Alteration of Cholesterol Metabolism in the Rat with Linoleic Acid

By Joseph M. Merrill, M.D.

When linoleic acid is added to the diet of the rat there is an average increase in incorporation of radiocarbon into liver cholesterol of 259 per cent. By contrast, the addition of coconut oil to the diet does not increase the incorporation of radiocarbon into liver cholesterol. The addition of linoleic acid to a stock diet is associated with an increased fecal excretion of Lieberman-Burchard chromogens, 3-β-hydroxy sterols and bile acids.

When unsaturated fats are substituted isocalorically for saturated ones in the diet of man, a fall in the serum cholesterol frequently occurs. In most instances of carefully controlled caloric intake, the substitution of saturated fats causes a rise in serum cholesterol. Since the mechanisms of these responses are unknown, a series of experiments have been performed in rats in order to gain additional information regarding the metabolic effects of unsaturated and saturated fats. The immediate objectives were to determine the effects of feeding saturated and unsaturated fatty acids on the incorporation of 1-\(^{14}C\)-acetate into liver cholesterol and the effects of these lipids on the excretion of certain fecal end-products of cholesterol metabolism.

Methods

Male rats of the Sprague-Dawley strain were fed a stock diet to which was added either 10 per cent of commercial grade linoleic acid (iodine value 134.0) or 10 per cent of coconut oil (iodine value 5.0); an equal number of control animals were fed the unmodified stock diet. After 8 to 19 days the rats were injected intraperitoneally with sodium acetate 1-C\(^{14}\) (6.0 μCi/100 Gm. body weight). Thirty minutes following injection the rats were killed by decapitation. The livers were removed and treated as follows for the isolation of cholesterol. They were homogenized in chloroform (35 ml./Gm. fresh liver tissue) with 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 to dryness with 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 digitonide was allowed to precipitate overnight. The solutions were centrifuged and the supernatants discarded. The precipitates were washed with 1:1 acetone-alcohol (twice), 1:1 acetone-ether (once), and 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 counts per second per milligram of cholesterol digitonide precipitate after correction for self-absorption.

For the in vitro studies, slices were prepared in a cold room (4.0 C.) from the livers of Sprague-Dawley, male rats using a Stadie slicer. Control slices were suspended in Krebs-Ringer phosphate buffer containing 12.5 μc. sodium acetate 1-C\(^{14}\). Incubation was made in a 100 per cent oxygen atmosphere at 37 C. The cholesterol was recovered from the slices and incubation medium and its radioactivity determined.

For the excretory studies, 24 hour fecal collections were made in the control and both experimental groups. The feces were frozen, homogenized and 1 Gm. samples obtained. These were extracted with chloroform in the manner described. Aliquots were taken and the Lieberman-Burchard chromogens measured according to the Kingsley and Schaffert\(^1\) technic. The 3-β-OH sterols were recovered with digitonin in the manner described and the dry weight recorded. The bile acids were measured using a modification of the spectrophotometric method of Mosbach et al.\(^2\) and are reported as total bile acids with maximum absorption at 3,200 and 3,850 A.

Results

In the 3 experiments, the addition of linoleic acid to the diet was associated with an average increase of 259 per cent in radioactivity of liver cholesterol (table 1).

To determine the effect of linoleic acid feeding on the rate of incorporation of labeled carbon into liver cholesterol, one group of

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TABLE 1.—Effect of Linoleic Acid On Incorporation of Radiocarbon Into Liver Cholesterol (in Viva). (Radioactivity of Liver Cholesterol Digitonide Precipitate, c.p.s./mg.)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Total Number of Rats</th>
<th>Control</th>
<th>Linoleic Acid</th>
<th>Percent Increase</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>2.80</td>
<td>5.30</td>
<td>89</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>1.00</td>
<td>6.30</td>
<td>232</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>1.74</td>
<td>9.62</td>
<td>458</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Rats was fed a control diet and a second group was fed the same diet plus 10 per cent linoleic acid. The animals were killed at 15, 30 and 45 min. intervals following the intraperitoneal injection of radiocarbon. Liver cholesterol was recovered and its radioactivity determined as described (fig. 1). Note the rapid rise to a peak within 15 min. or less for the rats fed 10 per cent linoleic acid and the fall in the next 30 min. The hepatic cholesterol of the control animals meanwhile did not reach maximum radioactivity until 30 min. following injection. These results suggest that linoleic acid increases the rate of incorporation of radiocarbon into liver cholesterol.

In an additional experiment in which the animals were killed 120 min. following injection, the specific activity of the liver cholesterol digitonide from control animals was 2.87 c.p.s./mg., while that from the animals fed linoleic acid was 3.97 c.p.s./mg. These differences were not statistically significant. Moreover, in another experiment when the animals were killed 240 min. following injection of the radiocarbon, the linoleic acid fed animals had 5.5 c.p.s./mg. of liver cholesterol digitonide while the controls had 3.0 c.p.s./mg. These differences were again not statistically significant.

To investigate the effect of a saturated fat on the incorporation of radiocarbon into liver cholesterol, 10 per cent coconut oil was fed to a group of rats while a second group was fed the stock diet. After 8 days the specific activity of the liver cholesterol digitonide from the rats fed coconut oil was 1.09 c.p.s./mg. while that from the animals eating the stock diet was 1.52 c.p.s./mg. The results of this experiment indicated that feeding an equivalent number of calories derived from fat as a saturated oil did not cause an increase in the specific activity of the liver cholesterol, but actually decreased it 30 per cent below control values.

To confirm these in vivo observations of the effects of an unsaturated fat versus a saturated fat on the incorporation of radiocarbon into cholesterol, in vitro studies were done. One group of rats was fed a stock diet plus 10 per cent linoleic acid and a second group was fed a stock diet plus 10 per cent coconut oil. After 1 week the animals were killed and liver slices prepared and incubated as described. The specific activity of the liver cholesterol digitonide recovered from the animals eating the linoleic acid was 71 per cent higher than the animals eating the coconut oil. The results of this in vitro study which confirmed the earlier in vivo observations indicate that feeding an unsaturated fatty acid enhances incorporation of radiocarbon into liver cholesterol while a saturated fat does not.

The average liver free cholesterol for 2 groups of animals eating the stock diet was 205 mg./100 Gm. fresh liver weight. Feeding
Table 2.—Average 24 Hour Fecal Excretion Containing 10 Per Cent Linoleic Acid or 10 Per Cent Coconut Oil

<table>
<thead>
<tr>
<th>Number of rats</th>
<th>Control (mg.)</th>
<th>Linoleic acid (mg.)</th>
<th>Coconut oil (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-B chromogens</td>
<td>19</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>3-β-OH sterols</td>
<td>125.3</td>
<td>235.0</td>
<td>114.5</td>
</tr>
<tr>
<td>Bile acids</td>
<td>45.7</td>
<td>97.0</td>
<td>54.1</td>
</tr>
</tbody>
</table>

10 per cent linoleic acid to two groups of rats for 8 days increased the average liver free cholesterol to 260 mg./100 Gm. Avigan and Steinberg previously reported an increase in esterfied cholesterol of livers of rats fed corn oil whereas coconut oil fed rats showed no significant changes in liver cholesterol.

Twenty-four hour fecal excretion of the major end-products of cholesterol metabolism were measured in the control group of animals and in both experimental groups. The results of this study are summarized in table 2. The addition of linoleic acid to the diet caused a 47 per cent increase in the excretion of Lieberman-Burchard chromogens; an 87 per cent increase in the excretion of 3-β-hydroxy sterols and a 100 per cent increase in the excretion of bile acids. When coconut oil was added to the diet there was a slight decrease below control values in the excretion of the Lieberman-Burchard chromogens and 3-β-hydroxy sterols, while the excretion of the bile acids increased 20 per cent over control values. These results show that the addition of linoleic acid to the diet of rats is associated with increased excretion of cholesterol when compared to animals eating a stock diet or a diet containing 10 per cent coconut oil.

Discussion

The results of these studies indicate that the rate of incorporation of radiocarbon into liver cholesterol is accelerated when linoleic acid is added to diet. Furthermore, this is associated with an increased fecal excretion of Lieberman-Burchard chromogens, 3-β-hydroxy sterols and bile acids with absorption maximum at 3,200 and 3,850 Å. Feeding an equivalent number of calories as a saturated fat did not produce these changes. These results support the idea that feeding unsaturated fats increases incorporation of radiocarbon into liver cholesterol and increases fecal excretion of the major components of cholesterol catabolism.

Summary

Rats fed linoleic acid incorporate more radiocarbon into liver cholesterol than do either control animals or animals fed an equivalent amount of fat as coconut oil. This effect was found both in vivo and in vitro. Rats fed linoleic acid have an increased fecal excretion of Lieberman-Burchard chromogens, 3-β-hydroxy sterols and bile acids when compared with rats eating stock diets or rats eating stock diet with coconut oil added.

Summary in Interlingua

Rattos recipiente acido linoleic in lor dieta incorpora plus radiocarbon in le cholesterol hepatic que rattos de controlo o que rattos recipiente un quantitate equivalentale de grasseia dietari in le forma de oleo de coco. Iste effecto esseva trovate in vivo e etiam in vitro. Rattos que recipie acido linoleic monstra un augmento del excretion fecal de chromogens de Lieberman-Burchard, do 3-beta-hydroxy-steroles, e de acidos biliar in comparation con rattos nutrite a dietas standard sin o con le addition de oleo de coco.

References

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