Quantitative Studies of Triglyceride Lipolysis after Heparin Administration

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With the technical assistance of Donu Sodensky

The extent of triglyceride lipolysis by postheparin lipemia clearing factor (lipoprotein lipase) was studied in vitro in 8 atherosclerotic individuals. Varying doses of heparin were injected intravenously and subcutaneously and blood was drawn at stated time intervals for analysis. The rate of splitting of human low density lipoproteins was then determined by measurement of the rate of release of unesterified fatty acids upon incubation of postheparin plasma plus lipoproteins in vitro at 37 C. The data obtained gives a range of the minimum in vivo removal of alimentary neutral fat following an injection of heparin.

In view of the clinical use of heparin in the therapy of atherosclerotic disease, 1 the accumulating evidence indicating that heparin may play a role in the serum transport phase of fat metabolism, 1 and the possibility that a deficiency of heparin production in the body may be etiologically involved in the accumulation of excess quantities of serum low density or \( \beta \)-lipoproteins, 2 it seemed important to quantitate the splitting of triglyceride by postheparin lipoprotein lipase. This could not be done in vivo since circulating lipids are in a dynamic state with lipids entering from the intestine and adipose tissue depots, and are constantly being removed by other tissues. Also optical density changes, using postheparin plasma plus fat substrates, are inadequate as they may be deceptive indicators of lipolysis. 3

Methods

Concentrated aqueous heparin* was injected using different doses and routes in 8 postabsorptive patients with old coronary atherosclerotic disease. Blood was drawn at varying time intervals thereafter. (Atherosclerotic individuals were used for this study since heparin is frequently therapeutically administered to such patients.) Chilled syringes were used and the plasma was separated in the cold to minimize lipolysis during this procedure. One milliliter aliquots of plasma were placed in 5 tubes and 0.2 ml of human low density lipoproteins 5 were added to each tube. Preliminary studies had shown that when less than 0.2 ml lipoproteins were added, the rate of lipolysis by postheparin plasma varied to some extent directly with the substrate concentration. When 0.2 ml or more lipoproteins were added to 1 ml of plasma, the initial rate of unesterified fatty acid release varied with the concentration of postheparin enzyme. Unesterified (free) fatty acids were then determined by the method of Borgstrom 4 at zero time, and after 5, 15, 30 and 60 min., incubation at 37 C. Duplicate analyses were frequently but not routinely performed. The method in this laboratory has, with rare exceptions, had a maximum deviation from the mean of 0.05 mEq/L. The calculation of the release of fatty acids per hour was based upon the maximal rate of release found in any incubation time period rather than from the same points in each experiment since the data reveal that the rate of hydrolysis in any one tube, although usually initially uniform, was not always so. This was probably due to several factors. The rapidity of chilling of the plasma during separation, and the rate of rewarming, may have varied somewhat. The end products of the reaction, glycerol and unesterified fatty acids, were not removed in this in vitro system as they are in vivo, and their accumulation inhibits further lipolysis. 5

*Low density lipoproteins were supplied through the cooperation of Mr. David Spector of the Institute of Medical Physics, Belmont, Calif. They were ultracentrifugally separated from the blood of patients who had large quantities of circulating \( \text{Sd} \) 100-400 lipoproteins. They represent a fivefold concentration of low density lipoproteins suspended in their original serum and consist of \( \text{Sd} \) 0-12 and \( \text{Sd} \) 12-400 lipoprotein classes with the latter predominating.
TABLE 1.—Lipolytic and Clearing Activity of Human Plasma after Various Doses of Heparin Subcutaneously and Intravenously

<table>
<thead>
<tr>
<th>Heparin dose and route</th>
<th>Time after heparin (hours)</th>
<th>O.D.* decrease of U.F.A. in one hour</th>
<th>Release of U.F.A. in mEq./L./hour</th>
<th>Equiv. total lipolysis of neutral fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.B., 65 yrs.</td>
<td>2 min.</td>
<td>0</td>
<td>1</td>
<td>0.06 Gm.</td>
</tr>
<tr>
<td>200 mg. i.v.</td>
<td>36</td>
<td>1</td>
<td>0.4</td>
<td>1.9 Gm.</td>
</tr>
<tr>
<td>100 mg. i.v.</td>
<td>24</td>
<td>0.2</td>
<td>0.2</td>
<td>0.6 Gm.</td>
</tr>
<tr>
<td>50 mg. i.v.</td>
<td>12</td>
<td>0.6</td>
<td>0.6</td>
<td>0.2 Gm.</td>
</tr>
<tr>
<td>200 mg. s.c.</td>
<td>24</td>
<td>0</td>
<td>0.0</td>
<td>0.0 Gm.</td>
</tr>
<tr>
<td>100 mg. s.c.</td>
<td>24</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2 Gm.</td>
</tr>
<tr>
<td>50 mg. s.c.</td>
<td>24</td>
<td>0</td>
<td>0.0</td>
<td>0.0 Gm.</td>
</tr>
<tr>
<td>B.M., 56 yrs.</td>
<td>2 min.</td>
<td>0</td>
<td>0.0</td>
<td>0.0 Gm.</td>
</tr>
<tr>
<td>200 mg. s.c.</td>
<td>36</td>
<td>1.1</td>
<td>1.1</td>
<td>0.2 Gm.</td>
</tr>
<tr>
<td>100 mg. s.c.</td>
<td>24</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1 Gm.</td>
</tr>
<tr>
<td>50 mg. i.v.</td>
<td>24</td>
<td>0</td>
<td>0.0</td>
<td>0.0 Gm.</td>
</tr>
<tr>
<td>H.U., 67 yrs.</td>
<td>24</td>
<td>0</td>
<td>0.0</td>
<td>0.0 Gm.</td>
</tr>
</tbody>
</table>

* O.D., Optical density.
† The release of unesterified fatty acids per hour was calculated from the maximum rate of release of U.F.A. determined after 5, 15, 30 and 60 min. of incubation in vitro.
‡ C, control.

Calculation of lipolysis of neutral fat is necessarily semiquantitative since blood was drawn only 3 to 4 times in 24 hours except in 2 cases. An arithmetic mean was taken and was weighted...
for the number of hours between observations. In most of the subjects, final figures are approximations and are therefore presented as a range rather than as an exact quantity. In the case of the first 2 subjects, however, a more exact determination of the total lipolysis of neutral fat was possible. In these calculations plasma volume was arbitrarily assumed to be 3 L. in males and 2.5 L. in females. The molecular weight of fatty acid used was 269. Thus fatty acids in milliequivalents per liter are converted to milligrams per cent by multiplying by 26.9. Original neutral fat in grams is slightly more than fatty acids released in view of glycerol component (molecular weight 92 per 3 fatty acid molecular weight 807) but this increment is partially reduced by molecular weight of 3 molecules of water (54) added to 3 fatty acid molecules upon hydrolysis of triglyceride. Final calculation of neutral fat is then the equivalent of complete lipolysis of triglyceride, and is the total released unesterified fatty acid in grams plus 4.7% \[
\frac{38(92-54)}{807}\]

An illustration of the calculation of total release of unesterified fatty acids in grams may be helpful at this point. Let us consider patient H.B., 100 mg. dose of heparin subcutaneously:

- First hour avg. release—.5 mEq./L./hr. = .5
- Next 2 hrs. avg. release—.8 mEq./L./hr. = 1.6
- Next 2 hrs. avg. release—.7 mEq./L./hr. = 1.4
- Next 7 hrs. avg. release—.5 mEq./L./hr. = 3.5
- Next 12 hrs. avg. release—.2 mEq./L./hr. = 2.4

Total 9.4 mEq./L. in 24 hrs.

\[9.4 \times 26.9 = 252.9 \text{ mg. per cent} = 2.53 \text{ Gm.} / \text{L.}\]
\[2.53 \times 3 = 7.6 \text{ Gm. in a plasma volume of 3 L.}\]
\[7.6 \times 1.047 = 8.0 \text{ Gm. neutral fat}\]

The question arises whether phospholipid or cholesterol ester may have contributed to the release of fatty acids. We have determined cholesterol esters before and after one hour in vitro incubation at least 25 times and have occasionally observed a small increase, but never a decrease. Phospholipid fatty acids* were measured on three occasions before and after incubation and no significant change was found. Furthermore, many studies of circulating lipids after the injection of heparin have shown rapid sharp decreases in the neutral fat fraction without any associated marked variation in phospholipids.

Clearing was determined by optical density readings using a Coleman junior spectrophotometer. One milliliter postheparin plasma was incubated at 25 C. with 0.5 ml. of 0.25 per cent suspension of coconut oil (Ediol).

| RESULTS |

The data presented in table 1 shows the in vitro lipolytic and clearing activity of human plasma drawn at various time intervals after the intravenous and subcutaneous injection of different doses of heparin. All the subjects showed lipolysis and clearing after heparin although the response is not uniform. Release of the fat-splitting enzyme after a 200 mg. subcutaneous dose of heparin gradually decreased over a 24 to 36 hour period. The total amount of neutral fat split in vitro during this period is appreciable, varying from 18 to 90 Gm., average 40 Gm.

When heparin was administered intravenously, the maximum lipolytic activity was found soon after injection, with a gradual decrease over the next 6 to 10 hours. The duration of activity persisted for a longer period with the larger intravenous dose. The average quantity of neutral fat lipolysis was 10.3 Gm. after 100 mg. heparin intravenously, 6.5 Gm. after 50 mg. heparin. The total in vitro lipolytic activity following a 100 mg. subcutaneous injection of heparin was studied in 2 subjects. It ranged from 8 to 10 Gm. of neutral fat split in 24 hours. The possible reasons for this surprisingly low figure as compared to the 200 mg. dose are considered below.

Decreases in optical density roughly paralleled the degree of lipolysis, although there was no exact relationship. It is apparent that a small amount of the splitting of triglyceride may occur without optical clearing. In one patient, E.H., an example of nonlipolytic clearing is seen in the control sample before the intravenous injection of 50 mg. of heparin. Although the data are not presented in the table, the clotting times (by the three tube Lee-White method) were concomitantly determined in several patients. The anticoagulant and lipolytic actions of heparin were closely parallel. However, a minimal degree of lipolysis was occasionally found when no anticoagulant activity was demonstrable.

| DISCUSSION |

The conditions under which these experiments were performed differ markedly from those existing in the body. In vivo the re-
TRIGLYCERIDE LIPOLYSIS AFTER HEPARIN

leased unesterified fatty acid is rapidly removed from blood; in the test tube it is not and it acts to inhibit further lipolysis.\(^5\) It is apparent that the maximum degree of neutral fat splitting demonstrated in vitro is the minimum which occurs in the bloodstream as a result of the activity of the same quantity of lipoprotein lipase (lipemia clearing factor). The quantities of neutral fat split in vitro by postheparin lipolytic factor are substantial, averaging 40 Gm. following a 200 mg. subcutaneous dose of aqueous heparin. It is highly probable that in vivo considerably more than 40 Gm. of neutral fat is removed from the bloodstream. Another observation indicates this. The unesterified fatty acid content of the lipemic plasma of patient D.R., prior to incubation, was 0.3 mEq./L. before heparin and 1.2 mEq./L. 2 min. after heparin. This represents a considerable degree of lipolysis in two min. in the body, or in the test tube in the 5 to 10 min. during which plasma was being separated in the refrigerated centrifuge, perhaps 5.4 to 27.0 mEq./L./hour. The rate of release of fatty acid from added lipoproteins by the same plasma upon in vitro incubation was much less (0.8 mEq./L./hour). This suggests that fresh chylomicra may be a better fat substrate for postheparin lipoprotein lipase than low density lipoproteins which have endured the involved process of ultracentrifugal separation, or that the in vivo rate of lipolysis is really much more rapid than in vitro.

An inspection of the results shows that the subcutaneous route is most efficient in terms of grams of triglyceride lipolysis per milligram of heparin. There was an average of 40 Gm. of neutral fat split after injection of 200 mg. subcutaneous heparin as compared to 10.2 Gm. split after 100 mg. and 6.5 Gm. after 50 mg. intravenous heparin. This is probably related to a more rapid excretion of heparin when higher blood levels are present. Lipolytic data suggest that above a certain level of heparin, little further increase in lipemia clearing activity is attained. Intravenous 100 mg. doses certainly result in far higher circulating heparin levels than 200 mg. subcutaneously, but there is little difference in the peak rate of lipolysis. It has previously been found in rats\(^6\) that above 0.5 mg./Kg. intravenously, increments in heparin dosage gave no further increase in clearing activity. Our data indicate that whereas the height of clearing activity was no different after 100 or 50 mg. intravenous heparin, the duration was more prolonged after the larger dose.

Data presented following a 100 mg. dose of heparin subcutaneously show lower values of triglyceride lipolysis than anticipated in view of the response following a 200 mg. dose. This may have been due to another complicating factor which would tend to minimize the extent of lipolytic activity demonstrable in vitro. There is evidence\(^7\) in rats that lipemia clearing activity is stimulated by fat intake and is altered or inactivated during the lipolytic process. Studies in progress in this laboratory of endogenous lipemia clearing factor following intravenous fat emulsions in humans are yielding results similar to those found in rats. It is possible that when lesser degrees of lipoprotein lipase activity result, such as that following smaller doses of heparin which are slowly absorbed from the subcutaneous tissue, inactivation of the enzyme during lipolysis almost keeps pace with its production. At any given moment, therefore, in vitro demonstration of activity might be misleading and considerably less than the degree of lipolysis which had occurred in vivo. When a high level of enzyme activity was being constantly produced in vivo, such as after larger subcutaneous doses of heparin or following intravenous injection, a larger amount would be left after in vivo lipolysis for detection by in vitro technics.

Several investigators have stated that the development of clearing factor following small doses of heparin is less in atherosclerotic individuals than in normals.\(^8\)\(^-\)\(^10\) No difference was found with 100 mg. doses between normal and atherosclerotic subjects.\(^11\) In elderly atherosclerotic patients used in this study, considerable lipolytic activity developed following each dose of heparin although most subjects had been receiving injections of heparin twice weekly for several years. Apparently heparin effectively clears lipemia.
regardless of the number of previous injections. In this small series no consistent difference was noted between sexes in their lipolytic response to heparin in large doses. It is also interesting that substantial fat-splitting enzyme activity developed in elderly subjects used in this study although it has been found that there is more postheparin clearing ability in younger age groups. Recent work indicates that neutral fat fatty acids function as the major vehicle for transport of fatty acids in plasma, and that there is a close relationship between removal of chylomicrons and hydrolysis of their constituent glycerides. The rate of removal of unesterified fatty acids from the blood of dogs is extremely rapid, with a half life of 2 min. Thus postheparin lipoprotein lipase clears fat from the blood via a mechanism very similar to or identical with that which normally functions in this phase of lipid transport.

SUMMARY

Triglyceride lipolysis by postheparin lipemia clearing factor has been investigated in vitro in 8 atherosclerotic patients. Approximately 40 Gm. neutral fat was split in vitro by lipolytic activity resulting from 200 mg. aqueous heparin injected subcutaneously, 9.1 Gm. after 100 mg. subcutaneously, 10.2 Gm. after 100 mg. intravenously and 6.5 Gm. after 50 mg. intravenously. Evidence is discussed which indicates that the maximum rate of in vitro lipolysis is probably the minimum which occurs in vivo.

REFERENCES

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