Plasma Heparin Levels
Correlation with Serum Cholesterol and Low-Density Lipoproteins

By H. Engelberg, M.D.

SINCE the original observations of the effect of heparin upon the clearing of alimentary lipemia,1 much data have accumulated that indicate that heparin functions physiologically to facilitate the exit of alimentary triglycerides from the blood. The available evidence on this important question has recently been summarized.2,3

The nature of the heparin lipemia-clearing reaction involves the activation of an enzyme, lipoprotein lipase, which splits the triglycerides of chylomicra and low-density lipoproteins. This activity has been demonstrated in plasma in vitro and in vivo, both after the injection of heparin and endogenously, and, at least in relation to postalimentary lipemia, apparently takes place within the bloodstream or at the capillary wall. The free fatty acids released after triglyceride lipolysis are bound to albumin and rapidly transported to the tissues. The distribution of the other triglyceride component, glycerol, has not been thoroughly investigated. There is much indirect evidence that heparin mobilizes the lipolytic enzyme (apparently present in the capillary wall), stabilizes its activity, and effects its rapid attachment to the chylomicron or lipoprotein substrate.

The heparin lipemia-clearing reaction thus circumvents the hitherto perplexing problem of the barrier that the capillary wall offers to the exit of lipid macromolecules from the bloodstream in all areas except in the liver. This organ may have a direct function in the clearing of alimentary lipemia. There is evidence in rats that the liver removes labeled triglycerides from chylomicra, but this may represent an exchange reaction rather than uptake of chylomicrons. Additional work is necessary to clarify this subject. It is unlikely, however, that the liver has the primary major role in chylomicon removal and dissolution in man, since it does not possess a true lipase and it inactivates lipoprotein lipase. Furthermore, abnormal liver function is not demonstrable in most persons with elevated serum cholesterol and lipoprotein levels.

The reticuloendothelial system is the third pathway which has been proposed for the removal of alimentary lipemia. The available evidence, however, indicates that it functions only in the removal of foreign lipid material, such as fat emulsions, but does not take up normal chyle fat.

Because of the probable physiologic relationship between heparin and fat transport we have been interested in the measurement of circulating endogenous heparin and its correlation with serum lipids. Applying toluidine blue4 and protamine titration5 procedures, other workers have also determined heparinoid-like substances in the blood of atherosclerotic patients but serum lipids were not analyzed, and the methods used are not so much an accurate index of heparin but rather indicators of the equilibrium between the systems of factors favoring or inhibiting coagulation. In our first study we reported an inverse correlation between circulating heparin and low-density lipoproteins.6,7 These findings have been corroborated8 but the data can be questioned, since the heparin values were far above normal,9 and probably represent heparin plus metachromatic sulfates that were not removed in the analytic procedure. Other papers10–13 have appeared in which the level of heparinoid substances in the blood of atherosclerotic patients was found to be lower than in normal individuals, but the results are only suggestive, since the methods applied are not specific for heparin.

Subsequent to our original report we pub-
Table 1

Summary of Average Data

<table>
<thead>
<tr>
<th>Heparin levels*</th>
<th>Age groups</th>
<th>Chol. mg.%</th>
<th>Standard low-density lipoproteins in mg. %</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sf 0-12 12-20 20-100 100-400 1200</td>
<td></td>
</tr>
<tr>
<td>Up to</td>
<td>5 &amp; over</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td></td>
<td>335 348 464 616 72 298</td>
<td>4 3</td>
</tr>
<tr>
<td>A</td>
<td>12.1 to 15</td>
<td></td>
<td>258 274 372 474 55 222</td>
<td>16 15</td>
</tr>
<tr>
<td>S</td>
<td>18</td>
<td></td>
<td>214 222 621 68 222 122 52 211 231</td>
<td>9 9</td>
</tr>
<tr>
<td></td>
<td>5 &amp; over</td>
<td></td>
<td>222 232 61 68 222 100 100 140 221</td>
<td>5 5</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td></td>
<td>241 241 671 52 110 43 205 18 18</td>
<td>4 4</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td></td>
<td>222 222 671 52 78 25 138 11 10</td>
<td>3 3</td>
</tr>
</tbody>
</table>

*Heparin levels in units/100 ml, 1 mg. = 100 units.

Table 2

Average Lipid Values at Varying Heparin Levels

<table>
<thead>
<tr>
<th>Heparin levels, units %</th>
<th>Cholesterol mg.%</th>
<th>Standard low-density lipoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sf 0-12 mg.% Sf 12-20 mg.% Sf 12-200 mg.%</td>
</tr>
<tr>
<td>Up to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>12</td>
<td>292</td>
</tr>
<tr>
<td>A</td>
<td>12.1 to 15</td>
<td>261</td>
</tr>
<tr>
<td>E</td>
<td>15.1 to 18</td>
<td>238</td>
</tr>
<tr>
<td>S</td>
<td>18</td>
<td>236</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>12</td>
<td>279</td>
</tr>
<tr>
<td>E</td>
<td>12.1 to 18</td>
<td>269</td>
</tr>
<tr>
<td>M</td>
<td>15</td>
<td>267</td>
</tr>
<tr>
<td>A</td>
<td>15.1 to 18</td>
<td>226</td>
</tr>
</tbody>
</table>

Established an improved technique for the extraction of endogenous plasma heparin. The product obtained contains heparin and chondroitin sulfuric acid. The final assay is based upon anticoagulant activity and therefore only the heparin content of the extract is determined, since chondroitin sulfuric acid has no, or only trace, anticoagulant activity. The use of a biologic assay (with previous dialysis to remove interfering salts) avoids the defects inherent in procedures that involve metachromatic assay. These have been previously discussed, and the reasons have been presented indicating the specificity and superiority of the heparin extraction method used in this study.

Methods

Approximately 50 to 60 ml of blood were taken from the arm veins of patients after they had been examined in my office, usually in the afternoon, after lunch. Heparin assay was performed in duplicate as previously described on 5-ml plasma samples, and cholesterol and ultracentrifugal lipoprotein determinations were made on serum aliquots. The results were discarded if the duplicate heparin determinations deviated more than 15 per cent from the mean, since this is the variation found in reproducibility studies of this method. Blood was not drawn post-absorptively, since there is no marked change after meals in the three parameters measured. Furthermore, we were interested in the post-alimentary relationships between heparin and lipids. It had also been previously established that the in vitro addition of varying concentrations of human low-density serum lipoproteins did not

*These were performed at the Institute of Medical Physics, Belmont, California.

†This occurred in 7.7 per cent of the analyses.
affect the extraction of heparin from aliquots of pooled plasma. Although both normal and atherosclerotic persons were used, certain categories were omitted from this study. These included subjects with liver or kidney disease, thyroid abnormalities, essential hyperlipemia, acute inflammatory or infectious diseases, and patients who were on restricted diets of any type or who were receiving hormone or drug therapy. These groups had variations of factors affecting fat transport other than heparin and the lipemia-clearing enzyme. The use of supplemental vitamins was ignored, as it is widespread, and we have not observed that it has any effect on circulating heparin levels. The age of the patients varied from 26 to 91 years.

Results*

A summary of the average data arranged according to increasing concentrations of circulating heparin, and separated according to sex and age groups, is shown in table 1. There were 147 males and 113 females who had heparin and cholesterol analyses, and 131 males and 77 females of the group also had the lipoprotein determinations. Inspection of the data reveals a decrease in the average lipid values as average heparin levels increase. A simplified set of average values is shown in table 2, and a statistical analysis of heparin versus each of the three lipid parameters in table 3.* All the correlation coefficients are negative. In males the inverse correlation between endogenous plasma heparin on the one hand and serum cholesterol and Sf 12-400 lipoproteins on the other is definitely significant, whereas the correlation of heparin with Sf 0-12 lipoproteins is not significant. In females the relationship between heparin and cholesterol is inverse but is just short of the 5 per cent level of significance. The correlation between heparin and the Sf 0-12 lipoproteins is probably significant, however, and the correlation with the Sf 12-400 lipoproteins is definitely statistically significant.

Discussion

These data were obtained in a fairly large group of people with a more reliable method of heparin extraction than that used in our

*The individual data of heparin levels, cholesterol, and lipoproteins have been deposited as Document number 6523 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C. A copy may be secured by citing the Document number and by remitting $1.25 for photoprints, or $1.25 for 35-mm. microfilm. Make checks or money orders payable to: Chief, Photoduplication Service, Library of Congress.

*I am indebted to Dr. Morton I. Grossman for help with the statistical calculations.
earlier study. They demonstrate a statistically significant inverse correlation between endogenous plasma heparin and serum low-density Sf 12-400 lipoproteins and cholesterol. This is physiologically important in view of the involvement of heparin in the enzyme system that probably has an important role in the removal of alimentary neutral fat from the bloodstream in man. Although the serum transport phase of fat metabolism is undoubtedly complex, with many dynamically interacting events, the results afford substantial evidence that a relative insufficiency of circulating heparin is one of the important etiologic factors leading to hyperlipoproteinemia, hypercholesteremia, and atherosclerosis.

The negative correlation coefficients between circulating heparin and lipids, though definitely significant, are, however, relatively low numerically. Therefore, there are other factors exerting powerful influences upon serum lipids. We may speculate upon these, since they are the basis for further investigation. Lipoprotein lipase, in addition to heparin, contains a tissue factor that is probably the true lipolytic moiety. The availability of this apoenzyme to the mobilizing action of heparin undoubtedly affects the resultant enzymatic activity, and apoenzyme deficiency has been reported. It could also be that adequate supplies of heparin and tissue factor exist but that other substances competitively bind heparin, so that lipoprotein lipase formation is inhibited. Lipoprotein lipase itself may be present in adequate quantity but inhibitors might interfere with its lipolytic activity. It is also well to remember that there is no evidence of impaired triglyceride clearance in subjects with cholesterol elevations of the essential hypercholesteremia type. Thus there is no reason to believe that heparin deficiency would be present in these individuals who constitute a substantial fraction of patients with increased serum cholesterol levels.

Other workers have reported a significant negative correlation between plasma heparin and Sf 0-12 and Sf 12-20 lipoproteins, but not between heparin and lipoproteins above Sf 20, nor with the high-density lipoproteins. Our findings, however, showed a statistically significant negative correlation between circulating heparin and Sf 12-400 lipoproteins in both sexes, whereas a probably significant relationship with Sf 0-12 lipoproteins was found only in females. In addition to their application of a method that is not specific for heparin, as discussed earlier in this paper, the discrepancy may lie in their use of fasting subjects who show a trend toward higher Sf 0-12 and lower Sf 20-400 lipoprotein levels. The more significant negative correlation of endogenous heparin with the Sf 12-400 lipoproteins, rich in triglyceride, which our data show, is consonant with all pharmacologic studies with injected heparin, which demonstrate that lipoproteins of Sf 20 and higher are those primarily affected by the heparin-activated lipase. Studies of endogenous plasma lipemia-clearing factor in non-fasting subjects have also shown a significant inverse relationship to the Sf 12-004 lipoproteins but not to the Sf 0-12 class.

It may be argued that the lipoproteins affect the level of circulating heparin rather than vice versa, but there is some evidence against this possibility. It has been found that intravenous fat infusions in man result in an increase in plasma heparin levels in the majority of subjects. Oral fat feedings do not decrease circulating heparin. Furthermore, individuals with nephrosis, hypothyroidism, or essential hyperlipemia, who have markedly elevated serum lipids, do not have low values of plasma heparin. Finally, the reduction of serum lipids by strict low-fat diets does not affect heparin levels. It is therefore more reasonable to consider that the heparin content of the plasma is the primary factor in the inverse relationship between heparin and serum lipids.

Summary

Endogenous plasma heparin was determined in 147 males and 113 females. Serum cholesterol was measured in the entire group, and low-density lipoproteins were ultracentrifugally analyzed in 131 males and 77 females.
Statistical analysis of the data showed a definitely significant ($p<.01$) negative correlation between heparin on the one hand and serum cholesterol and Sf 12-400 lipoproteins on the other in males.

In females the correlation between heparin and cholesterol was negative but fell short of the 5 per cent level of significance. The correlation between heparin and Sf 12-400 lipoproteins was negative and statistically definitely significant ($p<.01$). The correlation with the Sf 0-12 lipoproteins was also negative and probably significant ($p<.05$).

These results afford substantial evidence that a relative deficiency of circulating heparin is one of the important causative factors leading to elevated levels of serum lipid.

References

Plasma Heparin Levels: Correlation with Serum Cholesterol and Low-Density Lipoproteins
H. ENGELBERG

Circulation. 1961;23:573-577
doi: 10.1161/01.CIR.23.4.573
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/23/4/573

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/