Metabolic Heterogeneity of Lecithins in Intestine

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The incorporation of [2-3H] glycerol and lysolecithin labeled with [2-3H] glycerol into lecithin and its subspecies was investigated in epithelial and muscular layers of rat small intestine. The labeled compounds were administered in two ways: the intraduodenal injection and the injection into femoral vein. The incorporation of glycerol into lecithin was distinctly higher in epithelial layer than in muscular layer in both administration routes, but the incorporation of lysolecithin into lecithin showed no marked differences between both layers. Among lecithin species, dienoic and monoenoic species were highly labeled with the injection of labeled glycerol, and dienoic as well as tetraenoic species were markedly labeled with the injection of labeled lysolecithin. Anyhow, no marked differences were found on the incorporation pattern of any labeled compounds into lecithin between both layers and between both administration routes.

Akino et al. (1972) investigated the fate of labeled lysolecithin injected into rat femoral vein and showed the vivid conversion of lysolecithin to lecithin in several tissues. The conversion was most prominent in intestine as well as in liver. Among subspecies of lecithin, tetraenoic species showed a distinctly higher specific activity than other species in any tissues examined. However, Sato (1970) has presented a quite different pattern on the labeling of lecithin species of intestine after the injection of labeled lysolecithin into duodenal lumen, that is, no marked differences on specific activity were found among the subspecies. The detailed studies on the lecithin species newly formed by different pathways have been presented in lung (Akino et al. 1971) as well as in liver (Arvidson 1968; Kanoh 1969). However, only a few data have been presented on the metabolic heterogeneity of lecithin subspecies in intestine (Yurkowski and Walker 1971; Saito 1973).

In the present paper, we investigated the incorporation of [2-3H] glycerol as well as lysolecithin labeled with [2-3H] glycerol into lecithin and its subspecies in epithelial and muscular layers of rat small intestine, by two ways of administration: the intraduodenal injection and the injection into femoral vein.

Materials and Methods

[2-3H]Glycerol (specific activity of 420 mCi/m mole) was purchased from the Radio-
chemical Centre, Amersham, England. Lysolecithin labeled with $[2-\text{H}]$ glycerol was biosynthetically prepared in the same manner as reported previously (Akino et al. 1971), using 5 mCi of $[2-\text{H}]$ glycerol. The specific activity of the lysolecithin was $161.45 \times 10^3$ cpm/µmole. About 5 µmoles of lysolecithin were mixed with 0.5 ml of saline containing 7% bovine serum albumin treated by the method of Goodman (1957). After overnight fasting, male rats weighing $114.5 \pm 14.1$ g were injected 100 µCi or 500 µCi of $[2-\text{H}]$ glycerol in 0.5 ml of saline, or $384.24 \times 10^3$ cpm (2.38 µmoles) or $768.48 \times 10^3$ cpm (4.78 µmoles) of lysolecithin labeled with $[2-\text{H}]$ glycerol, into duodenal lumen or femoral vein, respectively. The animals were decapitated under light ether anaesthesia at 5 or 20 min after injection of the labeled precursors.

The small intestines were excised, cut into about 5 cm each of segments, turned inside out, and washed with cold saline under a gentle shaking in order to remove intraluminal materials. The small intestinal segments were put between filter papers to remove water and then weighed. The epithelial layer was collected carefully by a dull razor and the remained muscular layer was weighed. The wet weight of total small intestine was $5.6 \pm 1.6$ g, epithelial layer 3.25±0.8 g, and muscular layer 2.35±0.6 g.

The lipid extraction and isolation of lecithin were generally performed according to the manner described by Sato (1970). Purified lecithin was further subfractionated into the molecular species of different degrees of unsaturation by silver nitrate thin-layer chromatography. Radioactivity was determined by a Beckman liquid scintillation spectrometer (LS 200) with toluene scintilator. Phosphorus was determined by the method of Bartlett (1959).

**RESULTS AND DISCUSSION**

The pool sizes of lecithin and its subspecies in rat intestine were shown in Table 1. There were not so significant differences between epithelial and muscular layers in the pool sizes of lecithin and its subspecies except that somewhat higher values were found in dienoic species of epithelial layer and in monoenoic species of muscular layer. It was noted that oligoenoic species such as monoenoic and dienoic species showed much larger pool sizes than polyenoic species such as tetraenoic and hexaenoic ones, in intestine, while polyenoic species, especially tetraenoic species, predominant among lecithin species in liver (Arvidson 1968; Kanoh 1969).

Table 2 shows the incorporation of labeled compounds into lecithin of rat small intestine at 5 and 20 min after administration into duodenal lumen or femoral vein. Marked differences in the incorporation of $[2-\text{H}]$ glycerol were observed between epithelial and muscular layer. The incorporation of labeled lysolecithin into lecithin showed, however, little differences between both layers not only by the routes via femoral vein but also by the intraduodenal administration, as was unexpected. The latter phenomenon might be explained by the detergent effect of lysolecithin on the behavior of intestinal epithelial cells. It was also noted that the incorporation of lysolecithin into intestinal lecithin was much higher than that of glycerol, especially by the intraduodenal route. This phenomenon was not in conflict with the findings by other investigators. Koike (1970) has suggested from his turnover study of intestinal lecithin by the administration of $[14\text{C}]$ choline into duodenal lumen, that lecithin formation in intestine might be mainly ascribed to the pathways other than de novo synthesis via CDP-choline. The vivid formation of lecithin from lysolecithin administered into duodenal lumen
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**TABLE 1.** Pool size and molecular species composition of lecithin in rat small intestine

<table>
<thead>
<tr>
<th>Compound analyzed</th>
<th>Epithelial layer</th>
<th>Muscular layer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unfractionated lecithin (μmoles/wet g tissue)</strong></td>
<td><em>8.2±1.9</em></td>
<td><em>7.8±1.5</em></td>
</tr>
<tr>
<td><strong>Subspecies</strong></td>
<td>(％)†</td>
<td>(％)†</td>
</tr>
<tr>
<td>Monoenoic</td>
<td>27.2±4.2</td>
<td>36.6±4.4</td>
</tr>
<tr>
<td>Dienoic</td>
<td>38.3±5.6</td>
<td>29.1±5.6</td>
</tr>
<tr>
<td>Trienoic</td>
<td>5.1±1.4</td>
<td>6.4±1.9</td>
</tr>
<tr>
<td>Tetraenoic</td>
<td>18.6±3.9</td>
<td>17.4±3.4</td>
</tr>
<tr>
<td>Hexaenoic</td>
<td>12.9±2.4</td>
<td>17.5±3.2</td>
</tr>
</tbody>
</table>

* The content of lecithin is given as Mean±S.D. obtained from 12 cases.
† The percentage distribution of lecithin subspecies is given as Mean ±S.D. obtained from 5 cases.

**TABLE 2.** Incorporation of labeled precursors into lecithin of rat small intestine

<table>
<thead>
<tr>
<th>Way of Administration</th>
<th>Time after administration (min)</th>
<th>Percent incorporation into lecithin*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glycerol†</td>
</tr>
<tr>
<td>Duodenal lumen</td>
<td>5</td>
<td>0.61(0.14)†</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.31(0.62)</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>5</td>
<td>0.11(0.05)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.08(0.04)</td>
</tr>
</tbody>
</table>

* The values are % incorporation of administered dose per g wet tissue.
† Labeled substances administered.
‡ The values without parenthesis show percent incorporation into lecithin of epithelial layer and the values in parenthesis are those of muscular layer.

has been demonstrated by Sato (1970). It has been well known that lysolecithin in intestinal lumen could be derived from lecithin, which was supplied from bile and from diet, by the action of pancreatic phospholipase A2. It seems to be probable that the high content of dienoic species in bile lecithin shown by Sakamoto and Akino (1972) might affect the pattern of the lecithin species in epithelial layer, as shown in Table 1, although the expected low activity of Δ⁵-desaturase and Δ⁶-desaturase, which were responsible for the formation of arachidonic acid from linoleic acid may also make some contribution.

Fig. 1 shows the distribution of radioactivity among lecithin subspecies of small intestine at 5 and 20 min after the administration of labeled compounds. The highest incorporation of labeled glycerol was found in dienoic species, and the incorporation patterns into lecithin subspecies were not so different between epithelial layer and muscular layer as well as between the ways of administration. It shows that the main species of lecithin formed by the de novo pathway was the dienoic one in epithelial and muscular layers as well as in liver.

The highest incorporation of labeled lysolecithin among lecithin subspecies
Fig. 1. Distribution of radioactivity in lecithin subspecies of rat small intestine. The
digits in the figures indicate the percentage of the individual groups.
(a), [2-3H] glycerol administered into the duodenal lumen (D) or femoral vein (F).
(b), Lysolecithin labeled with [2-3H] glycerol administered into the duodenal lumen (D)
or femoral vein (F).
* from the data of Sato (1970); lysolecithin administered into the duodenal lumen.
† from the data of Akino et al. (1972); lysolecithin injected into the femoral vein.
- epithelial layer; —, muscular layer; —, intestinal wall; —, liver.
was found in tetraenoic species at 5 min after the administration, but the marked incorporation was also noted in the dienoic species especially in epithelial layer at 20 min after the administration. The labeling patterns of lecithin subspecies were little different between both layers as well as between two administration ways. The patterns were somewhat different from Sato’s data (1970) obtained after the intraduodenal injection of lysolecithin, but were in good agreement with the data of Akino et al. (1972) by the intravenous injection of lysolecithin. The higher incorporation into dienoic species and the lower incorporation into tetraenoic species in intestine than in liver, might be ascribed to the contribution of bile lecithin for the lecithin species formed in intestine, especially in intestinal epithelial layer, and also to the higher utilization of arachidonic acid in liver than in intestine, in view of the much higher activity of Δ5-desaturase and Δ6-desaturase in liver (Brenner 1971).

References