PARTICIPATION OF PHOSPHOLIPIDES IN LYMPHATIC TRANSPORT OF ABSORBED FATTY ACIDS*

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(Received for publication, October 11, 1950)

Recently, we investigated the path by which an absorbed, long chain fatty acid leaves the intestine (1). Unanesthetized rats, into whose thoracic ducts or lacteals cannulae had been introduced, were fed C¹⁴-labeled palmitic acid as either the triglyceride or the free acid. From 70 to 92 per cent of the absorbed, labeled fatty acid was recovered as fatty acid C¹⁴ from the thoracic duct lymph in nine of ten rats studied, and from 69 to 84 per cent was recovered from the intestinal lymph of four rats. The amounts recovered in intestinal lymph in the present investigation (Table II) were as high as 97 per cent. Such high recoveries indicate that the transport of absorbed, long chain fatty acids is a concern, almost exclusively, of the intestinal lacteals.

Plasma and lymph serve as avenues of transport between organs and tissues. The fatty acid transport function of plasma phospholipide was dealt with in an earlier report from this laboratory (2). When C¹⁴-labeled palmitic acid was injected into fasted, liverless dogs, significant amounts of the labeled fatty acid were incorporated into the phospholipide of the small intestine and its mucosa, the kidneys, lungs, heart, and skeletal muscles. But practically none of the isotopic fatty acids was recovered in the plasma phospholipides. This finding lends no support to the view that plasma phospholipides serve to transport fatty acids between organs and tissues.

The present study was designed to test the participation of lymph phospholipides in carrying absorbed fatty acids from the small intestine to the plasma.

EXPERIMENTAL

Treatment of Rats

Collection of Lymph—Male rats of the Long-Evans strain were used. They were fed a diet composed of 67.5 per cent whole wheat, 15 per cent

* Aided by a grant from the American Cancer Society as recommended by the Committee on Growth of the National Research Council.
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261
TRANSPORT OF FATTY ACIDS

casein, 10 per cent whole milk powder, 5.2 per cent hydrogenated vegetable oil, 1.5 per cent calcium carbonate, 0.75 per cent sodium chloride, and 16 cc. of sardilene (a fish oil rich in vitamins A and D) per kilo. The rats were not fasted before the operation. Lymph was collected from unanesthetized rats kept in restraining cages (3).

After recovery from the anesthetic, which was induced for the purpose of inserting the cannulae into the lymphatics, the animals had free access to the diet noted above and 1 per cent NaCl solution. The NaCl, by increasing the rate of lymph flow (4), reduces clot formation in the cannula. Once a free flow of lymph was established, the NaCl solution was replaced by tap water (except for two rats; see Table I).

Administration of Labeled Fatty Acids—Palmitic acid, labeled with C\textsuperscript{14} at the carboxyl position, was synthesized (5). Approximately 4 mg. of its triglyceride were dissolved in 0.5 cc. of corn oil. The mixture was then brought to body temperature and administered by intragastric intubation to lightly etherized rats.

Analytical Procedures

Lipides were extracted from each lymph sample and dissolved in petroleum ether (Extract A) as described earlier (1). Separate aliquots of this extract were used to determine the following: fatty acid C\textsuperscript{14} (1), phospholipide fatty acids, total fatty acids, and total cholesterol.

Phospholipide Fatty Acids—The phosphorus content of Extract A was determined by King’s method (6). Phospholipide phosphorus was converted to total phospholipide by the factor 25, and the latter to phospholipide fatty acids by the factor 0.68.

Total Fatty Acids and Total Cholesterol—Aliquots of the petroleum ether Extract A were saponified and acidified, and the lipides were dissolved in petroleum ether. Fatty acids were measured by the oxidative procedure (7), and cholesterol was determined by the colorimetric method of Sperry and Brand (8).

Separation of Acetone-Soluble from Acetone-Insoluble Lipides—Aliquots of Extract A were transferred to 50 cc. centrifuge tubes, and the solvent was evaporated to approximately 0.5 cc. The phospholipides were precipitated by the addition of 30 cc. of acetone and 15 drops of a saturated solution of MgCl\textsubscript{2} in absolute alcohol. The mixture was centrifuged, and the supernatant containing the acetone-soluble lipides was decanted. 30 cc. of acetone were now added to the precipitate, and the latter was thoroughly dispersed by vigorous stirring. The treatment with acetone was repeated twice more. The four supernatants were then combined; this acetone-soluble fraction contained the non-phospholipide fatty acids and cholesterol.
The acetone-soluble and insoluble fractions were used for the determination of non-phospholipide fatty acid C\textsuperscript{14} and phospholipide-fatty acid C\textsuperscript{14}, respectively.

The acetone-insoluble fraction (phospholipides) was saponified as follows: The precipitate was dissolved in methanol and to this was added 0.5 cc. of 90 per cent KOH. The mixture was refluxed for 1 hour on a steam bath, cooled, and acidified. The fatty acids were extracted by four washings with hot petroleum ether.

The acetone-soluble fraction was likewise saponified and acidified, and its fatty acids were extracted with four separate portions of petroleum ether. It has previously been shown that the cholesterol in the lymph collected under the conditions of this experiment does not contain detectable quantities of C\textsuperscript{14} (1).

The C\textsuperscript{14} contents of these fractions were measured by the direct mounting technique of Entenman et al. (9).

Lipide Analysis of Liver and of Feces Plus Gastrointestinal Contents—At the end of the experiment the animals were sacrificed and the gastrointestinal contents and feces, excreted from the time of administration of the labeled fat, were analyzed for lipide C\textsuperscript{14} as described in an earlier report (1), which also deals with the determination of the lipide C\textsuperscript{14} in the liver.

Results

Seven rats were studied. In three cannulae were introduced into the intestinal lymphatics, and in four, into the thoracic ducts. As a rule, rats so treated begin to drink about 6 hours after the operation, and to eat within 12 hours. After clotting had ceased to occur in the cannulae and lymph was flowing freely, the labeled triglyceride was administered by stomach tube. The interval between the insertion of the cannula and feeding of the test fat was about 25 hours (Table I). The animals had access to food throughout the entire period of observation. Rats 6 and 7 received 1 per cent NaCl for drinking purposes during the period of lymph collection. The other five rats received the saline for the first 18 to 24 hours and tap water thereafter.

Under the influence of the saline the rats excreted about 2 cc. of lymph per hour (Table I). The substitution of tap water reduced the rate to about 1 cc. per hour. This latter value is in agreement with the rates reported by Bollman et al. for the 200 gm. rat (10).

Lipides of Lymph—The lipide composition of the two types of lymph is recorded in Table I. There was no apparent difference between the lipide composition of the intestinal lymph and that of the thoracic duct lymph obtained from rats fed fat.
TRANSPORT OF FATTY ACIDS

0.7 to 1.6 gm. of fatty acids was recovered from the total lymph collected in 20 to 22 hours from thoracic duct or intestinal lacteals. This amount of fatty acids far exceeds that administered by stomach tube (less than 0.5 gm.), but this was to be expected since the rats had access to food during the experiment.

In the fed, unanesthetized rat, only about 7 per cent of the total fatty acids in either thoracic duct or lacteal lymph was phospholipide fatty acids. In the case of the anesthetized rabbit, Fröhlicher and Süßmann (11) reported that 7 to 13 per cent of the fatty acids in intestinal lymph is present as phospholipide fatty acids. Since Reinhardt et al. (12) have shown that, in the dog, plasma phospholipides are transferred to thoracic duct lymph, it is not possible to state how much of the phospholipide fatty acids recovered from intestinal lymph in our experiments was derived from ingested fat.

Lymph contained from 55 to 69 mg. per cent of cholesterol in six of the seven rats studied.

C14-Labeled Lipides in Lymph—The percentages of the administered labeled fat absorbed are recorded in Table II. The term absorbed as used here refers to the difference between the amount of C14-labeled fat

Table I

Flow Rates and Lipide Composition of Intestinal and Thoracic Duct Lymph Obtained from Rats

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Lymph collected from</th>
<th>Total amount of lymph collected</th>
<th>Composition of lymph collected after feeding labeled fat</th>
<th>Phospholipide fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before fat administration</td>
<td>After fat administration</td>
<td>1 per cent NaCl solution replaced by tap water after</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hrs. cc.</td>
<td>hrs. cc.</td>
<td>hrs.</td>
</tr>
<tr>
<td>1</td>
<td>Lacteals</td>
<td>25 43</td>
<td>22 22.5</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>25 49</td>
<td>22 25.4</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>24 61</td>
<td>22 31.2</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>Thoracic duct</td>
<td>29 79</td>
<td>20.5 22.2</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>27 74</td>
<td>20 18.1</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>27 29</td>
<td>20 57.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>22 21</td>
<td>21 34.3</td>
<td></td>
</tr>
</tbody>
</table>

* 210 to 300 gm. in weight.
† The stock diet was available to all rats throughout the experiment.
‡ The intervals during which these amounts were recovered are recorded in the fifth column.
administered and that recovered from the gastrointestinal tract and feces at the end of the experiment. In five of the seven rats studied, 70 to 83 per cent of the administered palmitic acid C\textsubscript{14} was absorbed. Rats 1 and 7 absorbed 41 and 53 per cent, respectively. The low absorption in Rat 1 probably resulted from an intestinal disturbance, for its stools became liquid after the administration of the test fat. Table II also shows, in confirmation of our earlier findings (1), that nearly all of the absorbed C\textsubscript{14} can be recovered in the fatty acid fraction of lymph obtained from either the thoracic duct or intestinal lymphatics. In five of the seven rats, 90 per cent or more of the absorbed C\textsubscript{14} was present as fatty acid C\textsubscript{14} in the collected lymph.

Not more than 4 per cent of the C\textsubscript{14}-labeled fatty acids recovered from both intestinal and thoracic duct lymph had been incorporated into phospholipides.

**DISCUSSION**

The finding that 96 per cent of the labeled fatty acids in lymph was present in non-phospholipide form again emphasizes the unimportance of phospholipides as vehicles for fatty acid transport. It is also of interest to note that very little of the lymph fatty acids is carried in the form of cholesterol esters. Even if we assume that all of the cholesterol in lymph

**Table II**

*Fatty Acid C\textsubscript{14} Recovered in Lymph after Enteral Administration of Palmitic Acid--l-C\textsubscript{14}*

The palmitic acid labeled in the carboxyl position with C\textsubscript{14} was administered as the tripalmitin dissolved in 0.5 cc. of corn oil at body temperature by intragastric intubation. Each rat received approximately 4 mg. of radioactive tripalmitin containing $1 \times 10^5$ c.p.m. per mg.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Per cent of administered</th>
<th>Per cent of absorbed palmitic</th>
<th>Per cent of lymph fatty acid C\textsubscript{14} recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>palmitic acid C\textsubscript{14} absorbed*</td>
<td>acid C\textsubscript{14} recovered</td>
<td>in lymph as fatty acid C\textsubscript{14}</td>
</tr>
<tr>
<td>1 \dagger</td>
<td>40.8</td>
<td>81.4</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>77.5</td>
<td>95.7</td>
<td>0.50</td>
</tr>
<tr>
<td>3</td>
<td>82.2</td>
<td>96.7</td>
<td>0.75</td>
</tr>
<tr>
<td>4</td>
<td>83.3</td>
<td>92.8</td>
<td>0.36</td>
</tr>
<tr>
<td>5</td>
<td>77.5</td>
<td>90.7</td>
<td>0.55</td>
</tr>
<tr>
<td>6</td>
<td>70.3</td>
<td>63.3</td>
<td>0.36</td>
</tr>
<tr>
<td>7</td>
<td>52.7</td>
<td>96.5</td>
<td>0.51</td>
</tr>
</tbody>
</table>

\* Absorbed refers to the difference between the amount administered and the amount recovered from the feces and intestinal contents.

\dagger This rat developed diarrhea after the administration of the labeled fat.
is esterified, carriage in this form could account for, at most, 1.5 per cent of the non-phospholipide fatty acids contained in lymph. It would appear from these observations, as well as from those made earlier by Goldman et al. (2), that fatty acids are transported chiefly in forms other than phospholipides.

The results obtained also bear on the site of formation of plasma phospholipides. Our earlier studies with fasted, liverless dogs indicate strongly that the liver is the chief site for the formation of plasma phospholipide (2, 13, 14). This was shown in experiments carried out with P32 and with C14-labeled palmitic acid. But the significance of the small intestine as a contributor to plasma phospholipides during fat absorption was not satisfactorily settled. The present findings show that, during the absorption of fat, it is possible for intestinal lymph to contribute phospholipides to plasma. Although the indications are strong that the intestinal lymph phospholipide containing the C14-fatty acids was synthesized in the small intestine, the possibility that it was formed in part elsewhere (liver or mesenteric lymph nodes) is, of course, not ruled out. The extent to which the small intestine serves as an extrahepatic site for plasma phospholipide formation during fat absorption is being investigated.

SUMMARY

1. The present study was undertaken to test the participation of lymph phospholipides as vehicles for the transport of an absorbed, long chain fatty acid from the small intestine to plasma. Palmitic acid, the carboxyl carbon of which was labeled with C14, was fed to rats in which cannulae had been introduced into the intestinal lymphatics or thoracic ducts. As much as 96 per cent of the C14-labeled fatty acids recovered in the lymph obtained from these two sources was present in forms other than phospholipides. The conclusion is drawn that phospholipides are not important transport forms for absorbed palmitic acid.

2. The contribution of the small intestine to the synthesis of plasma phospholipides during fat absorption is discussed.

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