Effect of Protamine on Alimentary Lipemia


With the technical assistance of J. A. Peters, D.M.T.

The effect of protamine on the serum lipids during alimentary lipemia was investigated in normal male students and this effect was compared with that in atherosclerotic and in nonatherosclerotic men of the same age group. In the student group protamine produced a highly significant increase in the total fatty acid level, whereas in the 2 older groups no significant change occurred. These results were interpreted as demonstrating that circulating heparin exerts a physiologic effect on fat turnover in the young individual, an effect that is reduced with aging. It is suggested that the lowered fat tolerance known to occur with aging is the result of deficiency of circulating heparin.

Since Hahn reported in 1943, that heparin reduced alimentary lipemia, a considerable volume of work has been done in an attempt to define the mechanism of this effect. In addition, the physiologic role of circulating heparin in lipid transport has been investigated. One line of approach has been the investigation of the effect of antiheparin substances such as protamine on the serum lipids. These substances have recently been shown to cause a rise in plasma lipid and lipoprotein levels in experimental animals under a variety of circumstances. In this present work the effect of protamine on serum lipids following fat ingestion in human subjects has been investigated. Further, since there is evidence that atherosclerosis may be associated with a disturbance in normally occurring blood heparinoids or in the response of lipids to them, the effect of protamine on the blood lipids of atherosclerotic patients was determined and the response compared with that of control subjects. While this work was in progress, Moeller and co-workers reported that protamine caused an elevation in total fatty acids and in visible lipemia following the ingestion of fat in normal subjects. Our results confirm these findings and in addition demonstrate the differing response to protamine of a group of young individuals to that of atherosclerotic and nonatherosclerotic individuals of the same age group.

Methods

Three groups with 9 individuals in each were investigated. The first group consisted of normal male medical students with ages varying from 19 to 35 years (average 25 years). The second group were atherosclerotic patients with a hospital record of myocardial infarction 3 to 12 months prior to the experiment; their ages varied from 40 to 68 years (average 58 years). The third group were non-atherosclerotic men of approximately the same age as the atherosclerotic subjects (43 to 64 years, average 51 years). They were hospital patients with no evidence of atherosclerosis as judged by a clinical assessment of the cardiovascular system, a normal blood pressure, and a normal electrocardiogram. These patients were convalescing from conditions without any recognizable bearing on fat absorption or its metabolism.

The procedure in each individual was as follows: the subject was fasted overnight and then given a standard fat meal consisting of 100 ml. of cream with cocoa and sugar to taste. Blood samples were taken in the fasting state and then at 135 and 150 minutes after the fat meal. Immediately following the 150-minute sample, an intravenous injection of 100 mg. of protamine sulfate was administered. Two further blood samples were taken at 15-minute intervals after the injection, i.e., at 165 and 180 minutes. The 5 samples were designated O, A, B, C, and D. Serum total esterified fatty acids, serum cholesterol, serum phospholipid, and the visible lipemia were determined in each sample. To provide control measurements for the assessment of protamine effect the procedure was repeated using 10 ml. of saline instead of protamine.

The student group was also used a third time, but 5,000 units of heparin were given intravenously instead of protamine.

Chemical Determinations. Serum total esterified fatty acids were determined by the method of Stern and Shapiro, serum cholesterol by the method of Zlatkis, Zak, and Boyle, and serum

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phospholipids by the method of Brown.12 These methods were modified in this laboratory as described in a previous communication.5
Visible lipemia was determined by directly reading optical density at a wave length of 650 mm. in a Unicam S.P. 600 spectrophotometer with a 1-cm. cuvette. The serum was diluted prior to reading with 2½ times its volume of a 20 per cent urea solution.

RESULTS
The response to protamine in the serum lipid fractions was measured for each individual by comparing the rise in serum lipids after the injection of protamine, with the rise after the injection of saline.

Protamine effect

\[
\frac{\text{Protamine}}{2} = \frac{(C+D)-(A+B)}{2} - \frac{(C+D)-(A+B)}{2}
\]

where A and B are the 2 samples taken before the injection, and C and D are the 2 samples following it.

Table 1 gives the mean response to protamine in the total fatty acids, cholesterol, phospholipids, and visible lipemia for each of the groups, together with the estimated standard errors of the means. Analyses of variance (not presented) showed that the groups differed significantly only in their response in the total fatty acid levels to the protamine injection. There was a significant over-all response to protamine in cholesterol and visible lipemia, but this was of the same magnitude for all 3 groups. The phospholipid levels showed no significant changes in any of the groups studied.

With regard to the fatty acids, the student group showed a large response to the protamine injection, 73 ± 19 mg. per cent, which was as great as the rise already induced by the ingestion of the fat meal 2½ hours earlier. Figure 1 illustrates the rise following the protamine injection and compares it with the normal fat curve, which showed no response to the saline. The striking response to protamine in the student group contrasted with that in the atherosclerotic group and in the group of nonatherosclerotic controls of the same age range, where the mean responses were much smaller and not significant. Figures 2 and 3 illustrate the similarity of the curves in these 2 older groups, following either protamine or saline injections. The shape of the fat tolerance curves were essentially similar, but differed from that of the younger student group. A comparison of the "saline curve" of figures 1, 2, and 3 shows that after 3 hours the total fatty acid level of the student group was falling, whereas in the older groups the levels continued to rise over the whole period of the experiment.

The effect of heparin on the level of the total fatty acids of the student group is shown also in figure 1. Compared with the saline injection, heparin caused a reduction of 134 ± 23 mg. per cent, a change that is highly significant (p < 0.001). No significant response to heparin was found in the serum cholesterol and phos-

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<tr>
<th>Table 1.—Effect of Protamine on Serum Lipids</th>
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<thead>
<tr>
<th>Fatty acids</th>
<th>Students</th>
<th>Atherosclerotic</th>
<th>Elderly control</th>
<th>Groups combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean diff.</td>
<td>72.61 (9)†</td>
<td>14.33 (9)</td>
<td>9.83 (9)</td>
<td>32.26 (27)</td>
</tr>
<tr>
<td>S.E. of mean</td>
<td>19.38 (8)†</td>
<td>17.53 (8)</td>
<td>15.23 (8)</td>
<td>10.08 (24)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Mean diff.</td>
<td>15.33 (9)</td>
<td>8.33 (9)</td>
<td>7.00 (9)</td>
</tr>
<tr>
<td>S.E. of mean</td>
<td>6.94 (8)†</td>
<td>2.80 (8)</td>
<td>6.24 (8)</td>
<td>3.25 (24)</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>Mean diff.</td>
<td>6.89 (9)</td>
<td>-3.44 (9)</td>
<td>3.78 (9)</td>
</tr>
<tr>
<td>S.E. of mean</td>
<td>7.11 (8)</td>
<td>4.90 (8)</td>
<td>7.62 (8)</td>
<td>3.80 (24)</td>
</tr>
<tr>
<td>Visible lipemia</td>
<td>Mean diff.</td>
<td>0.0181 (9)</td>
<td>0.0345 (8)</td>
<td>0.0323 (9)</td>
</tr>
<tr>
<td>S.E. of mean</td>
<td>0.0112 (8)</td>
<td>0.0098 (7)</td>
<td>0.0077 (8)</td>
<td>0.0056 (23)</td>
</tr>
</tbody>
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* N.
† Degrees of freedom.
‡ Significant at 1 per cent level (by t test).
§ Significant at 0.1 per cent level (by t test).
EFFECT OF PROTAMINE ON ALIMENTARY LIPEMIA

pholipid levels, the rise being $4.3 \pm 3.0$ mg per cent and $4.8 \pm 3.8$ mg per cent respectively.

**Discussion**

Protamine has been shown to produce a highly significant increase in the total fatty acids of students during alimentary lipemia, but to have no effect on patients in the older age groups irrespective of the presence or absence of atherosclerosis. The striking effect of protamine in the student group can most readily be explained on the basis of its opposing the physiologic action of heparin in fat transport; most workers who have shown lipid changes with protamine accept this viewpoint of its action. The observation also recorded in this work that heparin itself reduces total fatty acids during alimentary lipemia serves to highlight this interpretation. Spitzer, however, in view of his failure to obtain results with other antiheparin drugs and because of the relatively long latent period before the effect of protamine is manifested, suggested that the effect of protamine on lipemia is a "sui generis action," and not due to its antiheparin effect. Against this view are the findings of other workers who obtained lipid changes with other antiheparin drugs. Further, it is to be expected that protamine will have a delayed effect on lipids if it is antagonizing heparin. If one envisages heparin dealing with lipid entering the circulation from the alimentary tract, the sudden elimination of this heparin will not have any immediate effect on the blood lipids. Subsequently, however, with progressive absorption of more fat, it will not be dealt with adequately and the level will rise.

If one accepts the view that the action of protamine is due to its opposing circulating heparin, it follows that the failure to produce significant changes in fatty acids in the 2 groups of older individuals can be attributed to a deficiency of heparin in these persons. We conclude that this decrease in circulating heparin is a feature of advancing years. The observations of Hellström and Holmgren of a marked decrease with advancing age of heparin-producing mast cells would fit well with this concept. Further, the low fat tolerance known

**Fig. 1.** Top. The effect of heparin, protamine, and saline on the total fatty acids of normal students following the ingestion of fat.

**Fig. 2.** Middle. The effect of saline and of protamine on the total fatty acids of atherosclerotic subjects following the ingestion of fat.

**Fig. 3.** Bottom. The effect of saline and of protamine on the total fatty acids of nonatherosclerotic subjects following the ingestion of fat.
to be present in older subjects may be similarly explained. This tendency to low fat tolerance is seen in this present work when the "saline curves" for fatty acids of the 3 groups are compared. It is reasonable to conclude that this lowered fat tolerance is due to a reduced level of heparin in these subjects, with the result that fat entering from the alimentary tract is not dealt with adequately. It is possible in this regard to draw a rough analogy between the blood glucose level and insulin on the one hand, and circulating fat and heparin on the other.

The object of this work has been to determine the significance of a possible "heparin deficiency" in the pathogenesis of atheroma. On the evidence presented, however, the conclusion is that there is a deficiency of heparin with advancing age. This deficiency may represent a phenomenon that characterizes aging in the human individual, and is not necessarily related to the incidence of atherosclerosis. The difficulty of excluding atherosclerosis by clinical assessment is fully appreciated, and it is possible that unsuspected atheroma was present in the individuals thought to be free of this condition, and who were of a similar age to the cases of proved atherosclerosis.

It is worth noting in this regard that many workers who have investigated lowered fat tolerance in atheroma, and the effect of heparin on serum lipids in atherosclerotic individuals have used younger individuals as controls. It is therefore impossible to know whether their results were determined by age or by atheroma.

Summary

The intravenous administration of 5,000 units of heparin produced a highly significant reduction in the serum total fatty acids in a group of normal students following the ingestion of a fat meal. No changes in the serum cholesterol or phospholipids were recorded.

The administration of 100 mg. of protamine sulfate under similar circumstances, to the same group of subjects, caused a highly significant elevation in the total fatty acids. Protamine had no significant effect on the total fatty acids of a group of atherosclerotic subjects or on a similar age group of nonatherosclerotic subjects. Protamine significantly elevated the serum cholesterol and the visible lipemia of the 3 groups studied, but no difference could be detected among the 3 groups in these measurements. No effect of protamine on the phospholipids was found in any of the groups investigated.

Summario in Interlingua

Le administration intravenous de 5,000 unitates de heparina produceva un altemente significativa reduction del total acidos grasse del sero in un grupo de studentes normal post le ingestion de un repasto rie in grassia. Esseva notate nulle alterationes in le sero quanto al contento de cholesterol o de phospholipidos.

Le administration de 100 mg de sulfato de protamina al mesme grupo de subjectos e sub le mesme conditiones causava un alte-mente significativa elevation del total acidos grasse. Protamina habeva nulle effecto significativa super le total acidos grasse de un grupo de subjectos atherosclerotic e de un grupo de subjectos non-atherosclerotic de simile etates. Protamina causava in omne le tres gruppos studiate un elevation significativa del cholesterol seral e del lipemia visible, sed nulle differentias esseva notate inter le mesurationes pro le gruppos individual. Nulle effecto de protamina super le phospholipidos esseva trovate in ulle del gruppos studiate.

Acknowledgment

We are indebted to Dr. W. D. Brown of the Department of Biochemistry for advice and encouragement and to Mr. G. N. Wilkinson of the Division of Mathematical Statistics, Commonwealth Scientific and Industrial Research Organisation, for the statistical evaluation of the results. We are also grateful to the students and patients who cooperated in this investigation.

References


Of 426 patients with congenital heart disease seen during a 5-year period, 27 presented with a history of maternal rubella, 13 had patent ductus arteriosus, 4 ventricular septal defect, 3 atrial septal defect, 2 Fallot’s tetralogy, and 1 each suffered from aortic stenosis, pulmonary stenosis, coarctation of the aorta, the Eisenmenger’s complex, and transposition of the great vessels. One fourth to one third of those with patent ductus arteriosus had an additional cardiac lesion. Deafness, cataract, or both occurred in 9 with patent ductus arteriosus and in 3 with atrial septal defect. The incidence of patent ductus arteriosus in children born of mothers who had suffered from rubella during pregnancy was about 88 times and that of other congenital heart lesions was 11 times the normal incidence. The higher incidence of patent ductus arteriosus may be due to its longer normal duration in the fetus.

Soloff
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