Effect of Nicotinic Acid on the Incorporation of Radiocarbon into Cholesterol

By JOSEPH M. MERRILL, M.D.

Nicotinic acid when fed to rats as 0.8 per cent of their diet caused an 83 per cent increase in radioactivity of liver cholesterol-digitonin precipitate. In vitro studies using rat liver slices indicated that nicotinic acid increased the incorporation of radiocarbon into cholesterol by 46 per cent.

Following the lead of Altschul, a number of investigators have established that nicotinic acid depresses the serum cholesterol. A previous report indicated that inclusion of nicotinic acid in experimental diets fed to rabbits partially prevented the anticipated rise in serum cholesterol, prevented cholesterol deposition in the aorta and, to a lesser extent, cholesterol deposits in the liver. It was emphasized that the mechanism whereby nicotinic acid altered serum and tissue cholesterol was unknown. It was pointed out, however, that the large number of methyl groups required for the excretion of nicotinic acid might alter cholesterol synthesis.

The purpose of this study was to determine: (1) the effect of nicotinic acid on the incorporation of sodium acetate 1-carbon into liver cholesterol in the living organism, and (2) the effect of supplementary nicotinic acid on the incorporation of sodium acetate 1-carbon into cholesterol in rat liver slices.

METHODS

Male rats of the Sprague-Dawley strain were fed a stock diet containing 0.8 per cent nicotinic acid; an equal number of control animals were fed a stock diet. At the end of 8 days, the rats were injected intraperitoneally with sodium acetate 1-carbon (6.0 μc./100 Gm. body weight). At intervals of 30 min. to 4 hours following the injection the rats were killed by decapitation. The livers were removed and immediately homogenized in chloroform (35 ml. chloroform/Gm. fresh liver tissue) by a Servall Omnimixer for 3 min. at 14,500 r.p.m. The homogenates were filtered and the residue extracted with chloroform.

The combined filtrates were evaporated by a continuous air stream. Two milliliters of 1:1 acetone-alcohol and 12 ml. of 0.6 per cent digitonin in 85 per cent ethyl-alcohol were added to the residue. The cholesterol and digitonin were allowed to precipitate overnight. The solutions were centrifuged and the supernatants discarded. The precipitates were washed with the following: 1:1 acetone-alcohol (twice), 1:1 acetone-ether (once), ether (twice). After each washing, the solutions were centrifuged and the resulting supernatants discarded. The final precipitates were suspended in ether, filtered on sintered glass funnels, weighed and counted in a windowless flow counter. Final radioactivity was reported as c.p.s./mg. of cholesterol-digitonin precipitate after correction for self-absorption.

The in vitro studies, rat liver slices were prepared in a cold room (4.0 C.) from Sprague-Dawley male rats using a Stadie slicer. Control slices were suspended in Krebs-Ringer phosphate buffer (pH 6.8) containing 12.5 μc. sodium acetate 1-carbon. Nicotinic acid (1 X 10^{-5} mol) was added to the experimental flasks and the pH adjusted to 6.8. Incubation was made in a 100 per cent oxygen atmosphere at 37 C. After 5 mg. of carrier cholesterol were added, the cholesterol-digitonin precipitate was recovered from the slices and incubation medium and its radioactivity determined in the manner reported.

RESULTS

The in vivo studies are summarized in table 1. Three studies were done using 12 rats in each experiment. The nicotinic acid increased the radioactivity of liver cholesterol-digitonin precipitate by 75 to 90 per cent. The average increase for the 3 experiments was 83 per cent. Differences in radioactivity of the precipitate in the 3 experiments are partially due to the length of time between injection and sacrifice of the animals. In
NICOTINIC ACID AND CHOLESTEROL

TABLE 1.—Effects of 0.8 Per Cent Nicotinic Acid in the Diet on the Incorporation of Radiocarbon into Liver Cholesterol (in vivo)—Radioactivity of Liver Cholesterol-Digitonin Precipitate, c.p.s./mg.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control</th>
<th>Nicotinic Acid</th>
<th>Per cent increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>1.01</td>
<td>1.92</td>
<td>+90</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>4.50</td>
<td>7.88</td>
<td>+75</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>4.13</td>
<td>7.64</td>
<td>+85</td>
</tr>
</tbody>
</table>

* Twelve rats in each experiment.

In experiment 1, the average time interval was 240 min.; in experiment 2, 120 min.; in experiment 3, 45 min.

The rats eating the experimental diets gained 1.3 per cent in weight whereas the controls gained 6.7 per cent. At the time of sacrifice the weight of the liver for the control animals constituted 3 per cent of the total body weight, while the livers from the rats fed 0.8 per cent nicotinic acid were 3.02 per cent of the total body weight. It seems unlikely that the large increase in incorporation of sodium acetate 1-carbon 14 in the experimental animals is due to these minor changes in weight.

To confirm this finding of increased incorporation of sodium acetate 1-carbon 14 into cholesterol with nicotinic acid, in vitro studies were done which are summarized in table 2. In these studies the only difference in the contents of the incubation medium was the 1 X 10^-3 mol nicotinic acid which was added to the experimental flasks. In these studies nicotinic acid increased the radioactivity of the cholesterol-digitonin precipitate. The increase in radioactivity ranged from 30 to 55 per cent with an average increase of 46 per cent. The results of both in vivo and in vitro studies indicate that nicotinic acid increases the incorporation of radiocarbon into cholesterol.

DISCUSSION

The results of these experiments answered the purpose of the study. First, nicotinic acid increased the incorporation of radiocarbon into cholesterol by approximately 83 per cent in vivo. Second, nicotinic acid when added to rat liver slices in vitro increased the incorporation of radiocarbon into cholesterol by an average of 46 per cent.

These data suggest that nicotinic acid has a pronounced effect on cholesterol metabolism. This should not be surprising as the work of other investigators has established that nicotinic acid is important in the metabolism of other naturally occurring compounds related to phenanthrene. Nicotinic acid occurs as the free form, amide, and as 3 co-enzymes: diposphopyridine nucleotide, triphosphopyridine nucleotide, and co-enzyme III. These co-enzymes serve as oxidizing agents in the dehydrogenation of a great variety of compounds. DPN is important in the destruction of testosterone since it activates an enzyme which converts testosterone to androstenedione. In vitro, this degradation is accelerated by DPN. Further work has indicated that DPN is a required co-factor for the oxidation of the alcohol group on the C17-carbon atom of testosterone. In a crude system of liver homogenates, DPN and nicotinamide are required for the oxidation of the C20-carbon atom of labeled cholesterol.

The previously reported changes which nicotinic acid produces in serum and tissue cholesterol may be produced by altered metabolism of cholesterol similar to that reported here. However, differences in species used in these various studies do not permit this conclusion.
Summary
Nicotinic acid fed to rats increased the incorporation of sodium acetate-1-carbon\(^{14}\) into cholesterol-digitonin precipitate. Nicotinic acid added to rat liver slices in vitro also increased the incorporation of radiocarbon into cholesterol.

Summario in Interlingua
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References
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