Failure of Parenterally Administered Pyridoxine to Influence Serum Cholesterol Levels and Development of Atherosclerosis in Cholesterol-Fed Rabbits

By Fred W. Martens, M.D., and Donald W. Hoskins, M.D.

Pyridoxine administered parenterally was found to have no effect on serum cholesterol levels of rabbits fed a normal diet, nor did it inhibit the development of hypercholesterolemia and atherosclerosis in rabbits fed a cholesterol-rich diet.

A relationship between pyridoxine deficiency and arteriosclerosis was first suggested by the observation of Rinehart and Greenberg that monkeys fed a diet deficient in pyridoxine developed vascular lesions resembling in many respects those of naturally occurring arteriosclerosis in human beings. Little is known of the precise physiologic role of pyridoxine in human nutrition except that it has been shown to be essential for the conversion of linoleic acid to the more unsaturated arachidonic acid. It is of interest in this connection that the feeding of unsaturated fatty acids to normal and hypercholesterolemic human beings has recently been shown to result in a striking decrease in the cholesterol content of their sera. To learn whether pyridoxine influences serum cholesterol levels or the development of atherosclerosis in rabbits, an experiment was done in which large doses of pyridoxine were administered parenterally to rabbits fed a diet rich in cholesterol.

Materials and Methods

Animals. Thirty-six normal rabbits of mixed breeds were divided into 3 groups of 12 animals each. The animals in the 3 groups were evenly distributed with respect to sex and weight. They were weighed at the beginning of the experiment and at weekly intervals thereafter. They appeared well throughout the experimental period except for animal no. 6 in group 1 which died of a pulmonary infection during the fourth week of the experiment.

Diet. The animals were fed a stock diet of Rockland rabbit pellets which have been found previously in this laboratory to be free of cholesterol. A diet containing 1 per cent cholesterol was prepared by dissolving 10 Gm. of cholesterol in 400 ml. of ether; this solution was added to 1000 Gm. of rabbit pellets and mixed thoroughly in a shallow pan. The pellets were then allowed to dry for 24 hours, leaving a cholesterol coating on each pellet.

The feed cups in the individual rabbit cages held 200 Gm. of pellets, and were filled daily regardless of the amount consumed the previous day. The daily gross intake of the animals varied from 75 Gm. to 125 Gm. The diet was supplemented with lettuce twice a week. Water was available ad libitum.

Pyridoxine. Using ampules of pyridoxine hydrochloride (100 mg./ml.), the dose was arbitrarily set at 25 mg. given intramuscularly on alternate days, an amount considerably in excess of the daily human requirement of 2 to 3 mg. Another solution consisting of a phosphate buffer at pH 3, the pH of the pyridoxine solution, was given intramuscularly every other day to control animals. The control solution was sterilized by passage through a Zeitz filter and was kept refrigerated in 10 ml. sterile test tubes. This solution remained clear and free from visible bacterial growth, and the pH did not vary significantly on storage.

Plan. The 3 groups of animals were subjected to the following regimen for a 9 week period.

Group 1: Twelve animals on the stock diet without added cholesterol were given 0.25 ml. of pyridoxine hydrochloride (100 mg./ml.) intramuscularly on alternate days.

Group 2: Twelve animals on a 1 per cent cholesterol diet were given 0.25 ml. of the control phosphate buffer solution intramuscularly every other day.

Group 3: Twelve animals on the 1 per cent cholesterol diet were given 0.25 ml. of cholesterol to each pellet and mixed thoroughly in a shallow pan. The pellets were then allowed to dry for 24 hours, leaving a cholesterol coating on each pellet.

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Serum Cholesterol Determinations. The animals were bled from the marginal ear vein. Ten milliliters of blood was drawn just before the experimental period began and again after 3, 6 and 9 weeks. The serum cholesterol levels were determined by the method of Abell, Levy, Brodie and Kendall.

Anatomic Studies. At the end of the 9 we experimental period the animals were sacrificed by air embolism and examined immediately. The heart and aorta in each case were removed en bloc. The viscera were examined grossly, and blocks of tissue for microscopic studies were taken from the liver, kidney, spleen, psoas muscle, thyroid, and pancreas. The blocks of tissue were fixed in Zenker-formol solution and stained with hematoxylin and eosin. The hearts and aortas were placed

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TABLE 1.—Effect of Pyridoxine on Body Weight, Serum Cholesterol Levels and Atherosclerosis of the Aorta in Rabbits Fed a Normal and a High Cholesterol Diet.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tbody>
<tr>
<td>Animal 1</td>
<td>Animal 2</td>
<td>Animal 3</td>
</tr>
<tr>
<td>No.</td>
<td>Sex</td>
<td>Weight (Kg.)</td>
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<td>1</td>
<td>M</td>
<td>2.63</td>
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<tr>
<td>2</td>
<td>F</td>
<td>2.75</td>
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<tr>
<td>3</td>
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<td>M</td>
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<tr>
<td>6</td>
<td>M</td>
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<td>7</td>
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<td>8</td>
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<td>10</td>
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<tr>
<td>11</td>
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<tr>
<td>12</td>
<td>M</td>
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<tr>
<td>Mean</td>
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</tbody>
</table>
PYRIDOXINE AND ATHEROSCLEROSIS

in 10 per cent formalin for 2 weeks and then stained with Sudan IV which imparted a bright orange-red color to the atherosclerotic areas. The degree of atherosclerosis of the aorta observed grossly following staining with Sudan IV was estimated on a scale of 0 to 4 +; where 0 represents absence of visible atherosclerosis; 1 + represents minimal but visible plaques in the aorta; 4 + represents extensive coverage of most of the aorta with numerous raised, confluent plaques; and grades 2 + and 3 +, intermediate states.

RESULTS

The serum cholesterol levels and the degree of atherosclerosis of the 36 animals in this study are recorded in table 1.

The animals of group 1, fed a stock diet and given pyridoxine injections, had a mean serum cholesterol level of 51 mg./100 ml. at the start of the experiment and 56 mg. at the end of 9 weeks. There was thus no significant change in the serum cholesterol levels of these animals during the experimental period, and their aortas had no atherosclerosis.

The mean serum cholesterol levels at the beginning of the experiment of the rabbits in group 2 was 51 mg. per cent, and that of group 3 was 46 mg. per cent. The serum cholesterol levels of the animals in both groups rose progressively during the experimental period. At the end of 9 weeks, the mean serum cholesterol level of group 2 had increased to 965 mg. per cent with a range of 486 to 1,400, and that of group 3 was 1,000 mg. per cent with a range of 495 to 1,340. There was no significant difference between the 2 groups with respect to cholesterol levels at any of the three determinations. The degree of atherosclerosis of the aortas of these groups is shown in table 1. The animals of group 3 (cholesterol diet plus pyridoxine) had slightly more atherosclerosis than the control animals of group 2 (cholesterol diet without pyridoxine), but the difference between the two groups in this respect is not great enough to be considered significant.

Microscopic examination of the hearts revealed subintimal deposits of lipid in the coronary arteries of the animals in groups 2 and 3. There was, however, no significant difference between the two groups in this respect. The coronary arteries of the animals in group 1 were completely free of atherosclerosis. The livers of the animals of groups 2 and 3 showed minimal to moderate fatty infiltration. There was no significant difference between groups 2 and 3 with respect to the degree of fat deposition in the liver. The livers of the animals of group 1 were normal.

DISCUSSION

The present experiment was designed to show what effect, if any, parenterally administered pyridoxine would have on the serum cholesterol levels of rabbits fed either a normal or a high cholesterol diet and what influence it would have on the development of atherosclerosis in these animals. Although the amount of pyridoxine in the serum was not measured, it is reasonable to assume that a large part of the dose administered was available to the animals. The findings indicated clearly that pyridoxine, in the dosage employed, produced no change in the cholesterol content of the serum of rabbits on a normal diet and failed to prevent hypercholesterolemia and the development of atherosclerosis in rabbits fed a cholesterol-rich diet. The results of this experiment, however, do not exclude the possibility that pyridoxine plays an important role in the metabolism of cholesterol and of other lipids.

Pyridoxine is known to facilitate the biological conversion of the essential unsaturated fatty acids, linoleic and linolenic acids, to the more highly unsaturated arachidonic and hexanoic acids. It has also been shown that in human beings most of the cholesterol of the serum is esterified with unsaturated fatty acids. In addition, diets containing large amounts of unsaturated fatty acids have been shown to produce striking reductions in the serum cholesterol level. Taken together, these facts suggest a quantitative relationship between the amount of essential fatty acids and pyridoxine in the diet and serum cholesterol such that a relative deficiency of either of the first two results in abnormalities in
the metabolism of the latter. In the present experiment it is possible that although sufficient pyridoxine was given to balance the excess cholesterol, the amount of essential fatty acids in the diet was relatively inadequate and hypercholesterolemia and atherosclerosis resulted. It may well be that in order to reduce the cholesterol content of the serum or influence the development of atherosclerosis in hypercholesterolemic animals, sufficient amounts of the essential fatty acids must be supplied in addition to adequate amounts of the vitamin pyridoxine.

**SUMMARY**

The effect of pyridoxine on cholesterol metabolism and atherosclerosis in the rabbit was studied. Pyridoxine had no effect on the serum cholesterol levels of rabbits fed a normal stock diet and did not effect the degree of hypercholesterolemia in rabbits fed a cholesterol-rich diet. In addition, pyridoxine had no significant effect on the degree of atherosclerosis produced by cholesterol feeding.

**SUMMARIO IN INTERLINGUA**

Esseva studiate le effecto de pyridoxina super le metabolismo de cholesterol e le disveloppamento de atherosclerosis in conilios. Pyridoxina habeva nulle effecto super le uivellos seral de cholesterol in conilios e dieta rutinari e non alterava le grado de hypercholesterolemia in conilios recipiente dietas a augmentate contento de cholesterol. In plus, pyridoxina non exerceva un effecto significative super le grado de atherosclerosis producte per le ingestion de cholesterol.

**REFERENCES**

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Circ Res. 1958;6:159-162
doi: 10.1161/01.RES.6.2.159

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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