Hemolysis and Alimentary Lipemia

Effects of Incubation, Heparin, and Protamine

By ROY L. SWANK, M.D., PH.D. AND ESTHER S. ROTH, M.A.

LITTLE ATTENTION has been paid to the observation of Longini and Johnson\(^1\) that an alimentary lipemia may be responsible for increased fragility of erythrocytes. The possibility that the hemolysis may also occur in vivo is suggested by Loewy, et al.,\(^2\) who demonstrated that the output of bile pigment increased in dogs receiving high fat diets. More recently, Creditor\(^3\) has observed that hemolysis due to shaking whole blood increased after intravenous injection of two different fat emulsions.

Our interest in this phenomenon was aroused when it was observed that after shaking whole blood in a mechanical shaker to facilitate the solution of high molecular weight dextrans, the blood plasma of normal human subjects usually contained some free hemoglobin whereas the plasma of patients with multiple sclerosis contained none. Further investigation indicated that the degree of hemolysis in the blood of both patients and normal subjects paralleled the degree of the lipemia. The blood specimens were drawn three hours after having given the subject a breakfast containing 15 Gm. of fat. The patients, most of whom were maintained on a low fat diet, seldom had visible lipemia, whereas the normal subjects had varying degrees of visible lipemia. Subsequent experiments in dogs were done and these are now reported.

Methods

Ten mongrel dogs, 9 months of age or older, were used as the subjects. The same animals were used repeatedly with an interval of a week or longer between the experiments. They were fasted eighteen hours prior to each experiment. Most of the animals consumed the fat meals voluntarily; the others were fed by tube. The lipemic bloods were drawn three hours after the fat meal (unless otherwise indicated) into syringes containing enough heparin (Connaught Laboratories) to prevent clotting. The fat meals consisted either of 35 per cent cream, or of cod liver oil or glyceryl trioleate emulsified in skim milk. Two to 5 Gm. of fat per Kg. body weight were given; 4 Gm. per Kg. was the fat content of the usual meal.

In a number of experiments heparin was injected intravenously in order to observe the effect of the clearing of the lipemia on the degree of hemolysis. In other experiments protamine sulfate dissolved in .05 N HCl was injected intravenously.

To demonstrate fragility of red blood cells to mechanical trauma 3 ml. samples of blood were shaken in stoppered 15 x 100 mm. tubes held horizontally, and parallel to the line of shaking in rubberized Kahn racks. In later experiments after it was discovered that slight deviations from parallel gave poor duplication of results, a wooden rack in which tubes were held rigidly horizontal and parallel was used. In some experiments the number of strokes per minute, time of shaking, and temperature during shaking were varied. In all other experiments the tubes were shaken for 30 minutes at room temperature at a speed of 240 horizontal 1\(\frac{1}{2}\) inch strokes per minute in a Kahn variable speed shaker.

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The plasma turbidity due to lipemia was determined by chylomicon counts of whole blood and by optical density measurement at 650 µ of a 1:10 dilution of unshaken plasma in water. The readings were made within 30 seconds after dilution of the plasma with water, because it was noted that after standing the diluted plasma became progressively more turbid. To remove the turbidity so that the amount of hemoglobin could be determined, the plasma was diluted 1:10 with distilled water and centrifuged at 20,000 × g in a Servall angle centrifuge for 20 minutes at a temperature of 4 C. Six ml. of the clear subnatant was then removed without contamination with the floating lipid. It was shown by dark field examination of the plasma and by later check readings at 650 µ that practically all of the visible chylomicona had been removed by this procedure. The subnatant plasma to which one drop of 5 per cent ammonia was added was read against a blank of distilled water at a wave length of 550 µ in a Coleman Universal Spectrophotometer. Because of excessive hemolysis it was occasionally necessary to dilute the solution further. The amount of hemoglobin present in the plasma was estimated from a standard hemoglobin curve.

Osmotic fragility tests were done by adding 0.05 ml. whole blood or saline-washed cells to 10 ml. of sodium chloride in concentrations varying from 0.7 to 0.32 per cent. These tubes were then incubated 15 minutes in a 37 C. water bath, centrifuged, and the amount of hemoglobin in the supernatant determined by reading at 550 µ.

Results

Our experiments showed that shaking caused lipemic whole blood to hemolyze much more readily than nonlipemic whole blood. In general the degree of hemolysis due to shaking paralleled the degree of lipemia (fig. 1). There were variations from time to time in the individual dogs but these were slight compared to the differences usually noted between animals. The hemolysis due to shaking was frequently greater after the ingestion of cod liver oil than after cream or glyceryl trioleate (table 1). This may have been due to the fact that cod liver oil usually caused a greater lipemia than the other two fats.

![Fig. 1.—Relationship of the degree of plasma lipemia to the degree of hemolysis produced by shaking whole bloods. Blood samples were drawn three hours after meals consisting of 4 Gm. of fat per Kg. of body weight.](image-url)
HEMOLYSIS AND LIPEMIA

TABLE 1.—Hemolysis Due to Shaking Blood Drawn Three Hours After Fat Meal

<table>
<thead>
<tr>
<th></th>
<th>Gm. per Kg.</th>
<th>No. of exp.</th>
<th>Mg. hemoglobin per 100 ml. plasma</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Fasting</td>
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<td>48</td>
<td>21</td>
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<tr>
<td>Fat fed:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cream</td>
<td>4</td>
<td>47</td>
<td>378</td>
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<tr>
<td>Cod liver oil</td>
<td>5</td>
<td>2</td>
<td>3310</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>4</td>
<td>16</td>
<td>762</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>2</td>
<td>3</td>
<td>717</td>
</tr>
<tr>
<td>Glyceryl trioleate</td>
<td>4</td>
<td>4</td>
<td>408</td>
</tr>
</tbody>
</table>

Fig. 2.—Effect of duration of shaking on the degree of hemolysis in two experiments (cream fat 4 Gm. per kg.).

The Effect of the Duration of Shaking Whole Blood

This effect in two experiments is shown in figure 2. Shaking bloods from fasting animals as long as 60 minutes produced no hemolysis. The hemolysis in lipemic bloods showed a progressive increase with duration of shaking. In two experiments it was shown that decreasing the rate of shaking lessened the hemolysis, but significant hemolysis (1200 and 180 mg. hemoglobin per 100 ml. of plasma) still occurred during gentle shaking at 100 strokes per minute. No hemolysis occurred in the control bloods allowed to stand without shaking at room temperature for as long as 6 hours.

The Effect of Temperature of the Blood During Shaking

This effect is shown in table 2. In three cream experiments less hemolysis was obtained by shaking at 4 C. than at 24 C. In five cod liver oil experiments more
hemoysis was obtained by shaking in the cold. A blood specimen from a fasted
animal shaken with glass beads hemolyzed equally at the three temperatures of
4, 24, and 37 C. Two bloods shaken at 37 C. hemolyzed more than when shaken
at 24 C. Other studies to be described in a later section showed that the hemoly-
sis increased rapidly in bloods incubated at 37 C.

**Relationship of Lipemia to Hemolysis After Fat Feedings**

Repeated studies of bloods obtained from seven dogs at various intervals
ranging from 1 to 9 hours after cream and cod liver oil meals (4 Gm. per Kg.)
confirmed the general impression that the amount of hemolysis due to shaking
paralleled closely the degree of lipemia. The results of two of these studies (fig.
3) illustrate the magnitude of the individual variations which were noted in the
responses to fat meals. Dog I always developed more visible lipemia and his
blood hemolyzed more on shaking than was noted in dog II. Both dogs exhibited
more lipemia and more hemolysis after 4 Gm. per Kg. of cod liver oil than after
4 Gm. per Kg. of cream fat. There was a suggestion that increase in hemolysis
sometimes lagged behind the increase in the visible lipemia following the con-
sumption of cod liver oil (see circled points in fig. 3).

**Osmotic Fragility Tests**

In ten dogs the use of both whole blood and saline-washed red blood cells
failed to demonstrate any consistent difference between the osmotic fragility of
red blood cells from fasted and fat-fed animals. Furthermore, dog erythrocytes
when washed three times in 0.85 per cent saline, brought back to original volume
in saline, and shaken in the usual manner were always hemolyzed although no
consistent difference was noted between saline-washed red blood cells from non-
lipemic and lipemic bloods. These results, particularly the latter, indicate that
the hemolysis found after shaking whole lipemic blood is not due to an increased
fragility of the erythrocytes themselves.

**Plasma Substitution Experiments**

To determine whether the increased hemolysis was due to substances in the
plasma, whole blood (3 ml.) from a fasted animal was lightly centrifuged and
increasing amounts of the clear nonlipemic plasma were pipetted off and replaced with lipemic plasma from the same dog. In another experiment clear plasma from a fasting animal was substituted for lipemic plasma in lipemic blood in the same manner. The bloods were then remixed and shaken as usual. The addition of increasing amounts of lipemic plasma to red blood cells from fasting animals increased the hemolysis on shaking, and conversely, the substitution of clear plasma in blood from a lipemic animal decreased the hemolysis (fig. 4).

Centrifugation Studies

Centrifugation studies were done to help clarify the role of the plasma as a factor in hemolysis. By high speed centrifugation (20,000 × g.) 20 minutes at 4 C. the particulate fat was separated from the lipemic plasma. The clear subnatant plasma which contained no chylomicra caused little or no hemolysis when shaken with red blood cells from fasting animals. However, clear plasma (from fasting animals) containing resuspended chylomicra from animals fed either cream or cod liver oil caused hemolysis after shaking when added to red blood cells from either fasting or fat-fed animals. This hemolysis was of the same order of magnitude as that which resulted from shaking whole lipemic blood.

In an attempt to determine whether the chylomicra themselves or something adherent to them was the cause of the hemolysis, four experiments were done as follows: Separated chylomicra were washed twice with 0.85 per cent saline, once with homologous fasting plasma, and then resuspended in fasting plasma. These suspensions when added to red blood cells from fasted animals caused hemolysis during shaking which was equal to that caused by nonwashed chylomicra from the same experiments.
Fig. 4.—Effect on hemolysis of substitution of clear plasma from fasting animal and of lipemic plasma in lipemic and nonlipemic bloods.

Table 3.—Clearing of Lipemia After Heparin Injection

<table>
<thead>
<tr>
<th>Fat fed</th>
<th>No. of exp</th>
<th>Mean per cent decrease approximately 5 minutes after injection of heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Optical density</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>17</td>
<td>51</td>
</tr>
<tr>
<td>Cream</td>
<td>9</td>
<td>68</td>
</tr>
<tr>
<td>Glyceryl trioleate</td>
<td>4</td>
<td>82</td>
</tr>
</tbody>
</table>

Injection of Heparin and Protamine

Since the agent responsible for the hemolysis appeared to be in, or closely associated with, the chylomicra, further studies were undertaken to determine the influence on hemolysis due to shaking, first, of intravenous injections of heparin which cause dissolution of the chylomicra, and second, of injections of protamine which cause the lipemia to reappear after it has been cleared by heparin. Heparin (20 to 30 units per Kg. body weight) was injected intravenously into thirty dogs three hours after they were fed cream, glyceryl trioleate, or cod liver oil. In six of these dogs, protamine (2 mg. per Kg. body weight) was injected intravenously 5 to 7 minutes after the heparin.

Varying degrees of dissolution of the visible fat occurred after heparin injections in twenty-nine of the thirty dogs. The clearing was more pronounced in cream and trioleate-fed dogs as indicated by both turbidity measurements and chylomicron counts (table 3).

In all cream-fed dogs it was found that the hemolysis on shaking decreased as the chylomicron counts and plasma turbidity decreased. In dogs fed glyceryl trioleate and cod liver oil, however, a transient increase in the amount of he-
molysis due to shaking occurred in over half of the bloods taken during the first 3 minutes after injection of heparin. In three of four trioleate-fed dogs this increase was found in samples taken 1 minute after heparin injection and all subsequent samples of blood exhibited less hemolysis than the preheparin bloods (fig. 5). This transient increase occurred during the first 3 minutes after intravenous heparin in twelve of seventeen dogs fed cod liver oil, but the hemolysis, although it soon decreased again, still remained above the preheparin level for 5 to 15 minutes, in spite of the steadily decreasing turbidity.

In four cod liver oil-fed dogs showing an increase in hemolysis after injections of heparin the preheparin and three to five postheparin plasma samples obtained during the first 7 minutes were twice centrifuged at 20,000 × g. and 1 ml. of each clear subnatant plasma was added to fasting cells. These were then shaken, two experiments at room temperature and two at 4 C. All bloods containing the postheparin subnatant plasma exhibited slightly more hemolysis after shaking than those containing the preheparin subnatant plasmas (fig. 6).

Heparin injected into three fasting dogs did not appreciably affect the hemolysis caused by shaking. Additional heparin added to lipemic blood samples in vitro did not affect the amount of hemolysis on shaking.

The injection of protamine after dissolution of the chylomiera by heparin was invariably followed in two or more minutes by reappearance of the chylomiera and by increased plasma turbidity (fig. 5). This was also accompanied by an increase in the hemolysis of whole blood when shaken.

*Incubation Studies*

Incubation studies were undertaken as the result of a chance observation that the amount of hemolysis due to shaking with fasting cells was greater after pre-
heparin lipemic plasma had stood for 2 or 3 hours before shaking. On the other hand, after the injection of heparin, the amount of hemolysis due to shaking was less after the plasma had stood at room temperature.

When preheparin whole blood was incubated for periods of 15 minutes to 9 hours in a 37 C. water bath the amount of hemolysis upon shaking invariably increased, sometimes sharply. Similar incubation of postheparin whole blood was usually followed by a decrease in the hemolysis upon shaking. In a few instances, however, it resulted in a slight increase (much less than was observed in the preheparin incubated blood). In two experiments the incubation of postheparin blood was followed, during the first hour’s incubation, by a slight increase in the amount of the hemolysis due to shaking, and then by a decrease in hemolysis (fig. 7). Shaking at 4 C. after incubation at 37 C., to minimize changes which might take place during the 30 minute shaking at room temperature, produced a similar transient increase in hemolysis upon incubation of the postheparin blood in one of three experiments. The 3 hour incubation data are shown in table 4. Our studies indicate that the hemolysis trend during incubation of postheparin bloods was usually the same in any one dog regardless of the interval (from 1 to 15 minutes) after the injection of heparin before withdrawal of the sample (fig. 7 and table 4). Unfortunately, turbidity measurements were not made on all bloods after incubation. The measurements which were made indicated a progressive in vitro decrease of turbidity during incubation of all of the postheparin bloods as has been shown by others and very little change in turbidity readings during incubation of preheparin bloods.

To determine whether the preheparin and postheparin incubation patterns were due to differences in the amount of visible fat in the plasma an experiment was done in which equal amounts of washed chylomicra in saline were added to

![Graph showing the effect of heparin injection on hemolysis and lipemia.](image)
Fig. 7.—Incubation at 37 C. of whole blood obtained before and after injection of heparin (25 units per Kg.). After incubation blood was shaken at room temperature. Fat meal was 4 Gm. of cod liver oil per Kg.

A number of tubes of chylomicron-free subnatant plasma obtained from preheparin and postheparin bloods. These mixtures were incubated at 37 C. and at various intervals were mixed with fasting red blood cells and shaken.

The results (table 5) show that even when the same amount of visible fat was added to particulate-free subnatant plasma before incubation the changes in hemolysis were the same as those obtained with whole blood: an increase in hemolysis in plasma from preheparin bloods and either a sharp decrease in hemolysis (dog I), or an increase followed by a decrease (dog II), in plasmas from postheparin bloods.

When washed chylomicra in normal saline were incubated for three hours at 37 C. and added to fasting red blood cells and shaken, an increased hemolysis resulted. Also when preheparin washed chylomicra were heated to 65 C. for 15 minutes, a sharp increase in hemolysis on shaking with fasting red blood cells at room temperature resulted.

Bloods taken 2 or more minutes after the injection of protamine showed a progressive increase in hemolysis due to shaking when incubated at 37 C. (four experiments; table 4). Incubation of all postprotamine bloods was accompanied by no further in vitro increase in the turbidity of the plasma.

When preheparin and postheparin plasmas were incubated, then shaken with fasting cells, the changes in hemolysis were the same, although somewhat less marked, as those observed when whole bloods were incubated.

Little or no hemolysis occurred in bloods incubated as long as 9 hours, but not shaken. When whole fasting blood was incubated, slight hemolysis was produced by shaking.
Table 4.—Hemolysis Due to Shaking Whole Blood Before and After 3 Hours Incubation at 37 C.

<table>
<thead>
<tr>
<th>Dog</th>
<th>Fat fed</th>
<th>Before heparin</th>
<th>After heparin (1-2 min.)</th>
<th>After heparin (3-15 min.)</th>
<th>After protamine (5 min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0  3  0  3  0  3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cs</td>
<td>Cod liver oil</td>
<td>115 468 117 53 170 313</td>
<td>500 2750 276 182 1050 1880</td>
<td>65 528 127 54 138 115</td>
<td>94 614 62 51 147 73</td>
</tr>
<tr>
<td>JF</td>
<td>Cod liver oil</td>
<td>115 468 117 53 170 313</td>
<td>500 2750 276 182 1050 1880</td>
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<td>94 614 62 51 147 73</td>
</tr>
<tr>
<td>SI</td>
<td>Cod liver oil</td>
<td>115 468 117 53 170 313</td>
<td>500 2750 276 182 1050 1880</td>
<td>65 528 127 54 138 115</td>
<td>94 614 62 51 147 73</td>
</tr>
<tr>
<td>Cu</td>
<td>Cod liver oil</td>
<td>115 468 117 53 170 313</td>
<td>500 2750 276 182 1050 1880</td>
<td>65 528 127 54 138 115</td>
<td>94 614 62 51 147 73</td>
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<tr>
<td>Sa</td>
<td>Cod liver oil</td>
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<td>500 2750 276 182 1050 1880</td>
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<tr>
<td>To</td>
<td>Cod liver oil</td>
<td>115 468 117 53 170 313</td>
<td>500 2750 276 182 1050 1880</td>
<td>65 528 127 54 138 115</td>
<td>94 614 62 51 147 73</td>
</tr>
<tr>
<td>Ty</td>
<td>Cod liver oil*</td>
<td>115 468 117 53 170 313</td>
<td>500 2750 276 182 1050 1880</td>
<td>65 528 127 54 138 115</td>
<td>94 614 62 51 147 73</td>
</tr>
<tr>
<td>To</td>
<td>Cod liver oil*</td>
<td>115 468 117 53 170 313</td>
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<td>115 468 117 53 170 313</td>
<td>500 2750 276 182 1050 1880</td>
<td>65 528 127 54 138 115</td>
<td>94 614 62 51 147 73</td>
</tr>
<tr>
<td>Bu</td>
<td>Glycerol trioleate</td>
<td>115 468 117 53 170 313</td>
<td>500 2750 276 182 1050 1880</td>
<td>65 528 127 54 138 115</td>
<td>94 614 62 51 147 73</td>
</tr>
<tr>
<td>Ty</td>
<td>Glycerol trioleate</td>
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</tr>
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</table>

* Shaken at 4 C.

Table 5.—Effect of Incubation After Addition of Chylomicra to Plasma Subnatants

<table>
<thead>
<tr>
<th>Chylomicron suspension (0.1 ml.)</th>
<th>Particulate-free subnatant (0.9 ml.)</th>
<th>Hours incubated</th>
<th>Mg. hemoglobin per 100 ml. plasma after shaking with fasting cells</th>
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<td>Preheparin</td>
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</tr>
<tr>
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<td>Preheparin</td>
<td>0.5</td>
<td>Dog I: 303 114 Dog II: 334 135</td>
</tr>
<tr>
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<td>Preheparin</td>
<td>3.0</td>
<td>Dog I: 334 135 Dog II: 39 59</td>
</tr>
<tr>
<td>Preheparin</td>
<td>2 min. postheparin</td>
<td>0</td>
<td>Dog I: 408 100 Dog II: 249 162</td>
</tr>
<tr>
<td>Preheparin</td>
<td>2 min. postheparin</td>
<td>0.5</td>
<td>Dog I: 249 162 Dog II: 191 103</td>
</tr>
<tr>
<td>Preheparin</td>
<td>2 min. postheparin</td>
<td>3.0</td>
<td>Dog I: 191 103 Dog II: 374 96</td>
</tr>
<tr>
<td>Preheparin</td>
<td>5 min. postheparin</td>
<td>0</td>
<td>Dog I: 374 96 Dog II: 294 128</td>
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<tr>
<td>Preheparin</td>
<td>5 min. postheparin</td>
<td>0.5</td>
<td>Dog I: 294 128 Dog II: 166 92</td>
</tr>
<tr>
<td>Preheparin</td>
<td>5 min. postheparin</td>
<td>3.0</td>
<td>Dog I: 166 92 Dog II: 86 50</td>
</tr>
</tbody>
</table>

In Vitro Studies

The addition of .01 ml. of cod liver oil or glyceryl trioleate to 3 ml. fasting whole blood was followed by marked hemolysis when the bloods were shaken in the usual manner. Some, but less hemolysis resulted when salt-free butter fat was added, and very slight hemolysis resulted when mineral oil, tributyrin, or tristearin was added. Adding the fat directly to the blood or homogenizing it with the fasting plasma and then mixing this with the fasting cells gave essentially the same results. In four experiments, incubation at 37 C. of blood to which
cod liver oil had been added resulted in a rapid increase in hemolysis due to shaking during the first hour of incubation, and a progressive decrease of hemolysis after 3 and 6 hours incubation. The shaking of incubated blood to which glyceryl trioleate and butter fat had been added gave inconsistent results.

**Discussion**

Our studies have shown that the hemolysis caused by shaking lipemic whole blood is so closely associated with the chylomicra that removal of the chylomicra from the blood by high speed centrifugation or bringing about their dissolution by heparin injections (in cream-fed animals) reduces the amount of hemolysis. Furthermore, adding chylomicra to the blood, or causing them to reappear by injections or protamine, increases the amount of hemolysis when the blood is shaken. Earlier studies have shown that the chylomicra are composed almost entirely of neutral fat, and it seems likely that this material or something very closely associated with or derived from it is responsible for the hemolysis.

The observations that emulsified mineral oil causes no hemolysis when added to blood in vitro and shaken, and that a cream lipemia when chilled at 4 C. prior to and during shaking causes very little hemolysis, would indicate that the hemolysis is not due to the physical effect alone of the particulate fat in the blood. It seems unlikely that the hemolysis is due to changes in the plasma proteins of the blood which might have resulted from the lipemia since simple removal of the chylomicra by centrifugation greatly reduces hemolysis and the addition of washed chylomicra to fasting blood causes hemolysis to occur on shaking. It also seems unlikely that the hemolysis is due to agents such as bile salts and lysolecithins, which are known to cause hemolysis, since these substances would not be present in significant amounts in washed chylomicra. Our observations of lipemic and incubated bloods in dark field illumination have indicated that the hemolysis is not accompanied by spherocytosis to the point of rupture. Although spherocytes were seen in some very lipemic bloods, they were infrequently seen in shaken hemolyzed blood samples.

It seems more likely that the hemolysis is due to the neutral fat in the chylomicra or to its breakdown products, and that the agitation of the blood facilitates transfer of this (or these) substance(s) to the red blood cells. Many of the phenomena which we observed could be explained if one assumed that the kind of fatty acids in the neutral fats in a measure determines the amount of the hemolysis. This would help to explain why the injection of heparin was sometimes followed by a transient increase in hemolysis in cod liver oil and trioleate-fed dogs and not in cream-fed ones. This might be due to the greater chemical reactivity of the unsaturated fatty acids contained in cod liver oil and trioleate. It might also explain why some synthetic fat emulsions cause greater hemolysis than others.

The results of shaking blood at 4 C. lend support to the suggestion that the chylomicra may vary in composition depending on the fat consumed. If this is so, the decreased hemolysis following cream fat meals when the blood was chilled and shaken chilled would not be unexpected since cream fat is solid at 4 C. and one would expect the chemical reactivity to be decreased when in this state. On the other hand, cod liver oil is fluid at that temperature and the he-
Hemolysis was increased, possibly because the number of chylomicra also increased when the blood was cooled.

The results of injections of heparin and of incubation studies may help to clarify the question of the role of the particulate chylomicra. The decrease in hemolysis due to shaking which is usually found after heparin injection and after incubation of postheparin blood accompanies a decrease in turbidity of the plasma and in the number of chylomicra. The chylomicon size also is being altered during this clearing process. Incubation of preheparin blood, on the other hand, is accompanied by no marked change in number or size of chylomicra and therefore the rapid increase in hemolysis due to shaking must be due to changes within the chylomicra, possibly to products of breakdown of the neutral fat, or to changes of the protein components of the chylomicra.

Swank and Wilmot showed that in the first 7 minutes following heparin injection the fatty acid content of the plasma remained essentially unchanged, even though the fat became invisible. Their methods did not differentiate between free fatty acids and those present as glycerides, but Boyle, et al. have stated that during this clearing process triglycerides are hydrolyzed and one might presume that free fatty acids are liberated into the plasma. If free fatty acids were the main cause of hemolysis one would expect a greater degree of hemolysis to occur when supernatant clear postheparin plasma obtained by high speed centrifugation was shaken with fasting red blood cells. Actually only slightly more hemolysis occurred than with similarly prepared preheparin plasma, a result which suggests that if free fatty acids are present in the postheparin plasma they must contribute very little to the hemolysis in the absence of chylomicra. With the data at hand it is probable that the neutral fat in the chylomicra per se is chiefly responsible for the hemolysis which occurs on shaking. The transient increase in hemolysis which occurs after injections of heparin, and the sustained increase during incubation of preheparin blood are not readily explained, but the ultimate decrease in hemolysis after injections of heparin could be due to increases in the percentage of protein within the lipoprotein components of the chylomicra. Graham, et al. and Boyle, et al. have reported that shifts from low density to high density lipoproteins occur after injections of heparin.

The increase in hemolysis which resulted from heating washed chylomicra at 65 C. for 15 minutes could have been due to denaturation of a protein film around the chylomicra. In this way the neutral fat of the lipoprotein could more readily gain access to the red blood cells. A similar mechanism, change in the protein envelope of the chylomicra, might explain the increased hemolysis which resulted from incubation at 37 C. The effect of heat on the fat itself cannot be ruled out however.

Weld and Spitzer observed increased hemolysis in lipemic bloods drawn after the injection of heparin and demonstrated an increase in the volume of postheparin plasma required to prevent hemolysis by saponin. Their suggestion that this might be due to decreased cholesterol is not supported by the observation of Swank and Wilmot that the cholesterol levels were not changed by intravenous heparin. Although we observed occasional slight hemolysis in freshly drawn bloods this was not consistently related to preheparin or postheparin bloods.

The studies of Loewy, et al. suggest that in vivo hemolysis occurs during a
HEMOLYSIS AND LIPEMIA

Heavy alimentary lipemia. Others have observed that anemia may develop in animals receiving frequent intravenous injections of fat emulsions, a change possibly due to increased destruction of the red blood cells. In our studies of four dogs receiving frequent large fat meals over a period of several months no significant changes in hematocrit were noted.

SUMMARY

1. Following ingestion of cream fat, cod liver oil, and glyceryl trioleate, hemolysis occurred when lipemic whole blood samples were shaken. The degree of hemolysis was roughly proportional to the degree of visible lipemia of the plasma. Blood samples from fasting dogs similarly shaken were not hemolyzed. The amount of hemolysis increased with increasing speed or duration of shaking.

2. This hemolysis was not due to an altered fragility of the erythrocytes per se, but to the chylomicra in the plasma or to substances in the plasma associated closely with the chylomicra.

3. Injections of heparin were followed by a rapid disappearance of lipemia and in many instances by a parallel decrease in hemolysis. At times, however, an early and transient increase in the amount of hemolysis followed injection of heparin in cod liver oil and trioleate-fed dogs. It was also shown that following heparin injection there was a slight increase in the amount of hemolysis produced by the particulate-free, usually inactive, subnatant plasma. Protamine injection caused a return of visible lipemia and an increase in the amount of hemolysis on shaking.

4. Incubation studies with whole blood and with plasma showed that the amount of hemolysis on shaking rapidly increased with incubation of preheparin lipemic blood. After heparin injection, incubation brought about either a decrease in hemolysis on shaking, a slight increase, or an increase followed by a decrease.

5. Possible mechanisms of the hemolysis are discussed.

SUMMARIO IN INTERLINGUA

1. Post ingestion de grassia de crema, de oleo de ficato de gado, e de trioleato de glycerylo, hematolyse occurreva quando lipemic specimenes de sanguine integre eseva agitate in vitro. Le grado de hemolysate eseva approximatemente proportional al grado de visible lipemia del plasma. Quando specimenes de sanguine ab canes jejunante eseva similemente agitate illos non eseva hemo-lysate. Le grado de hemolysate se augmentava con augmentate velocitate o dura- tion del agitation.

2. Iste hemolysate non eseva debite a un alterate fragilitate del erythrocytas per se, sed al chylomicros in le plasma o a altere intraplasmic substantias que es intimemente associate con le chylomicros.

3. Injectiones de heparina eseva sequite per un rapide disparition de lipemia e in multe casos per un discrescimento parallel de hemolysate. A vices, totevia, un prompte e transiente augmento in le grado de hemolysate sequave le injection de heparina in canes alimentate con oleo de ficato de gado o con trioleato. Il eseva etiam monstrare que post injection de heparina, un parve augmento in le grado de hemolysate eseva produite per le plasma subnatante, le qual eseva generalmente inactive e libre de particulatos. Injectiones de protamina causava le
reapparition de lipemia visibile e un aumento in le grado de hemolyse produceibile per agitation.

4. Studios de incubation con sanguine integre e con plasma mostrava que le grado de hemolyse produceble per agitation se augmentava rapidamente in conseguencia del efectos de incubation in le caso de sanguine lipemic non tractate con heparina. Post injection de heparina, incubation resultava in o un discrescimento del hemolyse produceibile per agitation o un parve aumento o un aumento sequite per un discrescimento.

5. Possibile mechanismos del hemolyse es discutite.

REFERENCES

Hemolysis and Alimentary Lipemia: Effects of Incubation, Heparin, and Protamine

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