Lipid Synthesis by Dog and Rabbit Lung
Relation to the Pulmonary Surfactants

Iwao MORI *
Second Department of Internal Medicine,
Nagasaki University School of Medicine,
Nagasaki, Japan

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Lipid metabolism in the lung from mongrel dogs and white rabbits was investigated in this study: I) incorporation of acetate–1–14C into lipids of lung tissue and/or lung washing extracts from healthy dogs or rabbits, II) incorporation of acetate–1–14C into lipids of embolized lung tissue from dogs, III) incorporation of CDP choline–1, 2–14C into lipids of lung tissue and/or lung washing extracts from healthy dogs and rabbits, IV) incorporation of CDP choline–1, 2–14C into lipids of pneumonic lung tissue from rabbits. For comparison, similar study was performed in the liver.

The present study shows that 1) the lung is an active metabolic unit, 2) the pattern of incorporation of acetate and CDP-choline into the lung differs from that into the liver, 3) the lung tissue from dogs and rabbits incorporates acetate and CDP-choline into lecithin, an important component of pulmonary surfactants, 4) KENNEDY pathway is very active in the lung, 5) the lung tissue lecithin is secreted as surfactant lecithin, 6) the production of pulmonary surfactants in the lung is dependent upon adequate pulmonary circulation, 7) the impairment of pulmonary phospholipid metabolism is one of the cause which lowers pulmonary surface tension in pneumonia, and 8) there is a close relation between the lipid metabolism of the lung and pulmonary surfactants.

INTRODUCTION

By using isotope labeled fatty acids the absorption pathway of lipids was clarified and it was shown that the fatty acids with less than 10 carbons mainly get into the portal vein and the high class

The following abbreviations are used in this paper: P.L., phospholipids; P. C., phosphatidyl choline (lecithin); P. E., phosphatidyl ethanolamine; LPC., lyso-phosphatidyl choline; Sph., sphingomyelin; FFA., free fatty acids; TG., triglycerides; C. F., free cholesterol; C.E., cholesterol esters; CDP-choline, cytidine diphosphate choline.
fatty acids with more than 12 carbons, especially more than 16 carbons, mostly passed through the thoracic duct\textsuperscript{12,29,90}. Namely the lung is the first substantial organ for the fats to pass through. These physico-anatomical characteristics suggest that the lung has a special relation to lipid metabolism. Concerning the lipid metabolism of lung, Sieber\textsuperscript{3} and Saxl\textsuperscript{4}, in 1908, reported for the first time that the lung had a function to take up and hydrolyze triglycerides of the circulating blood.

On the other hand von Neergaard\textsuperscript{5}, in 1929, was the first to demonstrate that the surface forces contributed significantly to the retractive pressure of the lung and in 1955, Pattle\textsuperscript{6} was the first to demonstrate that mammalian lung contained a potent surface active material. The dipalmitoyl lecithin was first isolated from the lung homogenate in 1946\textsuperscript{7} and Veerkamp\textsuperscript{8} studied that phospholipids showed some tissue specificity, i.e., in all lungs the amount of palmitate was remarkably high. It was shown that the dipalmitoyl lecithin was an essential component of the pulmonary surfactants\textsuperscript{9,20,47,89,94,98}, which lowered alveolar surface tension, and the surfactants were produced in alveolar cells (Type II cells\textsuperscript{11,12}) or Clara cell\textsuperscript{13}.

Mammalian lung rapidly incorporates labeled acetate\textsuperscript{14,31,41,42,43,44,50,54,55} or palmitate\textsuperscript{16,17,18,19,49,50,51,54,58,60,87,88} into phospholipids, and the pulmonary surfactants is a lipoprotein complex\textsuperscript{15} of which 74 per cent is phospholipids.\textsuperscript{9}

Lipid metabolism in the lung from mongrel dogs and white rabbits with relation to pulmonary surfactants was investigated in this study: I) incorporation of acetate into lipids of lung tissue and/or lung washing extracts from healthy dogs or rabbits, II) incorporation of acetate into lipids of embolized lung tissue from dogs, III) incorporation of CDP-choline into lipids of lung tissue and/or lung washing extracts from healthy dogs and rabbits, IV) incorporation of CDP-choline into lipids of pneumonic lung tissue from rabbits. For comparison, in part, similar study was carried out in the liver.

**MATERIALS AND METHODS**

A) Treatment of Animals

(1) Healthy mongrel dogs: Dogs weighing from 8 to 15 Kg were fed with regular stock diet and used after fasting for 24 hours.

(II) Healthy white rabbits: Rabbits weighing from 2.0 to 3.5 Kg were fed with regular stock diet and used after fasting for 24 hours.

(III) Dogs with a pulmonary embolus: They were produced by introducing a clot, which was made from Blood, BaSO\textsubscript{4}, Thrombin and CaCl\textsubscript{2}, into external cervical vein, and after a embolus was revealed by X-ray of the chest and pulmonary angiography, they were
used at 6 and 24 hours; This is a modified Just-Viera's method\textsuperscript{78} by Shibata\textsuperscript{30}.

(IV) Rabbits with pneumonia: They were produced by introducing Diplococcus pneumoniae intratracheally, and pneumonia was microscopically confirmed.

B) Experiments in vitro

Animals were anesthetized with sodium pentobarbital (25–30 mg/Kg) intravenously and sacrificed by exanguination. The lung and/or the liver specimens were removed, and immediately rinsed in ice-cold Krebs-Ringer bicarbonate buffer (KRB buffer)\textsuperscript{79} (pH 7.4) to remove blood.

Tissue slices were made with the aid of a Stadie-Riggs tissue slicer, lightly pressed on a filter paper and weighed. Slices (500 mg wet weight) were added to the prepared incubation flask. These processes were performed within 20 minutes.

The incubations were carried out at 38°C. In the experiment of acetate, the main compartment of each flask contained 50 \( \mu \) moles of D-glucose, 10 \( \mu \) moles of sodium glutamate, 4 \( \mu \)C of sodium acetate–1–\( ^{14} \)C (Specific activity 4 \( \mu \)C/10 \( \mu \) moles) and 5 ml KRB buffer. In the experiment of CDP-choline, the main compartment of each flask contained 2 \( \mu \)C of CDP choline–1, 2–\( ^{14} \)C (Specific activity 10 \( \mu \)C/1 mg or 25 \( \mu \)C/1 mg) and 5 ml KRB buffer. The gas phase was air. The flasks were agitated in a water bath shaker at 60 cycles/min. during the incubation period.

After the incubations were carried out, tissue and medium were homogenized by glass homogenizers. Extraction of lipids was performed according to the method of FOLCH, LEES and SLOANE-STANLEY\textsuperscript{80}. Total lipids were measured by the gravimetric method.

Triglycerides were measured by the method of VAN-HANDEL and ZILVERSMITH\textsuperscript{82}, phosphorus by Allen's method\textsuperscript{81}, total cholesterol by the method of ZAK\textsuperscript{83} and HENLY\textsuperscript{84} and free fatty acid by the method of ITAYA and U\textsubscript{1}\textsuperscript{85}.

In a part of sample, phospholipids were fractionated by thin layer chromatography and phosphorus contents of their subfractions were determined by the micro-quantitative method of NOJIMA\textsuperscript{86}.

In some aliquot, lipids were separated on columns of using 4 g silicic acid for about 10 mg lipid according to BORGSTROM\textsuperscript{32}. Neutral lipids, cholesterol and free fatty acids were eluted with 150 ml chloroform and phospholipids with 150 ml methanol, and then non-phospholipids and phospholipids were separated into subfractions by thin layer chromatography\textsuperscript{76,77}.

The subfractions on thin layer plates were scraped off with a spatula, and then extracted with 50 ml of chloroform-methanol mixture (1:1) and 50 ml of methanol. From a part of this elutions, lipid
contents and radioactivity were measured to obtain the specific activity. The radioactivity was determined by means of Packard Model 3324 Tri-Carb Liquid Scintillation Spectrometer after they were dissolved in 10 ml solution containing 0.4 g of 1, 4-di[(4-methyl-5-phenyl-oxazolyl)benezene and 4 g of 2,5-diphenyl oxazole per liter of toluene.

C) Experiments in vivo

Healthy rabbits were injected with 100 μC of sodium acetate-1-14C (Specific activity 4 μC/10 μ moles) or 100 μC of CDP choline-1, 2-14C (Specific activity 200 μC/13 mg) intravenously. In the former, at 10, 20, 60 and 120 minutes, and in the latter, at 10 minutes, 6, 12 and 24 hours, they were anesthetized with sodium pentobarbital intravenously and sacrificed by exanguination. The lung and the liver were removed and were perfused with physiological salt solution via either the pulmonary artery or the portal vein to eliminate blood constituents sufficiently. Lipids of lung and liver tissue were extracted from 1 g of wet tissue. Lung washing extracts were gained by washing the lung with 50 ml of physiological salt solution through the trachea233,235. Recovered washing extracts were about 40 ml and were instilled into the teflon trough of the modified Wilhelmy Balance for the measurement of surface tension. Then, surface area was continuously compressed to one-fifth of its area and re-expanded at the rate of 1 cycle every 2.5 minutes after the first surface aging for 30 minutes. A determination was completed when the two successive surface-area diagrams were accomplished and recorded on an X-Y recorder. The stability index was calculated according to the formula of Clements and colleagues10 as follows: Stability Index = 2 (r max − r min) / (r max + r min), where r = Surface Tension. Lung washing extracts in the teflon trough was decanted completely into a flask for lyophilization after the measurement of surface tension and then their lipids were extracted. Lipid analysis and their radioactivity were described above.

RESULTS

A) Lipid Analysis of Lung and Liver from Healthy Dogs and Rabbits

Table I shows the lipid composition of the lung and the liver from dogs and rabbits. The total lipid contents were slided in scale to 100 per cent. Phospholipids occupied 63−71 per cent of total lipids, cholesterol 22−35 per cent, triglycerides 2−12 per cent. The amount of phospholipids and triglycerides was more in the liver than in the lung. Cholesterol contents were more in the lung than in the liver.
The amount of phospholipids in the lung was not significantly different between dogs and rabbits. Lung from rabbits contained more cholesterol than that from dogs. The amount of triglycerides was occupied more in dog lung than in rabbit lung. Phospholipids and cholesterol contents were more in rabbit liver than in dog liver. The amount of triglycerides was occupied more in dog liver than in rabbit liver. Phospholipids were separated into five subfractions: lecithin, phosphatidyl ethanolamine, sphingomyelin, lyssolecithin and unknown. Table 2 shows the proportion of phospholipid subfractions of the lung and the liver from healthy dogs. P.C. occupied 58–60 per cent of phospholipids, P.E. 22–26 per cent, Sph. 8–15 per cent, LPC. 2–4 per cent and unknown 1–5 per cent. The contents of P.C. were not significantly different between the lung and the liver. The amount of Sph. was occupied more in the lung than in the liver. The other contents were more in the liver than in the lung.

B) Incorporation of Acetate–$1^{-14}$C into Lung and Liver Slices from Healthy Dogs and Rabbits

i) Incorporation of Acetate–$1^{-14}$C into Total Lipids

Figure 1 shows the time course of incorporation of acetate–$1^{-14}$C into total lipids in lung and liver slices from a healthy dog. The radioactivity of the lung and the liver increased continuously throughout the incubation period. Incorporation of acetate into total lipids

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Lipid Composition of Lung and Liver (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Dog</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>62.9±1.9</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>7.5±1.4</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>29.6±1.8</td>
</tr>
<tr>
<td>Total Amounts</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are means of 5 animals±standard deviation

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Phospholipid Subfraction of Lung and Liver (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P.C.</td>
</tr>
<tr>
<td>Lung</td>
<td>59.7±4.3</td>
</tr>
<tr>
<td>Liver</td>
<td>57.5±1.8</td>
</tr>
</tbody>
</table>

Values are means of 5 dogs±standard deviation
was more active in the lung than in the liver. When the incubation was carried out till 12 hours in another experiment, the radioactivity increased continuously.

ii) Specific Activity of Phospholipids

Figure 2 shows the time course of specific activity of phospholipids

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**Fig. 1.** Incorporation of acetate-1-14C into total lipids in lung and liver slices (500 mg wet wt) from a dog. ○-○ Lung, Δ-Δ Liver. The data are based on the average of duplicate determinations.

**Fig. 2.** Specific activity of phospholipids in lung and liver from a dog and rabbit. (Acetate-1-14C was used) ○-○ Dog Lung, Δ-Δ Dog Liver, ●-● Rabbit Lung, ▲-▲ Rabbit Liver
in the lung and the liver from a dog and rabbit. The specific activity of phospholipids increased continuously throughout the incubation period. It was higher in the lung than in the liver. In rabbit liver it was the lowest. When it was studied till 12 hours in another experiment, it increased continuously.

iii) Specific Activity of Phospholipid Subfractions

The time course of specific activity of phospholipid subfractions in the lung and the liver from a dog and rabbit is illustrated in Figure 3. The specific activity of phospholipid subfractions increased during the incubation time. The specific activity of lung lecithin was higher than that of liver lecithin. In the lung, the specific activity of lecithin was higher than that of phosphatidyl ethanolamine. But in the liver there was no significant difference in the specific activity between lecithin and phosphatidyl ethanolamine. When the specific activity of lecithin was studied till 12 hours in another experiment, it increased continuously.

iv) Specific Activity of Non-phospholipid Subfractions

Non-phospholipids were separated into four fractions; triglycerides, free fatty acid, free cholesterol and cholesterol ester. Figure 4 shows the time course of specific activity of non-phospholipid subfractions in the lung and the liver from a dog and rabbit. The specific activity of non-phospholipid subfractions increased throughout the incubation period.

![Figure 3](image_url)

Fig. 3. Specific activity of phospholipid subfractions in lung and liver from a dog and rabbit. (Acetate-1-14C was used) ○−○ Dog Lung, △−△ Dog Liver, ●−● Rabbit Lung, ▲−▲ Rabbit Liver.
Fig. 4. Specific activity of non-phospholipid subfractions in lung and liver from a dog and rabbit. (Acetate-1-\(^{14}\)C was used) ○ ○ Dog Lung, △ △ Dog Liver, ● ● Rabbit Lung, ▲ ▲ Rabbit Liver.

Fig. 5. Specific activity of phospholipid subfraction in lung slices from a dog. (CDP choline-1, 2-\(^{14}\)C was used) ● ● P.C., × × LPC, ○ ○ Sph, ▲ ▲ Unknown, ■ ■ P.E.
period. TG and FFA was higher in the lung than in the liver. In the lung TG and FFA was higher in the specific activity than C.E. and C.F. In the liver, there was no significant difference as in the lung.

C) Incorporation of CDP choline–1, 2–¹⁴C into Lung Slices from a Healthy Dog and Rabbit

The time course of specific activity of phospholipid subfractions in lung slices from a dog is illustrated in Figure 5. The specific activity of lecithin was the highest during the incubation period. LPC was relatively high in the specific activity. The other fractions were very low in the activity throughout the incubation period. Figure 6 and Table 3 show the time course of specific activity of lecithin and

![Graph showing specific activity of phospholipid subfractions in lung slices from a dog.](image)

Fig. 6. Specific activity of lecithin and total phospholipids in lung and liver slice from a rabbit. (CDP choline–1, 2–¹⁴C was used) O–O Lung, Δ–Δ Liver. The data are based on the average of duplicate determinations.

<table>
<thead>
<tr>
<th>Time (min/h)</th>
<th>Lung (c.p.m/µg P)</th>
<th>Liver (c.p.m/µg P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10. min.</td>
<td>82</td>
<td>35</td>
</tr>
<tr>
<td>3 hr.</td>
<td>533</td>
<td>143</td>
</tr>
<tr>
<td>6 hr.</td>
<td>775</td>
<td>143</td>
</tr>
<tr>
<td>12 hr.</td>
<td>821</td>
<td>249</td>
</tr>
<tr>
<td>24 hr.</td>
<td>699</td>
<td>227</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (min/h)</th>
<th>Lung (c.p.m/µg P)</th>
<th>Liver (c.p.m/µg P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10. min.</td>
<td>145</td>
<td>49</td>
</tr>
<tr>
<td>3 hr.</td>
<td>897</td>
<td>199</td>
</tr>
<tr>
<td>6 hr.</td>
<td>1287</td>
<td>217</td>
</tr>
<tr>
<td>12 hr.</td>
<td>1347</td>
<td>246</td>
</tr>
<tr>
<td>24 hr.</td>
<td>1283</td>
<td>186</td>
</tr>
</tbody>
</table>

Specific activity is pressed as c.p.m per µg phosphorus.
The data are based on the average of duplicate determinations.
total phospholipids in lung and liver slice from a rabbit. The specific activity of lecithin and total phospholipids arrived at the peak at 12 hours. It was higher in the lung than in the liver throughout the incubation period. In the lung, the specific activity of lecithin was higher than that of total phospholipids. As lecithin is the main part of phospholipids, it is thought that this high specific activity of lecithin means the low specific activity of other phospholipid subfractions. In the liver, such a phenomenon was not observed.

D) Incorporation of Acetate−1−14C into Lung Tissue, Lung Washing Extracts and Liver from Healthy Rabbits after its Intravenous Administration

i) Distribution of Radioactivity between Phospholipids and Non-phospholipids

Figure 7 shows the distribution of the radioactivity between phospholipids and non-phospholipids in the lung and the liver from rabbits after intravenous administration of acetate−1−14C. The distribution of the radioactivity was different in the lung and in the liver. In the lung the radioactivity was predominantly recovered in phospholipids. The radioactivity of phospholipids occupied 82.8, 91.9 and 87.4 per cent at 10, 60 and 120 minutes, respectively. In the liver the radioactivity of non-phospholipids was higher than that of phospholipids. Namely acetate was rapidly preferentially incorporated into non-phospholipids. The radioactivity of phospholipids occupied 28.6, 42.4, 37.8 and 48.7 per cent at 10, 20, 60 and 120 minutes, respectively.

ii) Radioactivity of Total Lipids

Figure 8 shows the radioactivity of total lipids in lung tissue,
lung washing extracts and liver. Acetate was incorporated more avidly into lung tissue than into liver and lung washing extracts. The radioactivity of lung tissue was the highest at 10 minutes and then decreased till 60 minutes. Incorporation of acetate into liver and lung washing extracts increased gradually till 120 minutes.

Fig. 8. Radioactivity of total lipids in lung tissue, lung washing extracts and liver from rabbits after the intravenous injection of acetate–1–14C. ○–○ Lung (1 g wet wt) ●–● Liver (1 g wet wt) △–△ All washing extracts.

Fig. 9. Specific activity of phospholipids in lung tissue and liver from rabbits after the intravenous injection of acetate–1–14C. ○–○ Lung, △–△ Liver.
iii) Specific Activity of Phospholipids in Lung Tissue and Liver

The specific activity of phospholipids in lung tissue and liver was illustrated in Figure 9. In lung tissue the specific activity of phospholipids was the highest at 10 minutes and then it decreased. On the other hand, it was low in the liver during all course.

iv) Specific Activity of Lecithin in Lung Tissue, Lung Washing Extracts and Liver

Fig. 10. Specific activity of lecithin in lung tissue, lung washing extracts and liver from rabbits after the intravenous injection of acetate-$1^{-14}$C. ○–○ Lung Tissue, ●–● Lung Washing Extracts, △–△ Liver.

Fig. 11. Specific activity of total phospholipids and lecithin in lung tissue, lung washing extracts and liver from rabbits after the intravenous injection of CDP choline-$1,2^{-14}$C. ○–○ Lung Tissue, ●–● Liver, △–△ Lung Washing Extracts.
Figure 10 shows the specific activity of lecithin in lung tissue, lung washing extracts and liver. It was higher in lung tissue than in lung washing extracts and liver. Until 60 minutes it decreased in lung tissue, and increased gradually in lung washing extracts and liver till 120 minutes. On the other hand, the specific activity of phosphatidyl ethanolamine was low.

v) Surface Tension

The maximal and minimal surface tension of lung washing extracts were 34–38 dynes/cm and 2–2.5 dynes/cm. The stability indices were 1.73–1.80. They were normal value in our department\(^489\).

E) Incorporation of CDP choline–1, 2\(^{-14}\)C into Lung Tissue, Lung Washing Extracts and Liver from Healthy Rabbits after its Intravenous Administration

The specific activity of total phospholipids and lecithin in lung tissue, lung washing extracts and liver was illustrated in Figure 11. The peak was at 6 hours in lung tissue and at 12 hours in the liver. In lung washing extracts the specific activity increased continuously till 24 hours. It was higher in lung tissue than in liver. The specific activity of lung washing extracts and lung tissue crossed between 6 and 12 hours, and thereafter the former was higher than the latter. The specific activity of lecithin in each time was higher than that of

Fig. 12. Incorporation of CDP choline–1, 2\(^{-14}\)C into pneumonic lung slices from rabbits. □ Healthy Rabbits, □ Pneumonia. Incubation time: 3 hours
total phospholipids. The maximal and minimal surface tension were 32–49 dynes/cm and 2–5 dynes/cm. The stability indices were 1.53–1.81. They were normal value in our department.⁴⁸)

F) Incorporation of CDP choline−1, 2−¹⁴C into Pneumonic Lung Slices from Rabbits

Figure 12 and Table 4 show the incorporation of CDP choline−1, 2−¹⁴C into pneumonic lung slices. The radioactivity of total lipids was 126429 cpm in control group and 33293 cpm in pneumonic lung. The specific activity of total phospholipids was 404 cpm/μg phosphorus in normal lung and 117 cpm/μg phosphorus in diseased lung. The specific activity of lecithin was 762 cpm/μg phosphorus in control part.

Fig. 13. Lipid composition of embolized lung from dogs. □ P.L., ■ Cholesterol, □ TG.

<table>
<thead>
<tr>
<th></th>
<th>Radioactivity of Total Lipids</th>
<th>Specific Activity of P.L.</th>
<th>Specific Activity of P.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>126429±21573</td>
<td>404±87</td>
<td>762±186</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>33293±14866</td>
<td>117±56</td>
<td>223±87</td>
</tr>
</tbody>
</table>

Values are means of 5 rabbits±standard deviation.
Radioactivity is expressed as cpm 500 mg of wet lung tissue. Specific activity is expressed cpm per μg phosphorus.
and 223 cpm/μg phosphorus in pneumonic part. Incorporation of CDP choline−1, 2−14C into pneumonic lung was very low. Namely there was a significant difference between pneumonic lung and control lung.

G) Incorporation of Acetate−1−14C into Embolized Lung from Dogs

i) Lipid Analysis of Embolized Lung from Dogs

Figure 13 shows the lipid composition. Total lipid contents were slid in scale to 100 per cent. Phospholipids were the main part of total lipids. Triglycerides were the lowest of all. Phospholipid contents were more in embolized lung than in control lung. Cholesterol and triglycerides occupied more in control lung than in embolized lung.

ii) Specific Activity of Phospholipids

In phospholipid synthesis, the ratio of embolized lobe to normal lobe in each dog was used. Figure 14 shows the ratio of specific activity of phospholipids from embolized lobe to that from normal lobe in each dog. Incorporation of acetate−1−14C into embolized lobe phospholipids dropped to 65.0 per cent of control lobe at 6 hours and to 71.5 per cent at 24 hours.

iii) Incorporation of Acetate−1−14C Injected Intravenously into Embolized Lung

At 22 hours after pulmonary embolization, a dog were injected with 500 μC of acetate−1−14C intravenously and then at 2 hours it was sacrificed by exanguination. Total radioactivity of lung washing extracts of normal and embolized lobe were 455 cpm and 165 cpm, respectively.
DISCUSSION

In lipid composition of the lung and the liver from healthy dogs and rabbits, phospholipids occupied more parts of lipids than cholesterol and triglycerides. Namely they occupied from 63 to 71 per cent of total lipids. Phospholipids contents of the liver were more than that of the lung. The amount of phospholipids in the lung was not significantly different between in dogs and rabbits. Lecithin was a major component of phospholipid subfractions in the lung and the liver from healthy dogs and occupied from 58 to 60 per cent of phospholipids. Percentage of lecithin in the phospholipids was not significantly different between in the lung and the liver.

Veerkamp\(^8\) showed by the analysis of mammalian tissues that the fatty acid composition of phospholipids was specific in each tissue. Namely the amount of palmitate in lung phospholipids was remarkably high. It is accepted that dipalmitoyl lecithin is an essential component of the surface active substance which lowers alveolar surface tension\(^9\)\(^{20}\).

It has been argued whether the surfactants exist in the non-mammalian lung. Miller\(^{21}\), Klaus\(^{22}\), Harlan\(^{23}\) and Tyler\(^{24}\) reported that the surfactants do not exist in the non-mammalian lung, and Pattle\(^{25}\)\(^{39}\), Fujiwara\(^{26}\) and Lambson\(^{27}\) reported that the surfactants exist in the non-mammalian lung.

In considering the mechanism involved in the absorption of lipids, most of the injected lipids pass through the lung by the way of the thoracic duct. By using isotope labeled fatty acids, it was shown that the fatty acids with less than 10 carbons mainly get into the portal vein and the high class fatty acids mostly passed through the thoracic duct\(^1\)\(^{22}\).

Lipoprotein lipase was demonstrated in the lung\(^{28}\)\(^{29}\)\(^{37}\) and this enzyme catalyses the hydrolysis of the triglyceride moiety of chylomicrons and low density lipoproteins, and then the lung utilizes free fatty acids for producing surfactants. Namely the thoracic duct is the pathway by which the high class fatty acids is transported to the lung producing surfactants. Therefore it may be need to study about the relationship between the surfactants and the thoracic duct.

The mammalian lung rapidly incorporates labeled acetate into phospholipids\(^1\(^{43}\)\(^{44}\)\(^{45}\)\(^{46}\)\(^{50}\)\(^{54}\)\(^{55}\). In this experiment in vitro, it was shown that lung slices from dogs and rabbits could incorporate acetate into phospholipids and non-phospholipids, and CDP-choline into phospholipids, especially lecithin. The data do not reflect quantitatively the rate of lipid synthesis because the accumulation of intermediates and the reutilization of the end production may also take place.

In the interval observation of the incorporation of acetate, the specific activity of lecithin was the highest in phospholipids, and triglycerides and free fatty acid were higher in the specific activity
LIPID SYNTHESIS BY DOG AND RABBIT LUNG

than free cholesterol and cholesterol ester.

CDP-choline was mostly incorporated into lecithin.

It was noteworthy in this paper that phosphatidyl ethanolamine, which was thought to be converted to lecithin by transmethylation in the lung\textsuperscript{34,35}, was lower in the specific activity throughout the incubation period than lecithin.

Lecithin was the major component of phospholipid subfractions.

This indicated that the amount of lecithin converted from phosphatidyl ethanolamine in the lung was smaller than that from the pathway as KENNEDY\textsuperscript{36,37,46}, LAND\textsuperscript{38,39} and STEIN\textsuperscript{40} reported.

KENNEDY's pathway\textsuperscript{36,37,46} was very active in the normal lung, but the incorporation of CDP-choline into pneumonic lung slices was very low as compared with normal lung slices. Namely the activity of phosphoryl choline glyceride transferase\textsuperscript{46} was low in the pneumonic lung.

KIMURA\textsuperscript{48} and the others\textsuperscript{91,92} showed that the surface activity of lung washing extracts in pneumonia diminished. It was indicated in this experiment that the impairment of pulmonary phospholipid metabolism was one of the cause which lowered the pulmonary surface tension in pneumonia.

Lipid metabolism of the lung was thought to differ in several respects from that of the other tissues\textsuperscript{50}.

This study supports the above conclusion. In rabbits given an intravenous injection of acetate the pattern of incorporation of acetate into the lung differed from that into the liver. Namely the radioactivity was predominantly recovered in phospholipid fractions of the lung, and in the liver the radioactivity of non-phospholipids was higher than that of phospholipids. The lung mitochondria actively synthesizes long chain fatty acids\textsuperscript{55}, whereas in the liver the clear supernate incorporates acetate more avidly into fatty acids\textsuperscript{56}. The lung slices catalyzes the incorporation of fatty acids into phospholipids, chiefly into lecithin. On the contrary most of fatty acids are esterified in the liver as neutral lipids, primarily as triglycerides\textsuperscript{62,63}.

Phospholipids of the liver contain mainly unsaturated fatty acids\textsuperscript{64} and those of the lung contain mainly palmitate\textsuperscript{65}. All of these differences may be correlated with the role of the lung in producing the dipalmitoyl lecith in moiety of surfactants as Salisbury-Murphy\textsuperscript{50}, Buckingham\textsuperscript{54} stated.

Prior to incorporation into the lung phospholipids, acetate must first be removed from the circulation by the capillary endothelium\textsuperscript{57}.

It is unknown how acetate is transferred from the endothelial cell to the active phospholipid synthetic site of the large alveolar cells\textsuperscript{54}.

After the intravenous administration of acetate the radioactivity of total lipids and the specific activity of lecithin in the lung tissues
from rabbits were the highest at ten minutes, and then they decreased.
On the other hand, the radioactivity of the liver and the lung washing
extracts increased gradually till 120 minutes. Lung tissue was higher
in the specific activity of lecithin than washing extracts.

After the intravenous injection of CDP-choline the specific activity
of lecithin was the highest at six hours. In lung washing extracts it
increased till 24 hours. The specific activity of lung washing extracts
and lung tissue crossed between in six and twelve hours and thereafter
the former was higher in the specific activity than the latter. Namely
this result indicated that lung tissue lecithin was secreted as lining
layer lecithin.

Scarpelli51) was the first to document the rapid appearance of
phospholipids in lining layer and he suggested that phosphatidyl etha-
nolamine of lining layer was converted to surface active lecithin.

However, it was indicated in this experiment and FuJiwarA's data
52) that the lung synthesized lecithin very actively by CDP-choline
pathway.

The turnover of phospholipids in the lung is very rapid. 14C
labeled palmitate appears in the phospholipids within two minutes after
its intravenous injection in dog59) and the half life of 14C palmitate in
the dog lung was estimated to be two hours59).

However Tierney60) showed that in the rats injected with palmitate-
1−14C and uniformly labeled glucose-U, 1−3H the biological half life
of the radioactivity in the lecithin was approximately 14 hours and
calculated biosynthetic rate of saturated lecithin was 5.7 moles/20 hr.,
or 0.3 moles/g tissue per hour. The prolonged half life of radioactivi-
ity in polyenoic lebithin containg arachidonate is more slowly metaboli-
zed in the lung than lecithin with other fatty acdis60). The half life
of lecithin within the alveolar cells is probably shorter than that of
lining layer lecithin61).

In this experiment it is suggested that the half life of lecithin
within the alveolar cells is shorter than that of the lining layer
lecithin. The pathway of degeneration and removal of component of
the surfactants system are poorly understood.

The clinical, pathological and physiological consequences following
acute pulmonary emboli are yet poorly understood. Studies in dogs
have demonstrated a decrease of surface activity in the ischemic lung
tissues56)67)68)70)71). It is supported that interruption of the pulmonary
circulation cause a decrease in metabolic activity of surfactants72)73)74)
75)87)88).

It was shown by Shibata30) that up to six hours after embolisation
the minimal surface tension of the embolized lung was the the same
value as that of normal lung and that at 12 and 24 hours it was higher
than that of normal lung, i.e., the surface activity decreased. The
resumption of blood flow was confirmed by the method of pulmonary
angiography at 24 hours. At 48 hours the minimal surface tension was normal in value. In this study the specific activity of phospholipid dropped to 65 per cent of control lung at six hours and 71.5 per cent at 24 hours.

Namely the change of the lipid metabolism in the lung proceeded to the change of surface tension. This is evidence that the production of pulmonary surfactants is dependent upon an adequate pulmonary circulation and that the half life of the lining layer lecithin is longer than that of lung tissue lecithin.

The measurement of the radioactivity in the lung washing extracts from the embolized dog must make more clear the relation between the surface tension, the lipid metabolism of lung tissue and the surfactant metabolism.

SUTNICK showed that the synthesis of phospholipid dropped to 69.5 per cent of normal at 4 to 6 hours after embolization and that the surface tension remained relatively normal up to 6 hours and then decreased in 7 hours or longer.

In SUTNICK's experiment palmitate—$^{14}$C was used. On the other hand acetate—$^{14}$C was used in this study and the result was agreed with SUTNICK's data.

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