Plasma Lipoprotein Lipase after Subcutaneous Heparin

By William E. Connor, M.D., and Mark L. Armstrong, M.D.

In addition to its anticoagulant action, heparin initiates the release of lipoprotein lipase into the blood. This lipolytic enzyme has the capacity to reduce the turbidity of lipemic plasma and hence has become known also as "clearing factor." Its clearing action occurs from the hydrolysis of triglyceride contained in chylomicrons and other lipoprotein particles.

Studies in animals have suggested that the administration of heparin inhibited the development of atherosclerosis. Heparin may block atherogenesis because of its antilipemic property. Heparin has been used in the treatment of patients with coronary atherosclerosis because of these effects on lipid metabolism. Lipoprotein lipase resulting from the injection of heparin reduces lipemia and thereby may inhibit atheroma formation.

It is known that this enzyme appears in the blood promptly after the intravenous injection of heparin and begins to disappear rapidly. In order to obtain more prolonged activity, heparin has been given by subcutaneous injection.

The present investigation was undertaken to find out how long lipoprotein lipase remained in the blood of men after the administration of subcutaneous and intravenous heparin. If the use of heparin in the therapy of atherosclerosis is predicated on its antilipemic action, then it would seem of some importance to show the particular dose as well as the route of administration that can be expected to produce significant and prolonged plasma lipoprotein-lipase activity.

The influence of age, disease, and long-term heparin administration upon the lipoprotein-lipase response to subcutaneous heparin was studied also.

Materials and Methods

All subjects were men in good nutritional status. Pre-heparin blood studies were performed before breakfast. For subcutaneous administration, heparin was then injected into the upper outer arm through a 25-gage needle. Heparin* was given subcutaneously in all instances except as indicated. Intravenous heparin was given over a 5-minute interval. Subsequently, the subjects had their usual meals. Subjects were divided into three groups.

Nineteen men, aged 22 to 71 years with a mean age of 43, received 50 mg. (5,000 units) of heparin (group A). Six were healthy prison volunteers, aged 22 to 26 years. Six, aged 33 to 64, had known coronary atherosclerosis; four, aged 36 to 71, had cerebral vascular disease. Three, aged 24 to 64, were healthy or had minor disorders.

Seven other subjects, aged 40 to 63, received 200 mg. (20,000 units) of heparin in two different concentrations 1 week apart (group B). The concentrations used were 100 mg. per ml. and 400 mg. per ml. These men had a variety of illnesses: only one had coronary atherosclerosis, another had hypertension, and the remainder did not have vascular disease. No subject in any group had cardiac failure, clinically evident renal disease, or a metabolic disease besides atherosclerosis.

The effects of the same dose of heparin (50 mg.) given by the intravenous and subcutaneous routes were compared on different days in two subjects of group A.

Eight men who had received 50 mg. of subcutaneous heparin daily for periods of 9 to 17 months were tested for lipoprotein-lipase response to heparin (group C). They had either coronary atherosclerosis with a documented myocardial infarction or cerebral vascular disease. In preparation for the test they had omitted their usual heparin for 36 hours. In the morning each was given 50 mg. of heparin.

*Sodium heparin, The Upjohn Company, Kalamazoo, Michigan.
†Sodium heparin, Abbott Laboratories, North Chicago, Illinois.
Heparin stimulated tubes.1
lipase completely.

Comparison arin.
of detail.10
emulsion.

The white blood samples were obtained from forearm veins, and 9 ml. of blood were added to 1 ml.
of 1.85 per cent potassium oxalate solution in tubes chilled in an ice bath. Specimens were centrifuged at 4,000 rpm and 4 C. for 10 minutes. Subsequently, the plasma specimens were kept at 4 C. for a period not longer than 2 hours before enzymatic activity was determined. The enzyme remains stable in plasma for over 4 hours at 4 C.

Plasma lipoprotein lipase was measured by two methods: by the amount of glycerol produced by lipolytic action, and by the reduction of optical density or "clearing" of a plasma-coconut oil emulsion. These methods have been described in detail.10

The whole-blood clotting time was measured by the method of Lee and White with three clotting tubes.11

Results
Comparison of the Plasma Lipoprotein Lipase after the Intravenous and Subcutaneous Injection of Heparin
After 1 hour heparin given intravenously stimulated an initially greater lipoprotein-lipase response than did subcutaneous heparin. By 6 hours the effect had disappeared completely. In contrast, subcutaneous heparin of the same dosage caused an increase in plasma lipoprotein lipase, which persisted for at least 24 hours and probably up to 36 hours (fig. 1). A similar result was obtained in the other subject so tested.

Table 1

<table>
<thead>
<tr>
<th>Number</th>
<th>Hours</th>
<th>Glycerol (µM per ml.)</th>
<th>Change in optical density (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>of subjects</td>
<td>after heparin</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>0.017</td>
<td>0.021</td>
</tr>
<tr>
<td>8</td>
<td>0.543</td>
<td>0.198</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0.020</td>
<td>0.045</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0.263</td>
<td>0.067</td>
</tr>
<tr>
<td>16</td>
<td>0.334</td>
<td>0.080</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0.020</td>
<td>0.045</td>
</tr>
<tr>
<td>20</td>
<td>0.239</td>
<td>0.058</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>19</td>
<td>0</td>
<td>0.018</td>
<td>0.031</td>
</tr>
<tr>
<td>24</td>
<td>0.150</td>
<td>0.120</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*The values for lipoprotein lipase obtained before heparin (at 0 hour) are compared with those occurring after heparin at various intervals of time. Each of the 19 subjects had a pre-heparin determination and two or more post-heparin determinations.

Measurement of Lipoprotein Lipase

Duration of Action of Subcutaneous Heparin, 50 Mg. (5,000 Units)

In 19 subjects of group A, plasma lipoprotein lipase was determined before and after the subcutaneous injection of 50 mg. of heparin. Table 1 gives the mean values of the two indices for lipoprotein-lipase activity: glycerol production and the change in optical density of the incubation mixture. Both glycerol and optical density change were increased significantly at 8, 12, 16, 20, and 24 hours after heparin. Some decline in lipoprotein-lipase activity occurred between 8 and 12 hours, but there was little change from 12 to 20 hours after heparin. At 24 hours lipoprotein lipase was still present in the plasma at a significant concentration for both glycerol production and optical density change. The decline from 8 to 24 hours was 23 per cent for glycerol and 22 per cent for optical density change. Figure 2 shows graphically the net increases in plasma lipoprotein lipase after subcutaneous heparin as compared with the control level.

Influence of Disease and Age upon the Lipoprotein-Lipase Response to Subcutaneous Heparin (Group A)

Ten subjects had coronary or cerebral atherosclerosis. The lipoprotein-lipase response of these subjects at 16 and 24 hours after heparin was compared with the response in the eight healthy individuals (table 2). There
Circulation, Volume XXIV, July 1961

PLASMA LIPOPROTEIN LIPEASE AFTER HEPARIN

Figure 1

Plasma lipoprotein lipase (LPL) was measured after the injection of heparin in a 43-year-old man with clinically severe coronary atherosclerosis. Heparin in a dose of 50 mg. was administered intravenously on one day and then subcutaneously 1 week later.

were no significant differences in responses between the two groups.

When the subjects were divided by age, seven were over 60 and six were under 25 years of age. The two groups had similar plasma lipoprotein-lipase concentrations at 16 and 24 hours after heparin (table 3).

Comparison of Two Doses of Heparin

Table 4 shows the plasma lipoprotein lipase found at 1, 4, 24, 36, 48, and 72 hours after the subcutaneous injection of 200 mg. of heparin in seven men. At 24 hours after heparin both glycerol production and optical density change indicated lipoprotein-lipase activity. This persisted to 36 hours. At 48 hours after heparin the values for both glycerol and optical density change were very low.

When the plasma lipoprotein lipase was compared at 24 hours for the two doses of heparin, 50 mg. and 200 mg., the resulting enzymatic activities were statistically alike (fig. 3). Although the larger dose had no conclusively greater effect at 24 hours, it did produce more lipoprotein lipase at 1 and 4 hours after injection. Comparative mean figures with regard to optical density change were:

50 mg. 
200 mg.

(15 subjects) 
(7 subjects)

1 hour 0.273 ± 0.095 units 0.482 ± 0.059 units
4 hours 0.224 ± 0.097 units 0.429 ± 0.126 units

Figure 2

The net increase in plasma lipoprotein lipase (LPL) after the subcutaneous injection of 50 mg. of heparin. The values for glycerol and optical density change were obtained by subtracting the pre-heparin results (0 hour) from the post-heparin figures at the indicated times, thus giving the net increases.

Lipoprotein-Lipase Response to Two Different Preparations of Heparin

Heparin (Upjohn) 100 mg. per ml. and heparin (Abbott), 400 mg. per ml. were given subcutaneously in doses of 200 mg. to the same seven subjects. One week separated the two experiments. The lipoprotein-lipase responses were identical at 4 and 24 hours after heparin. For example, the 24-hour values were as follows:

<table>
<thead>
<tr>
<th>Heparin concentration</th>
<th>Glycerol optical density (200 mg. dose) (µM per ml.)</th>
<th>(units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg. per ml.</td>
<td>0.21 ± 0.19</td>
<td>0.11 ± 0.09</td>
</tr>
<tr>
<td>400 mg. per ml.</td>
<td>0.21 ± 0.13</td>
<td>0.11 ± 0.08</td>
</tr>
</tbody>
</table>

Thus these two different preparations, each in a different concentration, caused almost identical lipoprotein-lipase responses.

Lipoprotein Lipase after Prolonged Heparin Therapy

Eight patients with coronary or cerebral vascular disease who had been receiving 50 mg. of subcutaneous heparin daily for an average period of 12 months were tested for lipoprotein-lipase activity after a single 50-mg. injection of the drug. Despite the fact that these patients had not received heparin for a period of 36 hours before the test, they still had residual lipoprotein-lipase activity.
Anticoagulant net used daily after tested who men. heparin. already ered.

Subsequently, the increase in plasma lipoprotein lipase at 8 and 12 hours was as great as or greater than for the group A subjects so tested who had not been receiving heparin daily (table 5). At 24 hours there were no net increases, but this fact must be considered in view of the lipoprotein-lipase activity already present at the zero-hour time in these men.

**Anticoagulant Activity**

The clotting time of whole blood was measured in all of the subjects at 1, 4, and 24 hours after the subcutaneous injection of 50 mg. of heparin. The mean increase of the clotting time after heparin was 39 per cent at 1 hour and 28 per cent at 4 hours. At 24 hours the clotting time had returned to the pre-heparin level. Thus 50 mg. of heparin had a mild, but detectable, anticoagulant effect.

**Discussion**

A significantly increased concentration of plasma lipoprotein lipase occurred for as long as 24 hours after the subcutaneous injection of 50 mg. of heparin. When the same dose was given intravenously, the duration of action was only one fourth as long. A similar duration of action for intravenous heparin has been found by others.12 The 200-mg. dose of heparin given subcutaneously produced an initially greater quantity of lipoprotein lipase than 50 mg., but at 24 hours after injection, the lipoprotein-lipase responses after the two different doses were similar. The effects of the 200-mg. injection were dissipated almost completely 48 hours later.

Nikkila and Sirola12 reported that 20,000 units of heparin (200 mg.) given subcutaneously produced a clearing activity in plasma for as long as 24 hours after injection. Engelberg12 measured lipoprotein lipase after subcutaneous heparin in a dose of 200 mg. and found plasma lipolytic activity at 24 hours but little beyond this time.

Thus, the evidence suggests that, for the maintenance of significant plasma lipoprotein-lipase concentration on a long-term regimen, heparin should be given subcutaneously.
Once a day. The dose of 50 mg. per day increased lipoprotein lipase in plasma for 24 hours and did not cause a profound anticoagulant effect. A 200-mg. dose given daily causes a therapeutic anticoagulant effect; with prolonged therapy the risk of bleeding might be great. A recent study emphasized the hazards in terms of thromboembolic episodes when heparin therapy was terminated abruptly. These complications occurred 24 to 30 hours after the cessation of heparin therapy in patients with acute myocardial infarction. Such rebound phenomena might conceivably be expected from a regimen of intermittent subcutaneous injections of heparin.

As yet, the treatment of coronary atherosclerosis with heparin remains experimental. The use of heparin is advocated largely on the basis of its antilipemic effect. The most direct approach to a lessening of lipemia is dietary prudence; i.e., the avoidance of meals rich in fat and cholesterol. It is not known whether a combination of heparin therapy and dietary lipid restriction might be more effective than either therapy alone in ameliorating atheromatous disease.

Anticoagulant activity from a 50-mg. dose of heparin was present at 1 and 4 hours, but not at 24 hours after heparin. Such an in vitro finding at 24 hours does not necessarily indicate an absence of anticoagulant effect within the body. As Hartman and co-workers have indicated, an anticoagulant effect from a given dose of heparin may be measured with more sensitive technics long after the whole-blood clotting time in glass tubes has become normal. It has also been suggested that a heparin-induced anticoagulant effect is measurable in interstitial fluid for a longer time than in blood. Such studies provide additional evidence for the view that heparin continues to have an in vivo anticoagulant action after a return to normal of the whole-blood clotting time.

Our results support the information given by Baker that the plasma lipoprotein lipase resulting 8 minutes after intravenous heparin was not affected by age or the presence of atherosclerosis. Sixteen and 24 hours after subcutaneous heparin, plasma lipoprotein lipase was similar in subjects under 25 and over 60 years of age. Nikkila and Niemi,18

---

Table 4

<table>
<thead>
<tr>
<th>Hours after heparin</th>
<th>Glycerol (μM per ml.)</th>
<th>Change in optical density (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>0</td>
<td>0.029</td>
<td>0.024</td>
</tr>
<tr>
<td>1</td>
<td>0.482</td>
<td>0.059</td>
</tr>
<tr>
<td>4</td>
<td>0.429</td>
<td>0.126</td>
</tr>
<tr>
<td>24</td>
<td>0.210</td>
<td>0.192</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>0.053</td>
<td>0.035</td>
</tr>
<tr>
<td>72</td>
<td>0.029</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Table 5

<table>
<thead>
<tr>
<th>Hours after heparin</th>
<th>Glycerol (μM per ml.)</th>
<th>Change in optical density (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>0</td>
<td>0.069</td>
<td>0.125</td>
</tr>
<tr>
<td>8</td>
<td>0.670</td>
<td>0.107</td>
</tr>
<tr>
<td>12</td>
<td>0.530</td>
<td>0.178</td>
</tr>
<tr>
<td>24</td>
<td>0.100</td>
<td>0.059</td>
</tr>
</tbody>
</table>

*The eight subjects received 50 mg. of heparin subcutaneously on the test day. The values after heparin were compared with the 0-hour values.
Figure 3
Comparison of the lipoprotein lipase response at 24 hours post-heparin between two different doses given subcutaneously. Group A subjects were given 50 mg. and group B subjects, 200 mg.

however, found more lipoprotein lipase in younger subjects after the injection of heparin intravenously. They measured only optical density changes; the means were 0.060 units for the younger and 0.023 for the older group. In our experience both of these optical density changes are slight. Our data were based upon optical density changes of the order of 0.200 for the older men and 0.198 for the younger.

Summary
Plasma lipoprotein lipase was measured at intervals after the subcutaneous injection of heparin in 34 men. This enzyme was significantly increased in plasma for as long as 24 hours after a 50-mg. dose. While the initial response was greater after a 200-mg. dose, the 24-hour lipoprotein lipase was similar for both 50 mg. and 200 mg.

Plasma lipoprotein lipase produced after intravenous heparin was dissipated after 6 hours.

The lipoprotein-lipase response at 16 and 24 hours after the subcutaneous injection of heparin was similar in men with coronary and cerebral atherosclerosis and in healthy men. Age did not affect plasma lipoprotein-lipase response.

Patients who had received 50 mg. of heparin subcutaneously each day for an average time of 12 months did not have exhaustion of the lipoprotein-lipase response to a test dose of heparin.

It is suggested that the daily administration of heparin is necessary to produce a continuous concentration of plasma lipoprotein lipase. A dose of 50 mg. (5,000 units) given subcutaneously produced a prolonged lipoprotein-lipase response and only a mild anticoagulant effect, unlikely to induce the complication of bleeding.

References

Circulation, Volume XXIV, July 1961

When young men, not yet arrived at their full growth, are forcibly impressed into the military service, and thereby at once lose all hope of returning safe and sound to their beloved home and country, they become sad, silent, listless, solitary, musing, and full of sighs and moans, and finally quite regardless of, and indifferent to, all the cares and duties of life. From this state of mental disorder nothing can rouse them—neither argument, nor promises, nor the dread of punishment; and the body gradually pines and wastes away, under the pressure of ungratified desires, and with the preternatural sound of one side of the chest. This is the disease nostalgia. I have examined the bodies of many youths who have fallen victims to it, and have uniformly found the lungs firmly united to the pleura, and the lobes on that side where the obscure sound had existed callous, indurated, and more or less purulent. Some years ago this disease was very common, but is now rarely met with, since the wise arrangement has been adopted of limiting the period of military service to a certain number of years only.—From On the Percussion of the Chest. Published in 1761. Translated by John Forbes, M.D. In: Classics of Medicine and Surgery. New York, Dover Publications, Inc., 1959, p. 132.
Plasma Lipoprotein Lipase after Subcutaneous Heparin
WILLIAM E. CONNOR and MARK L. ARMSTRONG

Circulation. 1961;24:87-93
doi: 10.1161/01.CIR.24.1.87
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1961 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/24/1/87

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at: http://circ.ahajournals.org//subscriptions/