THE ABSORPTION OF C\textsuperscript{14}-LABELED EPICHOLESTEROL IN THE RAT\textsuperscript{*}

By H. H. HERNANDEZ, I. L. CHAIKOFF, W. G. DAUBEN, AND S. ABRAHAM

(From the Department of Physiology, School of Medicine, and the Department of Chemistry, University of California, Berkeley, California)

(Received for publication, August 7, 1953)

The present report deals with the absorption of cholesterol and its 3-\(\alpha\)-hydroxy isomer, epicholesterol, in the rat. It is shown that cholesterol is absorbed at more than twice the rate of its stereoisomer, and that, while 50 per cent of the absorbed cholesterol recovered in lymph is esterified, none of the epicholesterol is so bound. The difference in the absorption rates of these two isomers brings to light the importance of stereoconfiguration in sterol absorption, a phenomenon previously observed for carbohydrates (1) and amino acids (2).

EXPERIMENTAL

Synthesis—The preparation of cholesterol-4-C\textsuperscript{14} and epicholesterol-4-C\textsuperscript{14} has been described elsewhere (3). The unlabeled epicholesterol was prepared by the reduction of the enol acetate of cholestenone (3) with sodium borohydride (4). The pure compound was separated on an alumina column and repeatedly chromatographed and crystallized to a constant melting point of 140°.

Treatment of Rats and Collection of Lymph Samples—Twelve rats of the Long-Evans strain, weighing from 250 to 300 gm., were fed ad libitum an adequate stock diet. 48 hours before the experiment all food was removed from the cages, but, for the 24 hours just preceding the operation, the animals again had access to the stock diet. This feeding procedure insured the presence of food in the intestinal tract and, consequently, a copious lymph flow at the time the cannulas were inserted. Rats with empty stomachs or poor lymph flow were discarded at the time of operation. The thoracic lymph duct was cannulated (5), and immediately thereafter a solution of 0.25 cc. of corn oil containing 1 mg. of either 4-C\textsuperscript{14}-labeled epicholesterol or 4-C\textsuperscript{14}-labeled cholesterol was introduced into the rat by stomach tube. During the rest of the experiment the rats had access to the stock diet and 0.9 per cent NaCl solution.

At the intervals recorded in Table I, samples of lymph were collected in tared 50 cc. volumetric flasks containing 4 drops of Tween 20, 4 drops of heparin, and 2 drops of Tween 85. These three compounds served to keep

\textsuperscript{*} Aided by a grant from the United States Public Health Service.
the lipides from precipitating when water was later added to bring the mixture to volume.

**Total C<sup>14</sup> Content of Lymph Sample**—An aliquot of each lymph sample was mounted directly on an aluminum plate and its C<sup>14</sup> content was measured with a gas flow counter. The total C<sup>14</sup> recovered for each interval is recorded in Table I, and the cumulative C<sup>14</sup> recoveries are shown in Fig. 1. The difference in the C<sup>14</sup> recoveries for cholesterol and epicholesterol is quite pronounced. At an early interval, for example from 4 to 8 hours, the C<sup>14</sup> recoveries in the experiments with cholesterol were more than twice those observed with epicholesterol. At the end of 48 hours, the cumulative C<sup>14</sup> values were 8 to 9 per cent of the administered C<sup>14</sup> in the case of epicholesterol and 18 to 31 per cent in the case of cholesterol.

**Separation of Cholesterol from Epicholesterol**—It became necessary, at this point, to examine the efficiency of separation of cholesterol from epicholesterol by digitonin precipitation. Mixtures containing epicholesterol and cholesterol, as shown in Table II, were prepared. Each mixture was dissolved in 14 cc. of acetone-alcohol (1:1) in a centrifuge tube, an excess of 0.5 per cent digitonin in 95 per cent ethyl alcohol-water (1:1) was added, and the vessel and its contents were allowed to stand overnight at 37°. After centrifugation at 3000 r.p.m. for 15 minutes, the precipitate was washed and centrifuged successively, as above, first with about 5 cc. of acetone-alcohol (1:1), next with acetone-ethyl ether (1:1), and finally with

---

**Table I**

Recovery of C<sup>14</sup> of Enterally Administered Cholesterol-4-C<sup>14</sup> or Epicholesterol-4-C<sup>14</sup> in Thoracic Duct Lymph

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Sterol administered</th>
<th>0-2 hrs.</th>
<th>2-4 hrs.</th>
<th>4-8 hrs.</th>
<th>8-16 hrs.</th>
<th>16-24 hrs.</th>
<th>24-36 hrs.</th>
<th>36-48 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cholesterol-4-C&lt;sup&gt;14&lt;/sup&gt;</td>
<td>0.2</td>
<td>3.0</td>
<td>3.3</td>
<td>0.5</td>
<td>0.4</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>0.4</td>
<td>1.2</td>
<td>3.9</td>
<td>1.2</td>
<td>1.3</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>1.4</td>
<td>4.4</td>
<td>9.6</td>
<td>6.0</td>
<td>4.6</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>0.4</td>
<td>0.6</td>
<td>11.2</td>
<td>6.0</td>
<td>3.2</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>&quot;</td>
<td>0.1</td>
<td>1.7</td>
<td>6.8</td>
<td>5.5</td>
<td>1.5</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>&quot;</td>
<td>0.6</td>
<td>1.1</td>
<td>7.0</td>
<td>9.7</td>
<td>2.6</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Epicholesterol-4-C&lt;sup&gt;14&lt;/sup&gt;</td>
<td>0.2</td>
<td>3.0</td>
<td>3.3</td>
<td>0.5</td>
<td>0.4</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>0.4</td>
<td>1.8</td>
<td>3.6</td>
<td>2.3</td>
<td>0.8</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>0.3</td>
<td>0.9</td>
<td>3.0</td>
<td>3.7</td>
<td>0.7</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>&quot;</td>
<td>0.3</td>
<td>0.8</td>
<td>3.4</td>
<td>3.3</td>
<td>0.7</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>&quot;</td>
<td>0.2</td>
<td>0.7</td>
<td>2.8</td>
<td>3.5</td>
<td>1.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>&quot;</td>
<td>0.4</td>
<td>1.3</td>
<td>3.2</td>
<td>3.1</td>
<td>0.6</td>
<td>0.4</td>
<td>0.1</td>
</tr>
</tbody>
</table>

---

This table shows the recovery of C<sup>14</sup> in thoracic duct lymph at different time intervals after the administration of cholesterol or epicholesterol.
ethyl ether (6). The digitonide was dissolved in methanol, and a small aliquot of it mounted on an aluminum plate; the $^{14}C$ was then counted in a gas flow counter. The methanol solution was then taken to dryness, and the residue was redissolved in 0.5 cc. of redistilled pyridine; this treatment served to split the cholesterol digitonide. The free digitonin was then precipitated by the addition of 10 cc. of ethyl ether according to Schoenheimer and Dam (7).

![Figure 1](https://example.com/figure1.png)

**Fig. 1.** Cumulative $^{14}C$ recoveries in thoracic duct lymph of rats fed either cholesterol-$^{14}C$ or epicholesterol-$^{14}C$. The dotted areas show the range of values observed with six rats in each experiment.

The precipitate (free digitonin) was centrifuged as above, and the precipitate washed three times with ethyl ether. The combined supernatant fluids were taken to dryness on the steam bath. The dry material was dissolved in 14 cc. of acetone-alcohol (1:1) as before, and reprecipitated with 0.5 per cent digitonin. This whole procedure was repeated three times.

It will be noted in Table II that there is a certain amount of coprecipitation of epicholesterol with cholesterol digitonide, but, after successive splittings of the cholesterol digitonide with pyridine, the coprecipitated radioactivity was reduced to negligible quantities.
Digitonin-Precipitable $^{14}$C in Lymph of Rats Fed Either Cholesterol-$^{14}$C
or Epicholesterol-$^{14}$C—Each lymph sample from all twelve rats, collected in the tared flasks at two intervals of from 8 to 12 and 12 to 16 hours, was next analyzed for free and esterified cholesterol in the following manner.

A 2 cc. aliquot of the lymph mixture was added dropwise to 40 cc. of a boiling 1:1 mixture of acetone and alcohol. The mixture was filtered, and the protein precipitate was washed repeatedly with the boiling solvent mixture until the precipitate showed no further radioactivity. The combined filtrate and washings were taken just to dryness on a steam bath in a CO$_2$ atmosphere, and the residue was dissolved in a small volume (14 cc.) of alcohol-acetone (1:1) mixture. 1 mg. of cholesterol was added as carrier, and the solution was treated with digitonin as described in the preceding section (four precipitations).

A second 2 cc. aliquot of the lymph mixture was hydrolyzed with 10 cc. of alcoholic KOH (9 gm. per 100 cc.) on a steam bath for 6 hours. The saponified lymph mixture was extracted three times with petroleum ether, the combined extracts were taken just to dryness, and the residue was redissolved in 14 cc. of a 1:1 acetone-alcohol mixture. To this was first added 1 mg. of cholesterol as carrier, and the solution was treated with digitonin as described in the preceding section. The C$^{14}$ content of the

Table II

Digitonin* Precipitability of Cholesterol and Epicholesterol, Singly and Mixed
For an explanation, see the text.

<table>
<thead>
<tr>
<th>Starting material</th>
<th>Per cent added $^{14}$C recovered in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st ppt.</td>
</tr>
<tr>
<td>1 mg. epicholesterol</td>
<td>None</td>
</tr>
<tr>
<td>0.1 mg. epicholesterol</td>
<td>6.77</td>
</tr>
<tr>
<td>0.1 “ “</td>
<td>7.32</td>
</tr>
<tr>
<td>0.1 “ “</td>
<td>5.66</td>
</tr>
<tr>
<td>0.1 “ “</td>
<td>6.01</td>
</tr>
<tr>
<td>1 mg. cholesterol</td>
<td>99.03</td>
</tr>
<tr>
<td>1 “ “</td>
<td>97.08</td>
</tr>
<tr>
<td>1 “ “</td>
<td>100.33</td>
</tr>
<tr>
<td>1 “ “</td>
<td>99.39</td>
</tr>
<tr>
<td>1 “ “</td>
<td>100.09</td>
</tr>
<tr>
<td>1 “ “</td>
<td>99.34</td>
</tr>
<tr>
<td>1 “ “</td>
<td>98.99</td>
</tr>
<tr>
<td>1 “ “</td>
<td>95.66</td>
</tr>
</tbody>
</table>

* In all cases 4 cc. of 0.5 per cent digitonin in 50 per cent alcohol were added to the sterol solutions.
insoluble digitonin complex was also determined as described in that section.

Practically all of the C\textsuperscript{14} in the hydrolyzed lymph samples obtained from the rats fed labeled cholesterol was recovered as the digitonides (Table III). As previously shown (8), approximately 50 per cent of the lymph cholesterol is in the esterified form. In the case of the epicholesterol-fed rats, none of the lymph C\textsuperscript{14} was recovered in the digitonide-precipitable fraction after the fourth splitting with pyridine, as described above.

**Table III**

*Digitonin Precipitability of C\textsuperscript{14} in Thoracic Duct Lymph of Rats Fed Cholesterol-4-C\textsuperscript{14} and Epicholesterol-4-C\textsuperscript{14}*

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Sterol administered</th>
<th>Per cent of lymph C\textsuperscript{14} recovered in digitonin-precipitable fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8-12 hr. sample</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Before saponification</td>
</tr>
<tr>
<td>1</td>
<td>Cholesterol-4-C\textsuperscript{14}</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>47</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>56</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>49</td>
</tr>
<tr>
<td>19</td>
<td>&quot;</td>
<td>44</td>
</tr>
<tr>
<td>56</td>
<td>&quot;</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>Epicholesterol-4-C\textsuperscript{14}</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>18</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>58</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>59</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

**Identification of C\textsuperscript{14}-Containing Compound in Lymph of Rats Fed Epicholesterol-C\textsuperscript{14}**—A 2 cc. aliquot of the lymph mixture collected from 4 to 8 hours after administration of the labeled compound was added dropwise to a boiling alcohol-acetone (1:1) solution. The precipitate obtained was filtered and washed exhaustively with the same solvent solution. The combined extracts contained all the C\textsuperscript{14} in the lymph (Table IV). The combined filtrates were concentrated on a steam bath to a volume of approximately 2 cc. (but not to dryness). 8 cc. of 50 per cent ethyl alcohol solution were added, and the resulting solution was extracted three times, each with a fresh portion of 150 cc. of petroleum ether. The volume of the combined petroleum ether extracts was reduced to about 10 cc. on a steam bath. To remove the bulk of extraneous material, the 10 cc. of concentrate were treated as follows: a 5 gm. alumina column (0.5 × 10 cm.) was
first exhaustively washed with petroleum ether, and to this column were transferred the 10 cc. of concentrated material. The column was then washed with a small amount of petroleum ether. The C$^{14}$-containing ma-

**TABLE IV**  
*Accountability of C$^{14}$ in Lymph of Typical Rats Fed Cholesterol-4-C$^{14}$ and Epicholesterol-4-C$^{14}$*

<table>
<thead>
<tr>
<th>Material chromatographed</th>
<th>Rat 3 fed cholesterol-4-C$^{14}$</th>
<th>Rat 18 fed epicholesterol-4-C$^{14}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph sample</td>
<td>26,810</td>
<td>13,900</td>
</tr>
<tr>
<td>Acetone-alcohol extractions</td>
<td>27,210</td>
<td>14,000</td>
</tr>
<tr>
<td>Alumina column eluate</td>
<td>26,310</td>
<td>13,400</td>
</tr>
</tbody>
</table>

**TABLE V**  
*Paper Chromatographic Analysis of Lymph from Rats Fed Cholesterol-4-C$^{14}$ and Epicholesterol-4-C$^{14}$*

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Material chromatographed</th>
<th>RF$^*$ of radioactive spots 4 to 8 hr. sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before saponification</td>
</tr>
<tr>
<td>1</td>
<td>Cholesterol</td>
<td>0.56 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Cholesterol-palmitate†</td>
<td>0.56 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>Epicholesterol-4-C$^{14}$</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>Lymph extract from rat fed cholesterol-4-C$^{14}$</td>
<td>0 and 0.56</td>
</tr>
<tr>
<td>4</td>
<td>Cholesterol-palmitate†</td>
<td>0 and 0.56</td>
</tr>
<tr>
<td>5</td>
<td>Epicholesterol-4-C$^{14}$</td>
<td>0 and 0.56</td>
</tr>
<tr>
<td>6</td>
<td>Lymph extract from rat fed cholesterol-4-C$^{14}$</td>
<td>0 and 0.56</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>0 and 0.56</td>
</tr>
<tr>
<td>56</td>
<td></td>
<td>0 and 0.56</td>
</tr>
<tr>
<td>2</td>
<td>Epicholesterol-4-C$^{14}$</td>
<td>0.91</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.88</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>0.91</td>
</tr>
<tr>
<td>58</td>
<td></td>
<td>0.93</td>
</tr>
<tr>
<td>59</td>
<td></td>
<td>0.89</td>
</tr>
</tbody>
</table>

*RF is measured as the distance of the front of the compound from the origin, divided by the distance of the front of the solvent from the origin.
† Prepared according to Page and Rudy (10).
Kirk (11). Petroleum ether solutions of several known sterols, namely cholesterol, epicholesterol-4-C\textsubscript{14}, and cholesterol-palmitate, were also applied on the same paper. The other half of the eluates was saponified with alcoholic KOH on a steam bath for 6 hours. The resulting mixture was extracted with petroleum ether, and the extract was washed with dilute acid to make it neutral to brom cresol green. The saponified eluates were applied to the Quilon-treated filter paper. As a rule, saponified and unsaponified materials were chromatographed simultaneously. The known sterols were also saponified, extracted, and applied to the filter paper.

Chromatograms were developed with methanol, as described by Kritchevsky and Calvin (12). Radioautographs were made from the chromatograms with 14 × 17 inch "no screen" x-ray film. After development of the films, the spots were intensified with Kodak intensifier solution No. 77.

### Table VI

<table>
<thead>
<tr>
<th>Recrystallization</th>
<th>Solvent</th>
<th>Amount at start (mg.)</th>
<th>Amount recovered (mg.)</th>
<th>Specific activity (c.p.m. per mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Ethanol</td>
<td>53.7</td>
<td>43.1</td>
<td>140,000</td>
</tr>
<tr>
<td>2nd</td>
<td>Acetone</td>
<td>43.1</td>
<td>31.8</td>
<td>142,800</td>
</tr>
<tr>
<td>3rd</td>
<td>Ethyl ether</td>
<td>31.8</td>
<td>5.9</td>
<td>144,800</td>
</tr>
</tbody>
</table>

The filter paper chromatograms were next sprayed with a 1:1 solution of antimony trichloride and acetic acid. It was observed here that antimony trichloride is a more sensitive indicator than is antimony pentachloride (11). The chromatograms were allowed to dry in an oven at 95–105° for 2 minutes.

The chromatograms of the lymph extract obtained from the rats fed epicholesterol-4-C\textsubscript{14} showed a single radioactive spot with an \( R_f \) of 0.88 to 0.93; the \( R_f \) value for the reference compound, epicholesterol-4-C\textsubscript{14}, was 0.91 ± 0.05 (Table V). It was, therefore, considered probable that none of the epicholesterol recovered in lymph had been esterified.

Six chromatograms were prepared from the lymph of rats fed epicholesterol-4-C\textsubscript{14}. The six radioactive spots were cut out and thoroughly extracted with petroleum ether. The dried material, which weighed about 3 mg., was mixed with about 51 mg. of epicholesterol and the mixture was recrystallized three times, each time from a different solvent. The finding (Table VI) that the specific activity was the same after each recrystalliza-
tion indicates that the C\(^{14}\) recovered in the lymph of rats fed epicholesterol-4-C\(^{14}\) was present as free epicholesterol.

In the case of the extract prepared from the lymph of rats fed cholesterol, two radioactive spots were observed, one at the origin and the other at \(R_F\) 0.51 to 0.60. Upon hydrolysis, this extract yielded a single radioactive spot with \(R_F\) values of 0.52 to 0.59. The \(R_F\) values from the two reference compounds, cholesterol and cholesterol-palmitate, were 0.56 ± 0.05 and 0, respectively. It would appear that the radioactive compound at the origin is esterified cholesterol. These findings are in agreement with the data shown in Table III.

**DISCUSSION**

The results of the present investigation reveal a significant difference in the rates of absorption of cholesterol and epicholesterol, two compounds that differ only in the stereoconfiguration of the hydroxy group at carbon 3. In the rat, cholesterol is absorbed about twice as rapidly as epicholesterol. Two C\(^{14}\)-labeled fractions were identified in the lymph of rats fed cholesterol-4-C\(^{14}\) (free and esterified cholesterol), and approximately 50 per cent of the absorbed cholesterol in lymph was in the esterified form. The lymph of rats fed epicholesterol-4-C\(^{14}\) contained a single C\(^{14}\)-labeled compound which was identified as epicholesterol. No other C\(^{14}\)-labeled compounds were identified in the lymph of rats fed the cholesterol-C\(^{14}\) or epicholesterol-C\(^{14}\). Apparently, the OH group at carbon 3 of epicholesterol is not inverted in its passage from lumen to lymph.

Swell et al. have shown that extracts prepared from intestinal mucosa of normal rats possess the ability to esterify cholesterol (13). It would appear, from the results presented here, that the enzymes involved in the esterification of cholesterol during its passage from lumen to lymph are quite stereospecific. The finding that none of the epicholesterol recovered in lymph was esterified indicates that esterification may be responsible for the differences in the rates of absorption of cholesterol and epicholesterol.

**SUMMARY**

1. Cholesterol-4-C\(^{14}\) and epicholesterol-4-C\(^{14}\) were fed to rats that had cannulas inserted into their thoracic ducts. The rate of appearance of C\(^{14}\) in the lymph of each rat was measured, and the C\(^{14}\)-containing compounds in lymph were characterized.

2. Cholesterol was identified as the major C\(^{14}\)-containing compound in the lymph of rats fed cholesterol-C\(^{14}\). Approximately 50 per cent of the cholesterol recovered in lymph was esterified.

3. Epicholesterol was identified as the only major C\(^{14}\)-containing compound in the lymph of rats fed epicholesterol-C\(^{14}\). Practically none of this epicholesterol in lymph was in the esterified form.
4. Cholesterol is more rapidly absorbed than is epicholesterol. The C\textsuperscript{14} recoveries observed in the rats fed the cholesterol were about twice those found in the rats fed the epicholesterol. The possible relation between the rate of absorption of cholesterol and its esterification is pointed out.

BIBLIOGRAPHY

THE ABSORPTION OF C\textsuperscript{14}-LABELED EPICHOLESTEROL IN THE RAT


Access the most updated version of this article at http://www.jbc.org/content/206/2/757.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/206/2/757.citation.full.html#ref-list-1