Blood Lipids and Human Atherosclerosis

II. The Influence of Heparin upon Lipoprotein Metabolism

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Heparin administered to humans and rabbits causes profound reorientation in the distribution of low density lipoproteins, characterized by a shift of lipoproteins of high Sf rates to those of successively lower Sf rates. The observations appear to indicate that this agent has actually caused a transformation of the former group into the latter. Heparin administered to the rabbit prevents the usual buildup of high concentration of the Sf 10–50 lipoproteins during cholesterol feeding and retards the development of atherosclerosis. In man, accompanying the redistribution of lipoproteins, there was observed a marked reduction in angina pectoris in 55 of 59 patients studied who presented this symptom. The relation between the heparin effect upon lipoproteins and its effect upon angina cannot be assessed at present.

A HYPOTHESIS that atherosclerosis is a disease associated with or caused by an error in the metabolism of fat and other lipids has been previously presented.1 The evidence supporting this hypothesis consists in the demonstration of the existence of certain special classes of lipoprotein molecules associated with atherosclerosis in several experimental animal species and in the human developing or manifesting atherosclerosis.1–3

The further study of these lipoproteins as well as several other lipoprotein species has revealed4 that essentially all of the blood lipids (including fat, cholesterol, cholesterol esters, and phospholipids) exist in the blood only in the form of structural entities within several of these lipoprotein molecular classes. Current work is directed toward an understanding of the factors involved in the defect which results in the abnormal elevation of such molecules as the Sf 10–20 lipoproteins in certain individuals. Each lipoprotein, which can be identified in its native state by the ultracentrifugal analysis of serum, presumably has a different functional role in lipid metabolism. Its concentration in the blood is the resultant balance between formation and utilization at any particular steady state of physiologic activity. Physiologic factors already reported to influence serum lipoprotein levels include (a) dietary intake of fats and cholesterol, (b) thyroid function, (c) experimental adrenal cortical hyperactivity (rabbit only), (d) age, (e) sex, (f) pregnancy.5–5 Several errors in lipid metabolism have now been classified by the ultracentrifugal analysis of serum lipoproteins. Such are manifest in (a) atherosclerotic state, (b) nephrosis, (c) xanthoma tuberosum, (d) biliary obstruction, (e) acute hepatitis, (f) certain cases of diabetes, (g) hypothyroidism, (h) hypercholesterolemia, (i) xanthelasmas.5 In addition, there are certain bizarre, but characteristic, ultracentrifugal lipoprotein patterns which are seen in presumably normal individuals, but are as yet unclassified.

All individuals show some of the lipoprotein species in the serum. The individual variations encountered are derived from two variables, (a) the number of different lipoprotein species present, (b) the relative abundance of the different lipoproteins in a single serum. A variety of evidence5 suggests that the typical “normal” of humans shows one or more lipoprotein species in the range up to 6 Sf units (including the Sf 2, Sf 4, Sf 6 lipoproteins). An analogous situation exists in the rabbit, chicken and dog, with minor differences in the Sf rate of the normally occurring lipoproteins. In atherosclerosis and certain other lipid metabolic derangements there exist in addition varying concentrations

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This work was supported in part by the Atomic Energy Commission, by the United States Public Health Service, and by the Lederle Laboratories Division of American Cyanamid Corporation.

666 Circulation, Volume IV, November, 1961
of lipoproteins in the classes $S_t$ 8, $S_t$ 10, $S_t$ 13, $S_t$ 17, $S_t$ 17--20, $S_t$ 20--40, $S_t$ 40--40,000. In the range $S_t$ 40--40,000, the higher members of the group of lipoproteins make their appearance in serum only when appreciable levels of all lower members are present. However, the relative abundances of these various lipoproteins are variable from one disease category to another. In the range $S_t$ 40--40,000 there are also characteristic patterns associated with disease states, but lipoproteins of the $S_t$ 40--40,000 class also represent part of the alimentary "lipemia" of normals. Elevation of the $S_t$ 10--30 range of lipoproteins has been shown to be essentially universally a part of the development of atherosclerosis in the human as well as other species. However, at least certain of the higher members may also be involved in this disease, but as the variability in concentration in a given individual approaches the variability of groups, they have not been used as a correlative guide.

As an extension of our studies of the factors involved in the maintenance of the levels of the various serum lipoproteins, it has now been found that heparin can produce a profound alteration in the lipoprotein pattern in a manner which may prove of value in the further understanding and management of atherosclerosis. Heparin, of course, has long been used clinically in the therapy of thromboembolic disease, but not for any possible influence it might have upon metabolic factors leading to atherosclerosis.

Hahn reported that heparin abolished alimentary lipemia in vivo, but not in vitro. Weld showed that the phenomenon is not restricted to any particular vascular bed by perfusing various body regions with heparinized lipemic plasma. Recently Anderson has shown that the turbidity of lipemic plasma may be cleared in vitro by mixing lipemic plasma drawn before heparinization with that drawn five minutes after heparinization. He hypothesized an "anti-chylomicronic" factor which results from heparin injection and discussed the possibility of a heparin-phospholipid complex being a surface active agent responsible for the clearing of lipemic plasma. Waldron reported that other anticoagulants (sulfonated polysaccharides and pontamine fast pink B.L.) produced effects similar to heparin.

**EXPERIMENTAL**

A single injection of sodium heparin intravenously in the cholesterol-fed rabbit produces dramatic changes in the lipoprotein spectrum, characterized in general by a decrease in concentration of molecules of the high $S_t$ classes, with a concomitant increase in concentration in those of the lower $S_t$ classes.

The association of these two changes suggests a progressive conversion of the higher $S_t$ lipoproteins into those of lower $S_t$ rates. An immediate response is observed within five minutes in the lipoproteins of the highest $S_t$ classes, with successive changes in those of the lower $S_t$ classes, with maximum effect being observed at about three hours. Subsequently the pattern "reverts" to its initial state in approximately 24 hours.

To obtain some evaluation of the chronic effects of heparin administration upon the evolution of the pattern of serum lipoproteins and the associated aortic atheroma, 40 rabbits were fed cholesterol and cottonseed oil. These animals were divided into two groups, matched as closely as possible as to age, sex, weight, and strain. One group received heparin injections of 10 to 25 mg. per rabbit per day; the other group served as a control. Pairs of heparin and control animals were killed and autopsied after three to eight weeks of cholesterol feeding. Of 20 rabbits maintained on heparin, 17 showed no gross atherosclerosis and three did show atherosclerosis. Of the 20 controls, five showed no gross atherosclerosis and 15 did show gross atherosclerosis. The indicated suppression of atherosclerosis by heparin administration is significant (the probability of no significance of this result is less than 0.01). Heparin so administered suppressed the development of the $S_t$ 10--50 class of molecules to 15 to 50 per cent of the levels observed in the control animals fed the same amount of cholesterol and oil. It is highly likely that this explains the observed protection afforded these rabbits by the administration of heparin.

In man, even more striking effects are seen following heparin administration. The progres-
sive changes in the spectrum of lipoproteins following intravenous injection of 100 mg. of sodium heparin are shown in figure 1. This patient, a survivor of a myocardial infarction, showed initially high levels of lipoproteins in the S₁ 10–20 class, as well as in the S₁ 20–100 class. Within 15 minutes there was essentially a "wipe-out" of lipoproteins from S₁ 20–100, associated with an increase in the concentration of the S₁ 10–20 class. Then, during the next six hours there was an over-all shift within the S₁ 10–20 class, so that the S₁ 12–20 lipoproteins were markedly reduced in concentration below their original level, while the S₁ 10–12 and S₁ 6–10 lipoproteins increased in concentration over the initial level. Progressively over a period of 24 hours the entire pattern reverted toward the initial pattern, although there was still a lower S₁ 12–20 level than initially. This same general effect has been observed in each of 30 subjects (normal and myocardial infarction) studied in this manner, the only variations observed being in the degree of response and in the time relationships of the sequence of events which occur. Dosages ranging from 15 to 100 mg. of sodium heparin intravenously have proved definitely effective, although graded dosage in a single patient requires further evaluation.

In vitro attempts to alter the lipoprotein pattern by addition of heparin have failed in all of numerous trials. However, samples of plasma from heparinized patients have been found effective in altering the lipoprotein spectrum of serum drawn from the same individuals before heparin administration or of serum of other individuals. Thus it appears that the injection of heparin results in the in vivo production of an "active principle" capable of producing these conversions of lipoproteins in vitro. The result is similar to the studies of the "anti-chylomicronemic" factor of Anderson. In an effort to elucidate the mechanism of the effect on lipoproteins, we have found that the "active principle" appears to reside in the ultracentrifugal globulin region. The serum albumin fraction and the low density lipoprotein group both appear inactive. Globulin fractions from postheparin plasma when incubated with earlier postheparin plasma appear to cause further changes in the lipoprotein distribution in such plasma in vitro. We have as yet been unable to detect any of the "active principle" in serum from individuals who had not received heparin, although it is conceivable that such a factor may circulate at low concentration.

In an effort to determine the effect of a maintained heparin level on the serum lipoprotein pattern we have made some studies of individuals receiving repository heparin.* Figure 2 shows the effect of heparin repository in the same patient whose studies are shown in figure 1 with intravenous heparin. It is noted that in four hours with repository heparin this patient lost essentially all of her lipoproteins above S₁ 10, representing in essence a reversion to a "normal" lipoprotein pattern. After 24 hours there was a partial return of S₁ 10–20 molecules (50 per cent), but a much lower fractional reappearance in the S₁ 20–100 class of molecules. (Even after two and four days there is a significant depression of the S₁ 10–20 level.)

A series of 20 patients have received intravenous heparin at intervals varying from two to 14 days. There appeared to be no reduction of ability of patients to show the response described in figure 1, even after numerous heparin injections. A small proportion of the patients showed depression in S₁ 10–20 levels which persisted for the full three to 14 day intervals between intravenous injections of heparin, but in general the levels observed three to 14 days after injection showed no average trend toward reduction. In this particular series samples were drawn throughout just prior to each new heparin injection. From what has been observed in the 24 hour period following such injections, we may anticipate that, averaged over the entire period between injections, the S₁ 10–20 level may have been appreciably lower than its preheparin value. However, we do have direct evidence that with a suitable heparin dosage schedule, chronic lowering of the serum S₁ 10–20 levels can be obtained.

One patient studied over a one month period, who had shown a maintained S₁ 10–20 depression for the three day interval between heparin injections, showed a slow progressive

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* Lederle Repository Heparin.
rise approaching his original $S_1$ 10–20 levels over a one month period after cessation of heparin injections. In a patient with xanthoma tuberosum who had an initial $S_1$ 12–20 level of 500 mg. per 100 cc., there was a slow progressive reduction to 150 mg. per 100 cc. over a

![Fig. 1. The Effect of a Single Intravenous Injection of Heparin on Human Low-Density Lipoproteins. The accompanying ultracentrifugal flotation patterns show the progressive changes in the lipoproteins of a 63 year old female patient with coronary artery disease treated with a single 100 mg. dose of heparin given intravenously. In all the figures successive frames are taken at 0, 6, 12, 22, 30, and 38 minutes after full rotor speed of 52,640 rpm has been reached. Frames 2 and 5 in figure 1B, lower pattern, are ruled for the calculation of the $S_r$ rates of any peak appearing in these frames, respectively. These rulings can be used to calculate $S_r$ rates in the corresponding frames of the other figures. All stippled areas represent the measure of the $S_r$ 20–100 class of lipoproteins, all cross-hatched areas represent the measure of the $S_r$ 12–20 class of lipoproteins.

<table>
<thead>
<tr>
<th>Figure</th>
<th>Time after heparin</th>
<th>$S_r$ 12–20 (mg. %)</th>
<th>$S_r$ 20–100 (mg. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A upper pattern</td>
<td>Preheparin</td>
<td>167</td>
<td>530</td>
</tr>
<tr>
<td>1A lower pattern</td>
<td>20 minutes</td>
<td>239</td>
<td>50*</td>
</tr>
<tr>
<td>1B upper pattern</td>
<td>6 hours</td>
<td>128</td>
<td>52</td>
</tr>
<tr>
<td>1B lower pattern</td>
<td>26 hours</td>
<td>143</td>
<td>344</td>
</tr>
</tbody>
</table>

* The sample recorded in 1A lower was ultracentrifugally concentrated only to three-fourths the level in the other samples. Hence the measured area from the diagram was corrected for this in preparing the tabulation of results. It is seen that while the six hour postheparin sample shows an $S_r$ 12–20 level not over 25 per cent lower than the preheparin sample, there is a marked shift in distribution of the lipoproteins of the $S_r$ 12–20 class toward the lower ranges of this class.

An incidental and unexpected clinical observation made by Lyon and Yankley in the course of this investigation was that 55 out of 59 patients with moderate or severe angina pectoris reported marked relief from this symptom with a drastic decrease in nitroglycerin requirement soon after the period of initiating four week period of daily injections of 100 mg. of heparin. On cessation of the heparin there was a rise to 400 mg. per 100 cc. in the course of two weeks. On resumption of daily heparin injections the $S_r$ 12–20 level again fell progressively to 150 mg. per 100 cc.

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Forty-five patients in this group had previously had a documented myocardial infarction. Eight of the remaining 14 patients were hypertensives with cardiographic evidence of left ventricular hypertrophy. Four others showed cardiographic evidence consistent with coronary insufficiency, and the re-
FIGURE 2
remaining two cases showed normal records. These patients have been treated for periods of from one to eight months with one or two injections of 50 to 100 mg. of intravenous or intramuscular sodium heparin per week. The relief from angina usually was noted after the first few injections. In those patients who have been under treatment for over six months (17 cases) there has been no evidence of a loss of efficacy of the heparin. Reports on the effect of heparin in relieving pain of myocardial infarction and as a coronary vasodilator have appeared in the literature. However, the presently reported responses in angina pectoris have been observed with small, intermittent doses of heparin (50 to 100 mg.) given at intervals of several days in ambulatory patients. The size of the dose and the infrequency of injections speak against either a vasodilator or antithrombotic basis for the response. All of seven patients whose severe angina had been relieved by heparin injections complained of return of anginal symptoms when saline placebos were injected instead of heparin.

We are cognizant of the many difficulties in evaluating objectively efficacy of drug relief of angina. However, the striking character of the response in patients with relatively fixed anginal patterns and nitroglycerin requirements, plus the loss of response when saline placebos were used would appear to militate against the response being in any way psychogenically determined. Extended studies of this response are in progress now. We are not able at this time to provide any suggestion of a possible relation of the heparin effect on blood lipoproteins to that in relieving angina pectoris, although there may be such a relation.

**Discussion**

Heparin appears to act profoundly and rapidly in altering the blood lipoprotein spectrum. Shifts among the lipoproteins are observed both in man and the rabbit from molecules of the higher S₇ classes to those of successively lower classes in times of the order of minutes to hours following a single heparin injection. The decrease in concentration of a particular S₇ range

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**Fig. 2. The Effect of Heparin on Human Low-Density Lipoproteins.** The accompanying ultracentrifugal flotation patterns show the progressive changes in the lipoproteins of a 63 year old female patient with coronary artery disease treated with a single 200 mg. dose of repository heparin. In all the figures successive frames are taken at 0, 6, 12, 22, 30, and 38 minutes after full rotor speed of 52,640 rpm has been reached. Frames 2 and 5 have been ruled in figure 2D for calculation of the S₇ rates of any peak appearing in that frame. The ruling of frame 2 in figure 2D may be used to calculate S₇ rates in frame 2 of any of the other figures. Similarly the ruling of frame 5 in figure 2D may be used to calculate S₇ rates in frame 5 of any other figures. All patterns shown represent two different sera run simultaneously in the ultracentrifuge. In each figure only the upper pattern is involved in this study. The lower pattern is from another individual and is to be completely disregarded. All stippled areas represent the measure of the S₇ 20-100 class of lipoproteins, all cross-hatched areas represent the measure of the S₇ 12-20 class of lipoproteins.

<table>
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<th>S₇ 20-100 mg.%</th>
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<tbody>
<tr>
<td>2A</td>
<td>Preheparin</td>
<td>197</td>
<td>357</td>
</tr>
<tr>
<td>2B</td>
<td>50 min.</td>
<td>147</td>
<td>18</td>
</tr>
<tr>
<td>2C</td>
<td>3 hours</td>
<td>102</td>
<td>14</td>
</tr>
<tr>
<td>2D</td>
<td>4 hours</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>2E</td>
<td>6 hours</td>
<td>83</td>
<td>18</td>
</tr>
<tr>
<td>2F</td>
<td>24 hours</td>
<td>81</td>
<td>160</td>
</tr>
<tr>
<td>2G</td>
<td>72 hours</td>
<td>107</td>
<td>164</td>
</tr>
</tbody>
</table>

In addition the figures reveal a great increase in the concentration of lipoproteins of the S₇ 6-10 and S₇ 10-12 classes accompanying the decrease in concentration of lipoproteins in the classes above S₇ 12. The change in concentration of all lipoproteins between S₇ 12 and S₇ 100 in four hours after heparin administration is 533 mg. per cent. Assuming a plasma volume of approximately 2500 cc., this represents a minimum of 13 Gm. of lipoprotein cleared by the action of 200 mg. of heparin in the repository form.
of molecules, accompanied by an increase in the concentration of molecules in the next lower Sₜ classes suggests that the former molecules may be actually transformed into the latter by the influence of heparin. This appears to occur in several successive stages over a period of hours. This represents one of the first clues on the possible interrelationships of the various classes of lipoproteins of serum. Inasmuch as the lipoproteins of the Sₜ 17–100 classes represent in man the major glyceryl ester (fat) bearers of serum, the observed interconversion accentuated by heparin may represent steps in the normal pathway of transport and metabolism of fat. It is appropriate to consider the possibility that heparin itself, or some substance of similar properties, may normally be involved in the physiologic interconversion of lipoproteins. Thus, in individuals who usually show high levels of Sₜ 10–20 and Sₜ 20–100 lipoproteins, there may be a blockage in the utilization pathways of such molecules (possibly due to deficiency of a heparin-like substance), so that a piling up in concentration of such molecules occurs in the blood. In supposedly normal individuals (especially young adults and children of both sexes), all these molecules, if present, are in very low concentration, which would be expected if utilization pathways were greatly facilitated in these individuals.

As reported by Anderson for the in vitro clearing of alimentary lipemia by in vivo heparinized plasma, the present work indicates that heparin in free form does not directly induce any alteration in lipoprotein spectrum. However, plasma from heparinized patients contains a factor that is effective. This factor resides in the ultracentrifugally determined globulin fraction and induces changes in vitro which simulate, at least in part, the changes in lipoproteins which follow in vivo administration of heparin. Further search for such an "active factor" in the sera of "normal" individuals who show low levels of Sₜ 10–100 lipoproteins appears warranted. If present, such a factor might be anticipated at very low concentration, from the negative results of our searches for it to date. Again, such a factor might not normally reside in appreciable concentration in plasma, but might be called forth in response to lipid loading.

Heparin administered to rabbits protects the animal from development of atherosclerosis under circumstances which otherwise induce atherosclerosis. Since the protective effect of heparin is accompanied by a suppression of development of high levels of the Sₜ 10–50 lipoproteins, the observation strengthens the previously reported evidence linking these molecules with atherosclerosis, and further suggests the value of maintaining low levels of these molecules in prevention of experimental atherosclerosis. The effect of lowering similar classes of molecules in the human on progression of the clinical manifestations of atherosclerosis is being evaluated.¹²

The dramatic relief of angina pectoris by intermittent heparin administration parallels the profound effect of this substance upon lipoprotein metabolism. However, at this time we have been unable to demonstrate that these two simultaneous effects are actually related. It may be that the relief heparin provides in angina pectoris is due to its vasodilator or to its antithrombotic activity. The effect on angina pectoris is seen with small doses of heparin and persists much longer than the anticoagulant effect of the doses used. Currently under study are the effects of heparin in several diseases which are marked by extreme elevation of lipoproteins of the Sₜ 10–100 classes (such as nephrotic syndrome, hypothyroidism and xanthoma tuberosum) in 55 of 59 cases.

SUMMARY

1. Heparin administered to rabbits and man causes profound reorientation in the distribution of low density lipoproteins, characterized by a shift of lipoproteins of high Sₜ rates to those of successively lower Sₜ rates.

2. Heparin administered to cholesterol-fed rabbits prevents the development of high levels of Sₜ 10–50 lipoproteins and retards the development of atherosclerosis in such animals.

3. Following heparin administration the plasma contains an "active principle" associated with the ultracentrifugal globulins, which produces similar re-orientation of the lipoprotein spectrum in vitro.
4. Heparin added directly to serum is ineffective in vitro.

5. Intermittent heparin administration to patients with severe angina pectoris results in dramatic and uniform relief from this symptom for periods of several days beyond a single injection in 55 of 59 cases.

REFERENCES


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Circulation. 1951;4:666-673
doi: 10.1161/01.CIR.4.5.666

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