Effect of Small Doses of Heparin in Increasing the Translucence of Plasma during Alimentary Lipemia

Studies in Normal Persons and Patients Having Atherosclerosis

By William J. Block, M.D., Nelson W. Barker, M.D., and Frank D. Mann, M.D.

Heparin administered intravenously increases the translucence of plasma during alimentary lipemia. This apparently is solely a physical change. This article reports observations concerning this phenomenon in normal persons and atherosclerotic patients. For purposes of maximal sensitivity, exceedingly small amounts of heparin are used, and distinct differences between the normal and the atherosclerotic individuals are noted, with the atherosclerotic failing to clear well on the same empirical dose of heparin as the normal persons. An unexpected observation was that the normal men developed a significantly greater degree of lipemia than did normal women after ingestion of the same fat meal.

In 1943 Hahn1 made the observation that intravenous injection of heparin increases translucence of plasma during alimentary lipemia. This work has been confirmed and extended,2-5 but has received relatively little attention. This reaction occurs only in vivo, but once initiated in vivo it will continue in vitro, the effect lasting 18 to 24 hours.2,4 Apparently this is solely a physical change of blood lipids, quantitative determinations of blood lipids remaining unchanged. The work of Anderson and Fawcett4 suggests the possibility that the mode of action may be through the formation of a highly surface-active heparin-phospholipid complex.

Our interest in this phenomenon was aroused as the result of recent reports suggesting that the physicochemical state of blood lipids may be related to the development of atherosclerosis. These include the chylomicron hypothesis of Moreton6; the suggestive significance of the cholesterol:phospholipid ratio as indicated by the work of Ladd and co-workers7 and of Ahrens,8 and the possible significance of the giant particle observed by Gofman and co-workers9 in work with the ultracentrifuge. The recent work of Bragdon10 in experimental transfer of atherosclerosis by transfusion further suggests the importance of the physical state of plasma lipids. The present study was conducted to learn whether or not this peculiar effect of heparin occurred in equal degree in normal persons and patients who had atherosclerosis.

Method

In brief, the plan of study was to produce alimentary lipemia in our subjects and then observe the effect of intravenous injection of heparin on the translucence of the plasma.

Fat Meal. The subjects were given 240 cc. of 40 per cent cream (40 per cent butterfat by weight) to which 8 Gm. of cocoa and 16 Gm. of sugar had been added, making a total of 97.5 Gm. of fat. This proved to be a fairly palatable mixture. The subjects were required to fast after a normal supper the evening before. The fat meal was given in the early morning between 8 and 9 a.m. Smoking and drinking water were permitted on the morning of the test. The same quantitative fat meal was used for all subjects. The effect of fat on gastric motility and secretion11 and the variation in normal persons was appreciated.6,12 Our purpose was to observe the effect of heparin on the alimentary lipemia which developed three hours after the fat meal, whatever the degree.
Heparin. A solution of the sodium salt of heparin was diluted so that 1 cc. contained 100 Toronto units (approximately 1 mg.). This dilution was made by adding 10 cc. (10,000 Toronto units or approximately 100 mg.) of the original solution to 90 cc. of 0.85 per cent sterile sodium chloride solution with 10 capillary drops of liquefied phenol added as a preservative.

A dose of 3 mg. (3 cc.) injected intravenously just failed to restore completely the translucence of the plasma during alimentary lipemia in a pilot study of normal men. Two additional milligrams of heparin then did produce maximal translucence in the plasma of these persons. Accordingly this minimal dosage of 3 mg. was chosen on the presumption that it would probably be a more sensitive test to elicit possible differences between the various subject types than would a larger dose. This dose was given empirically to all subjects of this study three hours after the ingestion of the fat meal.

Blood Specimens. Three blood specimens were drawn as follows: just prior to the ingestion of the fat meal; three hours after the fat meal, just prior to administration of heparin; and three and one-fourth hours after the fat meal (15 minutes after the injection of heparin). For the purpose of spectrophotometric determinations, 4.5 cc. of each specimen of blood were mixed with 0.5 cc. tenth molar solution of sodium oxalate, and all these specimens were centrifuged simultaneously for 15 minutes at 2,500 rpm in a size 2 International Centrifuge, within one hour after the last blood specimen was drawn.

Quantitative plasma lipid determinations, including cholesterol, cholesterol esters, phospholipid, fatty acids and total lipids, were made on all fasting specimens.* Ten cubic centimeters of oxalated blood were used for this purpose. In agreement with previous investigations, the heparin was found to produce no significant change in the chemical values for blood lipids; therefore the chemical determinations were done only on the fasting specimens in most subjects.

Plasma cholesterol was determined by the method of Bloor13 adapted for use with the Coleman Spectrophotometer.

Plasma cholesterol esters were determined by the method of Bloor and Knudson14 adapted for use with the Coleman Spectrophotometer.

Plasma phospholipid was determined by the method of Youngburg and Youngburg15 as modified by Maclay.16

Plasma total lipids were determined by the method of Bloor.17

Plasma fatty acids were determined by subtracting the value of total cholesterol from that of the total lipids.

Measurement of Translucence of Plasma. This measurement was made one hour after the last specimen had been drawn. It was essential to observe accurately this one hour interval because, once initiated in vivo, this peculiar action of heparin continues in vitro.4 The plasma specimens were read in the Coleman Junior Spectrophotometer at 650 mμ against a water reference using a cuvette 10 mm. in diameter. Results were read on the optical density scale of the instrument. For the purposes of convenience and clarity the term “clearing” henceforth will be used to designate increased translucence of the plasma following injection of heparin.

Calculation of Percentage Clearing of the Lipemia. The value for the fasting specimen was subtracted from the values for the other two specimens, thus correcting the readings to indicate the decrease in translucence due to alimentary lipemia. The postheparin value was then subtracted from the preheparin value to obtain the difference due to clearing.

The ratio of this difference to the preheparin value X 100, thus the percentage clearing, was used as an expression of clearing of the lipemia. In a few instances the postheparin plasma became slightly clearer than the fasting specimen, or on the other hand slightly less translucent than the preheparin specimen. For simplicity, the former instances were recorded as 100 per cent clearing and the latter 0 per cent.

Subjects. The following groups were studied in this report: (1) 20 normal women of ages ranging from 19 to 42 years with no evidence of vascular disease; (2) 23 normal men of ages ranging from 27 to 46 years with no evidence of vascular disease; (3) 27 atherosclerotic male patients ranging in age from 33 to 72 years, all of whom had unequivocal evidence of atherosclerosis, which consisted of either clinical and electrocardiographic evidence of recent myocardial infarction or clearly demonstrable arteriosclerotic obliterans of the lower extremities; (4) five atherosclerotic female patients ranging in age from 50 to 66 years. Criteria for diagnosis were the same as under (3).

Results

The degree of translucence of plasma following a fatty meal is shown in table 1. The normal women in this study showed excellent clearing, averaging 84 per cent (table 2). The range was from 62 per cent to 100 per cent. Quantitative plasma lipids were all essentially within normal limits (tables 3 to 8). Cholesterol:phospholipid ratios were all less than unity, varying from 0.569 to 0.890, and averaging 0.715.
Normal men showed a degree of decreased translucence three hours after the fat meal that was significantly greater (mean difference 0.143 ± 0.034*) than that of normal women of the same age group (table 1). This was entirely unexpected, for, on the contrary, it would seem that in the women, who were almost all smaller than the normal men, a lesser degree of translucence would develop than in the men. The plasma of the majority of normal men cleared well, averaging 74 per cent as a group (table 2). Some cleared completely, but two cleared less than 40 per cent. One of these two men showed a reversal of the cholesterol:phospholipid ratio (1.04). Otherwise quantitative plasma lipids were essentially normal.

The group of atherosclerotic male patients showed no unusual decrease of translucence after the fat meal. The average fell between that for normal men and that for normal

* The value after the ± is the standard error of the difference. A difference which is two or more times its standard error is generally considered statistically significant.
Women. There was, however, a significant difference in the degree of clearing following injection of heparin. Sixteen of the 27 patients cleared less than 40 per cent, and the average clearance for the group was only 38 per cent, despite the fact that two patients cleared completely (100 per cent). Although there was no absolute correlation, and despite two exceptions, on the whole the patients that cleared best were in the older age group, more than age 55 years, younger patients showing poor clearance.

Although many in this group had normal plasma lipids, seven patients had elevated levels of plasma cholesterol and 10 patients had elevated total plasma lipids. The averages of all lipid determinations were greater than those of normal men and women. Six patients had reversal of the cholesterol:phospholipid ratio. The average approached unity, and was greater than that for the normal persons of this study. It can be readily seen that reversal of the ratio was infrequent, although a definite trend toward unity was seen. The fact that many atherosclerotic patients had normal plasma lipids shows that this finding is not at all inconsistent with the diagnosis of atherosclerosis.

There seemed to be no significant correlation between the percentage clearing due to heparin and the individual or total lipid content or the cholesterol:phospholipid ratio of the fasting samples. The results of scatter diagrams did not warrant further statistical survey of this matter.

The group of female atherosclerotic patients is too small to enable one to draw any conclusions. The degree of decreased translucence due to alimentary lipemia was not striking. One cleared less than 40 per cent after injection of heparin. Quantitative plasma lipids were normal in this patient, although elevated in another. Cholesterol:phospholipid ratios were less than unity. This group, because of its small size, will not be tabulated.

Comment

The mechanism of this peculiar action of heparin in changing the physical state of blood lipids during alimentary lipemia is not known. As stated before, the possible formation of a heparin-phospholipid complex has been suggested. This would mean a highly surface-active, hydrophilic substance similar to phospholipid alone.

It is interesting that in this study a significant difference in the response to intravenous injection of heparin during alimentary lipemia exists between normal male and female subjects and male atherosclerotic patients. This difference in clearing cannot be attributed to a difference in age, for nine of the 11 patients in this group who showed more than 40 per cent clearing (including one who showed 100 per cent) were more than 55 years of age. Actually there appeared to be, if anything, an inverse relationship between age and the abnormal resistance to clearing, but the size of the group was insufficient to test this statistically. Concerning other factors, two patients in the group had cardiac failure and both cleared well (100 per cent and 70 per cent). Several patients who cleared well and several who did not were receiving dicumarol.

The data appear to show a true relationship between atherosclerosis and resistance to clearing of lipemic plasma by heparin. This resistance in these patients could be an expression of an abnormal inhibition or neutralization of heparin. This may be consistent with variation in heparin tolerance and requirement in different individuals as regards anticoagulation; in this respect atherosclerotic patients have been found to require more heparin. The resistance may be due to a basic abnormality in the state of plasma lipids. Possibly both factors play a role.

The fact that normal men showed a significantly greater decrease of plasma translucence during alimentary lipemia than did normal women of the same age group following the same fat meal may be significant.

Quantitative determinations of plasma lipid showed variations. All normal subjects were essentially within normal ranges, although one normal man had a reversed cholesterol:phospholipid ratio. The majority of the atherosclerotic patients had essentially normal lipids, but some were elevated. Average values of those even with normal lipids were greater than in normal subjects. The cholesterol:phospholipid
ratio averaged lowest in normal women, next lowest in normal men, highest in the atherosclerotic patients, although still less than unity.

**SUMMARY**

The effect of intravenous injection of heparin on the transluence of plasma during alimentary lipemia in normal subjects, both male and female, and in male atherosclerotic patients has been observed. In this study, in normal men, a decrease in transluence of the plasma developed following a standard fat meal which was significantly greater than that which developed in normal women of the same age group. In the majority of instances male atherosclerotic patients showed much less clearing of alimentary lipemia following a small dose of heparin than did the normal male and female subjects.

Fasting quantitative lipid determinations were essentially normal in all normal subjects, although one showed a reversed cholesterol:phospholipid ratio. The majority of atherosclerotic men had normal lipids, although some were elevated. Several had reversed cholesterol:phospholipid ratios, and the average approached unity as compared with the normal.

**REFERENCES**

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