Postprandial</th>
<th>Cholesterol (mg.%)</th>
<th>Triglycerides (mg.%)</th>
<th>Serum lipoproteins</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>M</td>
<td>16.8</td>
<td>12.1</td>
<td>30</td>
<td>62</td>
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<tr>
<td>2</td>
<td>45</td>
<td>M</td>
<td>18.2</td>
<td>16.1</td>
<td>42</td>
<td>32</td>
<td>657</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>F</td>
<td>18.0</td>
<td>17.4</td>
<td>40</td>
<td>27</td>
<td>362</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>M</td>
<td>16.9</td>
<td>12.8</td>
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<td>29</td>
<td>1125</td>
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<tr>
<td>5</td>
<td>55</td>
<td>F</td>
<td>16.5</td>
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<td>35</td>
<td>17</td>
<td>1098</td>
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<td>6</td>
<td>40</td>
<td>M</td>
<td>16.5</td>
<td>14.2</td>
<td>29</td>
<td>10</td>
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<td>7</td>
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<td>15.6</td>
<td>12.5</td>
<td>25</td>
<td>15</td>
<td>753</td>
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<tr>
<td>8</td>
<td>38</td>
<td>M</td>
<td>15.0</td>
<td>12.7</td>
<td>22</td>
<td>15</td>
<td>362</td>
</tr>
<tr>
<td>9</td>
<td>44</td>
<td>M</td>
<td>10.8</td>
<td>9.5</td>
<td>26</td>
<td>16</td>
<td>154</td>
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</table>

Patients with idiopathic hyperlipemia (I.H.L.)

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yr.)</th>
<th>Sex</th>
<th>Serum lipids (mg.%)</th>
<th>Total lipids</th>
<th>Beta lipoproteins</th>
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</thead>
<tbody>
<tr>
<td>27</td>
<td>24.4</td>
<td>F</td>
<td>44.0</td>
<td>350</td>
<td>42.0</td>
</tr>
<tr>
<td>28</td>
<td>23.7</td>
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<td>28.0</td>
<td>42.0</td>
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</tr>
<tr>
<td>32</td>
<td>23.8</td>
<td>F</td>
<td>28.0</td>
<td>42.0</td>
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<tr>
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<td>18.0</td>
<td>42.0</td>
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<tr>
<td>34</td>
<td>15.0</td>
<td>F</td>
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Normal control subjects (N.C.)

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yr.)</th>
<th>Sex</th>
<th>Serum lipids (mg.%)</th>
<th>Total lipids</th>
<th>Beta lipoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>24.4</td>
<td>F</td>
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<tr>
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<td>28.0</td>
<td>42.0</td>
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<tr>
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<tr>
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<td>18.0</td>
<td>42.0</td>
<td>42.0</td>
</tr>
<tr>
<td>34</td>
<td>15.0</td>
<td>F</td>
<td>17.0</td>
<td>42.0</td>
<td>42.0</td>
</tr>
</tbody>
</table>

Fasting values

<table>
<thead>
<tr>
<th>Groups</th>
<th>Range (sec.)</th>
<th>Mean (sec.)</th>
<th>Fasting</th>
<th>Range (sec.)</th>
<th>Mean (sec.)</th>
<th>Postprandial</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.H.L.</td>
<td>10.8—18.2</td>
<td>15.9</td>
<td>69—93</td>
<td>83.5</td>
<td></td>
<td></td>
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<tr>
<td>P.H.</td>
<td>18.0—29.7</td>
<td>23.0</td>
<td>59—79</td>
<td>67.7</td>
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<td></td>
</tr>
<tr>
<td>N.C.</td>
<td>20.8—27.9</td>
<td>25.5</td>
<td>60—78</td>
<td>68.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F=Fasting, PP=Postprandial.

The postprandial shortening of the Stypven time was essentially similar in the 2 abnormal groups even though the patients with hyperlipemia had such short Stypven times in the fasting state (table 2).

The invariably shortened Stypven times in the fasting state in the patients with idiopathic hyperlipemia would suggest a possible association with the lassitude of the serum or the elevated levels of triglycerides present in this group, factors not encountered in the other 2 groups. However, careful scrutiny of the results of individual patients in this group (table 2) failed to show a strict correlation in the majority of cases between the fasting Stypven times and the serum triglyceride levels. A similar lack of strict correlation between the fasting Stypven times and the serum triglycerides was also evident in the other 2 groups of subjects, although here again there was a general relationship. Following the fatty meal, it is clear (table 2) that every individual in the 3 groups showed some increase in the serum triglyceride levels and at the same time almost always developed a varying degree of shortening of the Stypven time. However, analysis of individual cases again failed to show a strict correlation between the postprandial changes in the Stypven time and the serum triglycerides.
The data also show that the fasting fatty acid levels were moderately or markedly elevated in 8 of the 10 patients with hypercholesteremia, while in the control subjects they were all within normal limits. These levels seemed to a certain degree to parallel the mean values of the fasting Stypven times in the respective groups of subjects. Following the fatty meal, an increase in the serum fatty acids, as with the serum triglycerides, was observed in all subjects. However, analysis of individual cases showed no close correlation between the postprandial changes in the serum fatty acids and those of the Stypven time.

During this study it became apparent that the Stypven time could to a certain extent be predicted by simple inspection of the serum. Thus a shortened Stypven time could be anticipated and was in fact always found in patients with idiopathic hyperlipemia who exhibited a lactescent serum in the fasting state, as well as usually in other subjects when their serum became turbid after a fatty meal. Similarly a clear serum was almost always associated with a Stypven time well within the normal range. These gross observations were further substantiated when the serum turbidity values were subsequently determined with the spectrophotometer. Although this general impression was essentially correct, analysis of individual cases, particularly in the postprandial state, failed to support the assumption that there was in fact a strict relationship between the turbidity of the serum and the length of the Stypven time.

Table 2 indicates that there was no correlation in the majority of cases between the fasting Stypven times and the serum cholesterol or phospholipid levels. The serum cholesterol values showed a slight increase in a few cases postprandially and remained unchanged or decreased slightly in the remainder. The serum phospholipid levels increased after the fatty meal in approximately one third of the total number of subjects. The shortening of the postprandial Stypven times was independent of these lipid changes.

There was no correlation between the Stypven time and the beta-lipoprotein levels in any of the groups of subjects either in the fasting state or following the fatty meal.

**Blood Fibrinolysis Data**

**Method 1.** Determination of fibrinolytic activity by the Fearnley method.25 There was considerable individual variation in the fasting fibrinolysis times (i.e., between 3 and 13 hours), although both the range of values and the mean values of the fasting fibrinolysis times were essentially similar in the 3 groups of subjects. Following the fatty meal every subject in the series, with the exception of 1 individual in each group, showed acceleration of the fibrinolysis time to a greater or lesser extent, but again the 3 groups behaved alike.

Although this method has the advantage of measuring the fibrinolytic activity of freshly shed blood, it had certain limitations when employed in this series. Thus there were wide differences in the fasting fibrinolysis times among the different individuals. Furthermore, although there was a definite postprandial shortening of the fibrinolysis times in almost all individuals, it was difficult to assess the effect of the fatty meal inasmuch as these changes could have been the direct result of the fatty meal, or a reflection of the natural diurnal acceleration that was found by Fearnley et al.25 to exist in normal subjects.

**Method 2.** Measurement of the profibrinolysis activity (streptokinase-induced fibrinolysis). The mean values of the fasting streptokinase-induced fibrinolysis times were almost identical in the normal and hypercholesteremic groups (i.e., 68.1 and 67.7 seconds respectively) but were noticeably different in the hyperlipemic group (i.e., 83.5 seconds) (table 2). In contrast to the results with the method employing spontaneous fibrinolysis (method 1) the majority of individuals exhibited some prolongation of the streptokinase-induced fibrinolysis times after the fatty meal. However, even with this method (method 2) the degree of postprandial pro-
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...longation was considered to be of significance in only 4 subjects, inasmuch as the limits of normal variability were taken to be ± 15 seconds. According to the same criteria 2 subjects showed significant shortening postprandially (table 2).

In an attempt to establish the variability of the fasting and postprandial fibrinolysis times in the same individuals, this method was employed on 3 additional consecutive days in 9 of the 10 normal subjects. These repeat studies gave closely similar results for the range and mean values (i.e., 61 to 77 and 68.0 seconds respectively) as compared with the original values in the fasting state (i.e., 60 to 78 and 68.1 seconds respectively). There was a variation in the postprandial response not exceeding ± 5 seconds in any one individual from day to day.

Regardless of the degree of inhibition of fibrinolysis by hyperlipemia, it is still uncertain at which stage the fat acts upon the various components of the fibrinolytic system. Since hyperlipemia was not associated with a change in fibrinogen levels, any effect upon the fibrinolytic system could have been a result of any of the other 3 mechanisms stated above.

The distinctly different fasting fibrinolysis times in the hyperlipemic group suggest a possible association with the high serum triglyceride levels, a feature not encountered in the normal and hypercholesteremic subjects. Moreover, the slight postprandial lengthening of the fibrinolysis time observed in the majority of individuals in this series was always accompanied by a rise in the serum triglyceride levels (table 2). However, although there appeared to be some association between the length of the fibrinolysis time and the level of serum triglycerides in the fasting state and postprandially in all groups, no strict correlation could in fact be found when individual cases were analyzed. Similar observations were made with regard to the fibrinolysis time and the serum turbidity in all groups of subjects. There was also some association between the fibrinolysis time and the serum fatty acids in the control and hyperlipemic groups, but this did not hold true for the hypercholesteremic group in which 8 of the 10 patients had slightly elevated fasting fatty acid levels and yet showed normal fibrinolysis times. There was no evidence in any individual of an association between the fibrinolysis time and the serum cholesterol or phospholipid levels either in the fasting state or postprandially. This is well exemplified by the results in the hypercholesteremic group, in which high serum cholesterol or phospholipid levels were accompanied by essentially normal fibrinolysis times in the fasting state. Finally, inspection of table 2 shows that there was no association between the fibrinolysis time and the beta-lipoprotein levels in any of the groups of subjects either before or after the fatty meal.

DISCUSSION

The effect of hyperlipemia on blood coagulation is still the subject of much controversy. It is probable that the lack of uniformity of coagulation technics employed by various workers in this field is one reason for this difference of opinion, although in fact conflicting results have been reported even when identical methods have been used. Moreover, even if the serum lipids do exert some effect on coagulation, their actual mode of influence is still unknown. In this respect, some of the coagulation tests utilized in this series were those that are generally considered to reflect the over-all coagulability of the blood, while others were chosen because they are known to measure specific factors that participate in the various stages of coagulation. It is therefore of interest that almost all the coagulation tests employed in this study failed to provide any definite evidence of a constant trend toward increased coagulability of the blood either in the fasting state or after the ingestion of fat, even in those patients with idio-pathic hyperlipemia or primary hypercholesteremia. One of these tests, the silicone clotting time, has been a particular source of disagreement. Thus a number of workers, including Waldron and Nichols,7 Fullerton et al.,8 O'Brien,9 Buzina and Keys,10 and
Mustard\textsuperscript{11} contended on the basis of their experiments that alimentary hyperlipemia produces shortening of the whole blood clotting time in nonwettable glassware in normal subjects while others such as Tulloch et al.,\textsuperscript{32} Merskey and Nossell,\textsuperscript{20} although O’Brien\textsuperscript{9} and Borrero et al.,\textsuperscript{34} were unable to confirm these results. Another test essentially uninfluenced by alimentary hyperlipemia in this series was the coagulation time of recalciﬁed plasma. This is in accord with the work of Merskey and Nossell,\textsuperscript{20} although O’Brien\textsuperscript{9} and Pilkington\textsuperscript{35} using this test were able to demonstrate acceleration of coagulation following the feeding of fat to normal subjects. A technic which is being increasingly used in blood coagulation studies is that of thrombelastography, and according to some authors it may be of considerable value in the detection of thrombophilic states.\textsuperscript{36, 37} When thrombelastography was employed in this study, however, there was no evidence of a trend toward hypereagulability of the blood in any of the groups of subjects, either before or after the ingestion of fat. In this regard, Nitzberg et al.\textsuperscript{33} were previously unable to demonstrate consistent acceleration of the whole blood clotting time in normal subjects during alimentary lipemia, when this method was used. Recently Sheehy and Eichelberger,\textsuperscript{38} employing this technic in 30 healthy soldiers, were likewise unable to find consistent evidence of hypereagulability following the ingestion of fat.

The Stypven time was the only coagulation test in this series that was constantly altered in the presence of hyperlipemia. Thus there was acceleration of the Stypven time to a greater or lesser extent in all groups of subjects following the fatty meal. This is in agreement with the results of several workers including Fullerton et al.,\textsuperscript{8} O’Brien,\textsuperscript{39} Merskey and Nossell,\textsuperscript{20} and Mustard.\textsuperscript{11} Furthermore, there was marked acceleration of the Stypven time in the fasting state in all the patients with idiopathic hyperlipemia when compared with the fasting values in the normal subjects and in the patients with primary hypercholesteremia, a finding similar to that encountered by Sohar et al.\textsuperscript{15} It might therefore be suggested that the unique behavior of this coagulation test in the fasting state in the group of patients with idiopathic hyperlipemia was related to the high serum triglyceride levels found in this group, inasmuch as these lipid values were normal in the fasting state in the other 2 groups of individuals. Moreover, the acceleration of the Stypven time after the fatty meal was always accompanied by a rise in the serum triglycerides. Indeed, Sohar et al.\textsuperscript{15} concluded that there is in fact a close correlation between the Stypven time and the concentration of triglycerides in the serum. These observations could not be fully substantiated in our series, however, since analysis of the results of individual subjects clearly demonstrated that there was no strict quantitative relationship between the Stypven time and the serum triglycerides in the majority of individuals either in the fasting state or postprandially. Similarly, there was some association, though not a strict correlation, between the Stypven time and the serum turbidity. This is not surprising when it is realized that the turbidity of the serum is a reﬂection of the concentration of chylomiera, which in turn are mainly composed of triglycerides and to a lesser extent of phospholipids and lipoproteins. When the serum fatty acids are considered, it is obvious that, as with the serum triglycerides, there was some association between accelerated Stypven times and the concentration of these lipids in the serum, although again there was no strict quantitative relationship. There was clearly no association between the Stypven time and the serum cholesterol or phospholipid levels in any of the groups of patients either in the fasting state or following the fatty meal. The absence of an association between the Stypven time and the fasting serum cholesterol levels is further suggested by the demonstration of essentially similar Stypven times in a group of 20 hospital patients with either high or normal cholesterol values.\textsuperscript{40} However, the lack of association in this series between the Stypven time and the serum phospholipids
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does not necessarily exclude the possible effect of ingested phospholipids on blood coagulation. O'Brien\textsuperscript{41} was able to demonstrate appreciable acceleration of the postprandial Stypven time after feeding small quantities of phospholipids to normal subjects even though the actual levels of serum phospholipids remained essentially unchanged.

It is difficult to determine the significance of the acceleration of the Stypven time by hyperlipemia, since the test itself is highly artificial and hence unphysiologic. Thus, Russell viper venom is a thromboplastic agent which rapidly converts prothrombin to thrombin in the presence of optimal concentrations of labile and Stuart factor. In addition platelets, platelet lipid fractions, phospholipids, and perhaps fatty acids accelerate this reaction. For example, Poole\textsuperscript{42} using platelet-poor plasma was able to demonstrate acceleration of the Stypven time by various fatty acids. O'Brien\textsuperscript{43} confirmed this observation, but found that the phospholipids were even more potent in this respect. Subsequently, Poole and Robinson\textsuperscript{44} showed that chylomicra led to shortening of the Stypven time, and they attributed this effect to the presence of small quantities of ethanolamine phosphatide contained within them. Recently, Kingsbury and Morgan\textsuperscript{45} found that the recalcification time of plasma was influenced by the chylomicra and they were further able to show that the factor responsible for the actual shortening of the calcium clotting time consisted of the phospholipid fraction derived from the chylomicra. All this evidence certainly suggests that phospholipids, or one of the components thereof, such as ethanolamine phosphatide, may play an important role in the acceleration of blood cloting. Further evidence is provided by O'Brien,\textsuperscript{41} who reported that ingested phospholipids were about 5 times as active as triglycerides in causing acceleration of the Stypven time. He\textsuperscript{41} suggested that phospholipids inactive with respect to the Stypven time when added in the test tube, become hydrolyzed and activated after ingestion. Indeed O'Brien\textsuperscript{69} claimed that there is in fact a strict quantitative relationship between the concentration of "active phospholipids" in the blood and the Stypven time. He concluded\textsuperscript{49} that a postprandial increase in the serum of ethanolamine phosphatide, or similar substances such as phosphatidyl-serine or inositol phosphatide, is probably responsible for the maximal shortening of the Stypven clotting time. Since these alterations in the serum phospholipids are not apparent when, as in this series of patients, the usual chemical methods are used, it would seem essential that more sensitive procedures be introduced for the detection of such changes. It is possible that paper chromatography will be useful in isolating and identifying the various phospholipid fractions after a fatty meal. In this regard Troup and Reed\textsuperscript{47} using such a method were unable to demonstrate the presence of these substances in the plasmas of normal individuals. In any event, the fact that it was not possible to demonstrate a strict quantitative relationship between the Stypven time and the serum triglycerides in this series could well be due to the presence in hyperlipemic blood of other factors, such as for example, a cephalin fraction, which may play an important role in the development of hypercoagulability of the blood.

Since the role of platelets in blood coagulation is well established, it was decided to employ platelet-free systems in this study. It was considered that the presence of thromboplastic factors, introduced into the plasma as the result of a fatty meal, could be evaluated more clearly if platelet fractions of a similar thromboplastic nature were excluded. However, platelet-poor systems in vitro are unphysiologic, inasmuch as platelets or their fractions undoubtedly play an essential part in the development of intravascular thrombosis. The fact that platelet fractions, namely cephalin and leicithin, even in very small quantities possess a marked activating effect upon blood coagulation was shown by Gaffet al.\textsuperscript{48} in 1936. More recently, O'Brien\textsuperscript{49} has reported that one of the active clotting factors in platelets is similar to ethanolamine phosphatide while Wallach et al.\textsuperscript{50} have ac-
tually demonstrated the presence of this substance in the platelets by means of paper chromatography. On the other hand, Trup and Reed\textsuperscript{17} have found that weight for weight serine phosphatide is much more active than ethanolamine phosphatide as a platelet thromboplastic factor. Be that as it may, it is obvious that platelets do contain potent thromboplastic materials which must be considered.

Although the fibrinolytic activity of the blood, as measured by the Fearnley\textsuperscript{25} technic, was not decreased in this series in the presence of hyperlipemia either in the fasting or postprandial states, it is of considerable interest that hyperlipemia appeared to be associated with some reduction of the profibrinolytic activity of the plasma. This was observed primarily in patients with constant hyperlipemia, although there was suggestive evidence of this occurring even in normal subjects after ingestion of the fatty meal. It might therefore be assumed that this diminished profibrinolysin level was in some way related to the high levels of triglycerides (or the other constituents of chylomicra), which are responsible for the increased serum turbidity found in such states of hyperlipemia. In fact there appeared to be some association, although not a strict correlation, between the length of the streptokinase fibrinolysis time and the serum triglyceride levels as well as with the serum turbidity. In spite of this trend, however, the prolongation of the fibrinolysis time in this series was not considered to be of significance in the majority of these individuals. These results, which suggest some association between hyperlipemia and reduced fibrinolysis, were based upon a method that measured the fibrinolytic activity of the plasma only indirectly (i.e., fibrinolysis was activated by streptokinase). Moreover, the mere inhibition of fibrinolysis by fat in such an artificial system does not explain the exact mechanism of the interaction between the fat and the various fibrinolytic precursors.

Only a few reports have appeared with reference to the effect of ingested fat on blood fibrinolysis. The prevailing opinion is that fats do inhibit fibrinolysis, although there is no agreement as to which of the serum lipids is directly responsible for this phenomenon. Thus Greig and Runde\textsuperscript{13} have emphasized the role of the beta lipoproteins and the chylomicra in the inhibition of fibrinolysis, while in an earlier communication Greig\textsuperscript{12} was able to find a direct association between fibrinolysis and the serum turbidity. Admittedly, it was not possible in the present study to demonstrate such conclusive evidence of inhibition of fibrinolysis by fats as was obtained by Greig\textsuperscript{12} but this failure may be due in part to the different methods employed in assessing fibrinolytic activity. In this respect, Greig\textsuperscript{22} measured fibrinolysis by a modification of the method of Biggs and MacFarlane\textsuperscript{31} and Bidwell.\textsuperscript{22} Recently, however, Scott and Thomas\textsuperscript{33} reported significant prolongation of the streptokinase fibrinolysis time in rabbits following the ingestion of butter fat. Yet another approach has been introduced by Kwaan and MacFadzean,\textsuperscript{24} who found that feeding cholesterol to rabbits inhibited fibrinolysis in vivo. It is not clear from their communication what effect the ingested cholesterol had on the various serum lipids, but certainly in our own study the patients with primary hypercholesteremia did not show any inhibition of fibrinolysis in the fasting state.

At the time of these studies 3 patients with idiopathic hyperlipemia (cases 1, 3, and 5) and 3 patients with primary hypercholesteremia (cases 10, 17, and 18) had clinical and electrocardiographic evidence of ischemic heart disease, amounting in 3 patients (cases 1, 10, and 17) to acute coronary artery disease. In none of these patients was there evidence to suggest that the behavior of the blood clotting or fibrinolytic systems differed from that of the other patients within the same group, either in the fasting state or in response to a fat-loading meal. It is possible of course that the close association between primary hypercholesteremia and coronary artery disease does not depend upon the presence of hypercoagulability of the blood or diminished blood fibrinolysis. Indeed it still remains likely that the increased incidence
of coronary artery disease in these patients is primarily due to the formation of plaques in the intima, which in turn lead to an increased risk of secondary occlusive thrombi in the coronary arteries. Another point is that patients with primary hypercholesteremia may be more liable to coronary atherosclerosis than those with idiopathic hyperlipemia who do not have elevated levels of cholesterol in the serum. (The 3 hyperlipemic patients with concomitant ischemic heart disease in this series had high serum cholesterol levels.) If this is so, the effect of hyperlipemia on coagulation and fibrinolysis, as observed in this series, may not be of major importance in the development of ischemic heart disease.

Recently, other mechanisms that may help to account for the role of the blood lipids in the development of arterial disease have been suggested by the introduction of new and ingenious technics. Thus Goldberg and Morantz25 were able to demonstrate actual infiltration of the connective-tissue cells of the elastic lamina in the aortas of young chicks with lipid particles from a perfusing stream of lipid-enriched blood, while Rutstein et al.56 showed that lipids (cholesterol) were engulfed by the cells of human aortic intima grown in artificial culture media. For the present, however, the exact mode of development of atherosclerosis either in patients with abnormal lipid metabolism or in other individuals must remain conjectural. Moreover, in assessing the results in this study, it must be remembered that conditions in the test tube do not necessarily reflect those present in vivo. For example, the majority of coagulation tests in current use are primarily measuring factors that govern the speed of coagulation rather than control its initiation. Be that as it may, it is clear that in this series hyperlipemia (i.e., lactic acid serum with elevated levels of serum triglycerides) was usually associated with accelerated Stypven times and frequently also with prolonged prothrombinin times. These results suggest that there may be some relationship between hyperlipemia, increased coagulability of the blood and reduced blood fibrinolytic activity. However, the real significance of these in vitro findings with regard to clinical coronary artery disease remains obscure.

**Summary**

The clotting and fibrinolytic activity of the blood were studied before and after a meal containing 85 Gm. of fat. There were 3 groups of subjects; 9 patients with idiopathic hyperlipemia, 10 patients with primary hypercholesteremia, and 10 normal subjects. Simultaneous lipid studies were performed in an attempt to correlate the coagulation and fibrinolysis values with changes in the various serum lipid levels.

The results of the coagulation studies were divided into 2 categories. The first was composed of the data obtained by measuring specific coagulation factors. The values obtained for these factors were within normal limits before and after the fatty meal in the 3 groups of subjects.

The second category comprised the results of those procedures that measure the over-all coagulability of the blood (or plasma) with or without the addition of thromboplastic material. The results of all these determinations, with the exception of the thromboplastin generation test and the Stypven time, were within normal limits in the fasting state and remained essentially unchanged after the fatty meal in the 3 groups of subjects.

The thromboplastin generation test gave essentially inconclusive results although there appeared to be a tendency for the hyperlipemic and hypercholesteremic groups to develop a more potent thromboplastin in the fasting state.

The Stypven time (Russell viper venom test) was the one coagulation test that almost always showed significant changes in the presence of hyperlipemia. Thus it was markedly accelerated (i.e., shortened) in the fasting state in all the patients with idiopathic hyperlipemia. In addition, the Stypven time was accelerated to a greater or lesser extent in all groups of subjects following the fatty meal. Comparison of these results with the serum lipid levels showed that there was some
degree of correlation, although not a strict quantitative relationship, between the Stypven time values and the serum triglyceride levels (and the fatty acid levels to a lesser extent) as well as with the turbidity of the serum in all groups of subjects before and after the fatty meal. No correlation was found between the Stypven time and the serum cholesterol or phospholipid levels.

Two methods were employed to assess the fibrinolytic activity of the blood. Method 1, which measured blood fibrinolysis directly, gave variable and inconclusive results. Method 2, which determined the fibrinolytic activity of plasma after activation with streptokinase (in vitro), showed that prolonged streptokinase fibrinolysis times occurred under circumstances similar to those associated with accelerated Stypven times. Thus there appeared to be an association of the fibrinolysis time with the serum triglyceride levels as well as with the serum turbidity.

The behavior of the blood clotting and fibrinolytic systems in the 6 patients in this series who had evidence of coronary artery disease did not differ from that of the other individuals within the same groups either in the fasting state or after the fatty meal.

Summary

Hyperlipemia in general leads to acceleration of blood clotting, as measured by the Stypven time, as well as to inhibition of blood fibrinolysis.

The inability to demonstrate a strict quantitative relationship between the Stypven time and the serum triglycerides in this series suggests that other factors, such as a cephalin fraction, may also play an important role in the development of hypercoagulability of the blood. The same may hold true for the association between hyperlipemia and reduced fibrinolytic activity.

The development of coronary atherosclerosis in patients with primary hypercholesterolemia may depend on factors other than those of increased blood clotting or decreased blood fibrinolytic activity.

The conclusions with regard to the effect of serum lipids on coagulation and fibrinolysis in this series have been based essentially on highly artificial tests. The final elucidation of the exact relationship between the serum lipids and the coagulability of the blood, and its possible connection with coronary artery disease, must await further studies.

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Summary in Interlingua

Hyperlipemia, a generalmente parlar, resulta in acceleration del processo coagulatori del sanguine secundo le mesuration del tempore a stypven e etiam in inhibition del fibrinolys de sanguine.

Le impossibilitate de demonstrar in le presente serie de casos un relation strictemente quantitative inter le tempore a stypven e le triglyceridos del sero suggere que altere factores, como per exemplo un fraction de cephalina, ha etiam un rolo importante in le disveloppamento de hypercoagulabilitate de sanguine. Le mesmo es possibilmente ver pro le association inter hyperlipemia e un reduceite activitate fibrinolytic.

Le disveloppamento de atherosclerosis in patientes con hypercholesterolemia primaria depende de factores altere que le augmentate coagulation de sanguine o le reduceite activitate fibrinolytic del sanguine.

Le conclusiones con respecto al efecto de lipidos sereal super le coagulation e le fibrinolys in le presente serie es basate essentially super tests de character multo artificial.

Le ultime elucidation del relation exacte inter le lipidos del sero e le coagulabilitate del sanguine e su connexion possibile con morbo de arteria coronari debe attender studios additional.

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