Effect of Chelation on Phosphorus Metabolism in Experimental Atherosclerosis

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Interest in this laboratory has been directed toward the pathogenesis of plaque formation and general tissue change in the aortae of rabbits with induced atherosclerosis. Specifically, components of the tissue involving sulfate and phosphorus in relation to localization and chemical composition have been dealt with in some detail. In this communication, the action of the chelating agent ethylenediaminetetra-acetic acid (EDTA) on plaque formation is described with respect to phosphorus. While this report concerns animal experimentation and induced atherosclerosis, there have appeared in the literature accounts of beneficial effects of EDTA on the course of sclerosing diseases in humans. Several reports have cited beneficial effects of this compound on various manifestations of atherosclerotic origin. Hence it appeared worth while to test the effect of EDTA in animal experimentation on the phosphorus moiety of sclerosed arterial tissue.

In the course of earlier animal investigations, it was learned that subcutaneous administration of MgNa₂EDTA markedly lowers hepatic cholesterol and phospholipid concentrations as well as aortic cholesterol levels in rabbits with induced atherosclerosis. Spectrographic analysis demonstrated that a series of cations were grossly reduced in the livers of atherosclerotic rabbits following MgNa₂EDTA. While no effect on sodium content was observed, sharp decreases in potassium and iron concentrations were encountered as well as significant decreases in magnesium and copper levels. The same technique revealed a marked decrease in total hepatic phosphorus. Calcium, magnesium, iron, and copper were also investigated spectrographically in atherosclerotic rabbit aortae. While no change occurred in the iron content, a sharp reduction of the other three elements was noted. As indicated above, this communication demonstrates the effect of subcutaneous MgNa₂EDTA on the phosphorus content and distribution in the aortic wall of the rabbit with induced atherosclerosis.

Methods

A. PREPARATION OF MgNa₂EDTA SOLUTION

To a 3-L. beaker, add 200 Gm. of Na₂H₄EDTA·2H₂O and 1,750 ml. of deionized water. Heat to 45 C., stirring constantly. Filter with suction. Reheat the filtrate to 50 C., and with constant stirring add a solution containing 162 Gm. MgCl₂·6H₂O dissolved in 100 ml. of deionized water. The MgH₂EDTA·6H₂O precipitate formed by this reaction is allowed to stand for several hours. It is then filtered by suction and air dried on a porous plate. This procedure will give a 96 per cent yield of MgH₂EDTA·6H₂O (theoretical, 227 Gm.).

Place 167 Gm. of the dried salt in a 1-L. beaker. Add dropwise, and with constant stirring, a solution containing 30 Gm. of NaOH in 60 ml. of deionized water. The agitation must be vigorous, or localized concentration of NaOH will precipitate out Mg(OH)₂, which redissolves with some difficulty. Add 160 ml. of deionized water. If the solution is not clear at this point, add dropwise more NaOH of the same strength. The pH at this point should be about 6.0. Then neutralize the solution to a pH of 7.25 with 1 N NaOH. Dilute the solution to exactly 500 ml. with deionized water, mix well, and filter by suction through a 1-µ glass filter. Bottle and autoclave. This solution will contain 1 Gm. of MgH₂EDTA·6H₂O as the disodium salt in 3 ml. of water.

*Personal communication from R. E. Mosher.
CHELATION ON PHOSPHORUS METABOLISM

TABLE 1

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
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<tbody>
<tr>
<td>Normal chow</td>
<td></td>
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<tr>
<td>Corn oil plus cholesterol</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(15 weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus - +</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Subcutaneous MgNa₂EDTA</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>(weeks 12 through 15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacrificed 15th week</td>
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<td></td>
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</tr>
</tbody>
</table>

B. EXPERIMENTAL

Twenty-two rabbits divided into three groups were used. Group A animals served as the control. The eight animals of this group were fed normal rabbit chow. The seven animals in each of groups B and C were fed the same chow with the addition of 10 ml. of corn oil and 1 Gm. of cholesterol daily. Group C animals received 20 subcutaneous injections of 200 ing. each of MgNa₂EDTA converted to the soluble disodium salt (MgNa₂EDTA) over the last four weeks of the experiment. All three groups were injected with 13 C of P₃² once a week for the 15 weeks of the experiment. The last injection of P₃² was given 72 hours before sacrificing the animals (table 1).

At the conclusion of the experiment, blood was drawn from the heart and the aortae removed from the aortic valve to just below bifurcation of the renal arteries. Each aorta was sectioned longitudinally, washed thoroughly in water, and then dipped momentarily in acetone and air dried. The acetone wash was subsequently added to the arterial phospholipid extract. Each artery was placed intimal side down on no-screen x-ray film for 48 hours to obtain an autoradiogram. Individual aortae were then macerated in alcohol-ether (3:1) with the aid of a Virtis-45 homogenizer. The mixture was brought to the boiling point, filtered by suction through a no. 42 Whatman paper, and the filtrate analyzed for phospholipid phosphorus, the remaining solid for residue phosphorus. In all cases, both chemical analysis and radio counts were made. The phospholipid extract, to which the acetone wash referred to earlier had been added, was evaporated to dryness. Two drops of 10 per cent NaCl solution and 10 ml. of HNO₃-HClO₄ (9:1) acid mixture were added. This digestion was taken to incipient dryness. The salts were then dissolved in 25 ml. of water, and 2.0-ml aliquots were used for colorimetric phosphorus analysis. The phosphorus from a 20-ml aliquot was precipitated as magnesium ammonium phosphate using 8 mg. of NaH₂PO₄ as a carrier. The precipitate was filtered, dried, and the P₃² counted. Analogous procedures were used on the extracted tissue to determine phosphorus both colorimetrically and by radio count. Results of these determinations are tabulated in tables 2 and 3. All calculations for the aortae were made on the weight of the dried tissue obtained from the phospholipid extraction. Serum was analyzed for phospholipids and total phosphorus colorimetrically as well as for the corresponding P₃² counts. Cholesterol determinations were also made on the serum (table 4).

The results and discussion are as follows. Figure 1 is a photographic reproduction of representative aortae and their respective

A glance at these shows that the plaque formation in the MgNa₂EDTA-treated group C animals (nos. 20, 24) is markedly less than that of the nontreated cholesterol-fed group B animals (nos. 11, 12). This is reflected in the autoradiographs as well since the plaque formations are distinguished by the high phosphorus content and hence by a high P⁸² level. A visual assessment of the extent of atherosclerosis in each artery was made, and this appears in column 1 of table 2. Zero represents no visible atherosclerosis while + 4 implies that heavy plaquing was almost continuous throughout both the arch and descending portion of the aorta. Selection of the pictures was made by laying all the photographs of each group side by side in increasing order of visible atherosclerosis. The two pictures representing the median of each of the groups B and C were then chosen for publication. In group A, all reproductions were equivalent, and no such criteria were necessary. Table 2 shows that at the end of 15 weeks, the cholesterol-corn oil regimen had tripled the phospholipid content in the aortae. Administration of MgNa₂EDTA for the last four weeks of this regimen in group C animals shows the phospholipid content...
values to rise only twice above normal. However, it appears to be significant that the radioactivity per milligram of phosphorus (specific activity) is statistically the same for the aortic phospholipids in groups B and C. Also, the specific activity of serum phospholipid is statistically the same for group B as for group C animals (table 4).

In the serum with highly elevated phospholipid levels and the same $^{32}$P administration for all animals, the specific activity of groups B and C phospholipids is significantly below that of group A. However, in the aortae this is not the case. The increased specific activity of group B as compared to group A suggests that as the disease progresses, the rate of phospholipid deposition in the aorta increases. Since the animals are under continuous stress in terms of an ever-increasing lipid load, this appears reasonable.

Group C animals have lower aortic phospholipid content than group B, but as indicated above they have the same specific activity. This specific activity presents difficulties in interpretation. In view of the fact that the last $^{32}$P administration was made 72 hours before sacrifice, one or more of the following situations may have been operative to produce this similarity: (1) a more rapid destruction of phospholipids in group C animals, (2) a slower rate of synthesis in this same group, (3) a more rapid turnover of available phosphate ion. In the latter event, this action may be mediated by the parathyroids through chelation activity of MgNa$_2$EDTA on extracellular calcium. This has been postulated elsewhere.$^3$ The sera of groups B and C show similar findings. From animal experiments in this laboratory and from information through personal communication, it appar-
ent that CaNa₂EDTA, unlike MgNa₂EDTA, has no effect on sclerosing diseases.

Qualitatively, the residue phosphorus in the aorta presents a picture similar to that of the phospholipids. Again, one finds group A animals with the least phosphorus, group B with the most, and group C with an intermediate value. However, there are quantitative differences. While the phospholipid content tripled in group B aortae as compared to those of group A, residue phosphorus only increased 1.6 times. The analogous comparison for groups A and C shows doubled phospholipids while the residue phosphorus increased by about 1.5 times. A comparison of the corresponding specific activities of the control group A and the two experimental groups presents the most marked differences. The residue specific activity is lowest for the normals and highest for group B (cholesterol-oil fed, untreated animals), suggesting a slower phosphate turnover in the latter group. The difference in the residue phosphorus levels of the aortae in groups B and C is too small to be considered statistically significant; however, the difference in the specific activities of these two groups is significant. Unless one accepts an increased phosphorus-turnover in group C animals, this decrease in specific activity from 801 c.p.m. in group B to 670 c.p.m. per mg. of phosphorus is difficult to explain.

It is perhaps interesting to point out once more that under MgNa₂EDTA administration, there is marked liver cholesterol and aortic cholesterol lowering. However, since cholesterol continued to be given throughout the period of MgNa₂EDTA treatment, there is only a 12 per cent lowering of serum cholesterol level. Serum phospholipid phosphorus levels were lower in group C than in group B. They were 26.6 and 33.8 mg. per cent, respectively.

Summary

MgNa₂EDTA is shown to have an ameliorating effect on fat-induced atherosclerosis in rabbits. The compound was shown to decrease not only the general extent of atheromatosis, but to influence phosphate metabolism in induced atherosclerosis. Some credence is lent to a previously outlined hypothesis suggesting that the parathyroids mediate this effect.

References

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