Enhancement of Phosphatide-Induced Hypercholesteremia by Prior Ingestion of Cholesterol and Triglyceride

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ABSTRACT

FRIEDMAN, MEYER AND SANFORD O. BYERS. Enhancement of phosphatide-induced hypercholesteremia by prior ingestion of cholesterol and triglyceride. Am. J. Physiol. 192(3): 546-548. 1958.—Rats fed stock diet enriched with 2% cottonseed oil and 2% cholesterol for 3 days prior to intravenous infusion with phosphatides showed a hypercholesteremic response to the infusion which was significantly greater than that of rats fed a diet enriched in cottonseed oil only. The latter animals showed the same hypercholesteremic response as rats maintained on a fat and sterol-free diet. During infusions, the average plasma phospholipid concentrations of the three groups was the same.

Following the continuous infusion of certain phosphatides into either the normal or the liverless rat (1, 2), hypercholesteremia results if the plasma concentration of phosphatide is continuously elevated. The extent of the hypercholesteremia induced is dependent in part upon the degree of phosphatide elevation maintained. The degree of hypercholesteremia appeared to be influenced by the animal’s diet in that an animal fed large quantities of fat and cholesterol for 72 hours prior to infusion, generally exhibited a greater hypercholesteremic response to the infusion of comparable quantities of phosphatide than did a rat on stock diet.

This heightened response of the rat fed a high fat-cholesterol diet to phosphatide infusion appeared to warrant further study to determine whether both or but one of the two lipids fed were involved in this intensification of the hypercholesteremic effect of infused phosphatide. Also whether this dietary enhancement of the hypercholesteremia was mediated by a primary effect upon the phospholipid or upon the cholesterol metabolism of the rat infused with phosphatide. Our results indicate that the enhancement of phosphatide induced hypercholesterolemia by prior feeding of a lipid rich diet, is due primarily to the latter’s cholesterol content. This dietary enhancement occurs without any change of plasma phospholipid concentrations from those to be expected in the absence of dietary supplement.

METHODS

To determine the possible effect of fat alone, and of fat plus cholesterol, in intensifying the hypercholesteremia induced by continuous phosphatide infusion, three groups of adult, male rats (Long-Evans strain) were employed. Group A (12 rats) was placed upon a sterol- and fat-free diet for 72 hours immediately prior to the rapid intravenous injection of 3 ml of a 3% suspension of crude phosphatides in 5% dextrose-normal saline solution followed by continuous infusion of the same fluid at approximately 1.0 ml/hr. for 12 hours. Group B (6 rats) was placed upon a stock diet enriched with cotton seed oil (2%) for 72 hours and then infused as described above. Group C (11 rats) was placed upon a stock diet enriched with both cotton seed oil (2%) and cholesterol (2%) for 72 hours and then infused as described.

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2 Sucrose—7100, Na caseinate—2500, NaCl—150, methyl linoleate—50, salt vit. mixt. 200.

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above. Plasma samples were obtained from all rats before and at the end of the infusion and analyzed for total cholesterol (3) and phospholipid (4).

A second series of experiments also were done to study the effect of a fat-cholesterol enriched diet upon the rate of disappearance of administered phosphatide. The first group of eight rats was fed a stock diet enriched with cotton seed oil (2%) and cholesterol (2%) for 72 hours prior to a single intravenous injection of 3 ml of a 5% suspension of the above described phosphatides. The second group of eight rats was given a sterol- and fat-free diet for 72 hours and then injected with a comparable amount of the phosphatides. Plasma samples obtained before and immediately, 30 and 60 minutes after the single injection were analyzed for phospholipid.

RESULTS

The ability of the infused phosphatides to induce hypercholesteremia even in the rats prefed the sterol- and fat-free diet is well illustrated in table IA. A similar degree of hypercholesteremia and hyperphospholipidemia was observed (table II?) in the rats prefed the diet enriched with cotton seed oil. The rats prefed the diet enriched with both cholesterol and cotton seed oil exhibited a higher plasma cholesterol before infusion and a distinctly greater degree of hypercholesteremia at the end of the infusion (table IC). Despite this enhancement of the hypercholesteremia by the diet enriched with cholesterol and oil, no significant difference was observed between the plasma concentration of phospholipid attained at the end of the infusion in this group and that of the other two groups. The hypercholesteremic enhancement by diet then did not appear to be due to any effect upon the plasma phospholipid level.

The results of the second series of experiments confirmed this last finding. The eight rats prefed the cholesterol- and oil-enriched diet had an average plasma phospholipid of 96 mg/100 ml (S.E. mean: ±4.4) before, and 731 (S.E. mean: ±65.2), 629 (S.E. mean: ±50.8) and 424 (S.E. mean: ±29.8) mg/100 ml, immediately, 30 and 40 minutes, respectively, after injection. The eight rats prefed the sterol- and fat-free diet had an average plasma phospholipid of 102 mg/100 ml (S.E. mean: ±65) before, and 871 (S.E. mean: ±60.7), 776 (S.E. mean: ±68.5) mg/100 ml, at comparable periods, respectively, after injection. Although the deviations of the average values obtained after injection are large, prefeeding with cholesterol and oil certainly did not retard but hastened the disappearance of injected phosphatide from plasma.

DISCUSSION

Previous studies (1, 5) from this laboratory indicated that a primary elevation induced in either the plasma phospholipid or triglyceride content of the intact animal quickly leads to a hypercholesteremic state. Furthermore, this hypercholesteremic potential of either lipid appears to be present and indeed responsible for the hypercholesteremia observed in other types of hypercholesteremic derangements. Thus in biliary obstruction (6), the hypercholesteremia results from an initial rise of plasma phospholipid; in experimental nephrosis (7) and after Triton injection (8), the hypercholesteremia stems from the initial rise of plasma triglyceride.

The source of the excess cholesterol appearing in plasma after its mobilization or retention therein by a preceding plasma accumulation of phospholipid or triglyceride has not been determined precisely. However, it is certain that lipid-induced hypercholesteremia does not depend solely upon hepatic metabolism of cholesterol for the following reasons: first, during

<p>| Table 1: Effect of Prior Feeding on Phosphatide-Induced Hypercholesteremia |</p>
<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>Av. Wt., gm</th>
<th>Av. Amount Phosphatide Infused, mg</th>
<th>Average Plasma Total Cholesterol, mg/100 ml</th>
<th>Average Plasma Phospholipid, mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before infusion</td>
<td>12 Hours after infusion</td>
</tr>
<tr>
<td>A. Rats prefed with sterol- and fat-free diet</td>
<td></td>
<td></td>
<td>307</td>
<td>418</td>
</tr>
<tr>
<td>B. Rats prefed with cotton seed oil</td>
<td></td>
<td></td>
<td>315</td>
<td>444</td>
</tr>
<tr>
<td>C. Rats prefed with cotton seed oil and cholesterol</td>
<td></td>
<td></td>
<td>300</td>
<td>435</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
infusion of phosphatide, the liver of the rat exhibits no apparent change either in its rate of cholesterol synthesis or degradation as determined by bile assay methods (2). Secondly, in the fasted animal given phosphatide, concomitant with the rise of plasma cholesterol, a similar increase in hepatic cholesterol occurs exactly similar to the situation occurring when excess lipoprotein cholesterol is injected (2). Thirdly, the liverless animal also exhibits a hypercholesteremic response to phosphatide (2) and triglyceride (8). These observations of course show that the excess cholesterol is capable of arising also from extrahepatic sources.

The present observations not only tend to confirm this last finding but also suggest that this extrahepatic cholesterol can come in part from exogenous sources. Certainly the intensification of the phosphatide-induced hypercholesteremia observed after prior feeding of excess cholesterol could not be due to any increase in the rate of endogenous synthesis of cholesterol for this latter process has been reported (9) to be depressed by dietary intake of excess cholesterol. It is of interest, too, that the hypercholesteremia observed in nephrosis (10), after biliary obstruction (11) and after Triton injection (9) also can be intensified by prior feeding of excess cholesterol. In other words, hypercholesteremia in these various states appears to be dependent not upon any intrinsic or endogenous derangement of cholesterol metabolism per se but primarily upon the level of plasma phosphatide and secondarily, and to a far lesser extent, upon the availability of body cholesterol whatever its initial source.

Such a concept of course readily explains the failure heretofore to observe in any human hypercholesteremic state an actual increase in the endogenous rate of cholesterol synthesis.

It is undoubtedly an over simplification of the matters at hand to consider the plasma phospholipid and triglyceride levels as sole determinants of the plasma cholesterol content but apparently they play a more direct role in such regulation than the plasma proteins. Even the profound loss of albumin, as observed in nephrosis, induces hypercholesteremia only by initiating first an accumulation of plasma triglyceride and in all likelihood it is this prior occurring lipid excess which then induces the hypercholesteremic state.

REFERENCES