Arch Jap Chir 48 (5), 571～586, Sept., 1979

原　　著

Experimental Study on the Effect of Essential Fatty Acid Deficiency on Pulmonary Surfactant

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Received for Publication, June 22, 1979

Introduction

Studies in this laboratory have shown that the leakage of fluid from the vascular system into extravascular spaces can be prevented by the administration of essential fatty acids (EFA)[25], which may help to reinforce the gap between capillary endothelial cells. Essential fatty acid supplementation before operation might, therefore, be effective in preventing the sludging of blood and post-operative pulmonary edema[29] and might maintain good circulatory conditions during operation. It was also found that pre-operative essential fatty acid administration prevented lethal ventricular fibrillation during surface cooling[33] by decreasing the dissociation of oxidative phosphorylation in the mitochondria. On the basis of these experimental results, a method of open heart surgery in infants with large pre-operative oral supplements of essential fatty acids and surface induced deep hypothermia combined with limited cardio-pulmonary bypass was developed[20]. This method, now utilized throughout the world and called the "Kyoto Technique"[5], has produced excellent results in the corrective surgery of infants with transposition of the great arteries, total anomalous pulmonary venus connection, tetralogy of Fallot, large VSD and other severe congenitial heart diseases[2)3)4)5)7)11)12)24)28)31)37)38)39)42)43)].

However, post-operative respiratory complications in infants are more frequent and their management is more difficult than in older children. Thus, the care of the respiratory system after the operation is a major point in the success of the operation, especially in infants. Respiratory system care after surgery consists of removal of tracheo-broncheal secretions, and prevention or treatment of pulmonary edema and atelectasis. It is considered that the last two problems are related to the function of surfactant which has been reported to be indispensable in maintaining respiratory function and normal structure. Phosphatidyl-

Key Words : Essential fatty acid deficiency, Surfactant, Minimum surface tension, Decrease of palmitic acid content, Respiratory function.
索引語：必須脂肪酸欠乏，表面活性物質，最小表面張力，パルミチン酸含有量減少，呼吸機能.
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choline is the responsible surface active substance. In this study the influence of essential fatty acids on respiratory function was examined, since it was suspected that disturbances of lipid metabolism, such as essential fatty acid deficiency, might influence surfactant function and cause difficulties in the management of respiratory problems after operation.

Experimental procedures

Materials

Two month-old male Wistar rats were supplied by the Animal Center, Laboratory of Kyoto University, Kyoto. Rats in the control group were fed Standard rat chow (Oriental Co.). Those in the essential fatty acid deficient group were a diet containing 50% glucose, 20% milkcasein, 20% butter (Yukijirushi Nyugyo Co.), 5% salt mixture, 3.5% carboxymethyl cellulose sodium salt, 1% Panvitan Vitamins (Takeda Seiyaku Co.), and 0.5% choline chloride in solid form[41]. This essential fatty acid (EFA) deficient diet contained a small amount of EFA; the reason for using this diet is explained later. Glass plates were coated with 0.25mm of Silica-gel H (Merk, Darmstadt, West Germany) for thin layer chromatography (TLC). Diethylene glycol succinate (DEGS) in 10% solution was coated on chromosorb W (acid washed) 60-80 mesh (Wako Chem. Co.) and packed into a 2 m× 3 mm glass column for gas-liquid chromatography (GLC). Other chemical reagents (Nakarai Chem. Co., Kyoto) were of guaranteed chemical grade.

Apparatus

A gas-liquid chromatography, Type GC-4BM with flame ionization detector and digital integrater (Shimadzu Seisakushyo Co., Kyoto) was used for fatty acid analysis. A modified Wilhelmy balance (Acoma Medical Industry Co., Osaka) was used to measure surface tension. For the photometric measurement of phospholipids, a Model 111 spectrophotometer (Hitachi Co., Tokyo) was used. To measure pressure, a pressure transducer Model P23-BB (Statham Lab. Inc., Hato Rey, Puerto Rico) and an electrical amplifier (Model PMP-3004, Nihon Koden Co., Tokyo) were used. An electric X-Y recorder, Model BW-133 (Rikadenki Kogyo Co., Tokyo) was used.

Methods

Preparation of surfactant and phospholipids. Rats were divided into two groups. The control group was given tap water ad lib and standard rat food for two months. The other was fed with EFA deficient diet for the same period. They were then anesthetized with sodium pentobarbital (Pitman-Moore Inc.), perfused with physiological saline at approximate 20 cm H_2O pressure via the inferior vena cava as blood was washed from the aorta. Immediately the lungs were resected en block, and the large vessels and the main bronchi were removed. After weighing, the lung was minced into approximately 1 × 1 mm pieces with scissors. Physiological saline buffer with 0.01M Tris-HCl (pH 7.0), and containing 1 mM (EDTA) as homogenizing medium was added to the minced lung, 10 ml for each gram of tissue. The suspension of minced lung was placed under alternating negative and positive

pressure to exchange alveolar air with saline and stirred with a magnetic stirrer for 1.5 hours. The suspension was centrifuged at 130 G for five minutes; the supernatant contained alveolar surfactant and the precipitate tissue fragments. The supernatant was centrifuged by the 0.25M and 0.68M sucrose density gradient method at 10,000 G for one hour, as described by FROSOLONO et al.[16], and we defined the intermediate layer between these two sucrose layers as alveolar surfactant. The surface tension of this alveolar surfactant was measured with a Wilhelmy balance and lipids were extracted by the method of GODISH and RHOADES[18]. The liver was also excised and weighed and the lipids extracted by the method of FOLCH et al.[15] Lung tissue fragments were homogenized in a Teflon homogenizer and the cellular lipids extracted by the FOLCH's method.

Lipid analysis. The lipids extracted from surfactant or lung and liver tissues were divided into each component by thin layer chromatography. Development was carried out with chroloform-methanol-water (65:25:4) for phospholipid analysis. The spots were detected under ultraviolet light after spraying with Rodamin G or charring the plate at 180°C for 30 minutes after spraying with 50% H_2SO_4. For the quantitative analysis of phospholipids, the charred spots with H_2SO_4 were scraped and measured photometrically as inorganic phosphate by the method described by KAHOVKOVA et al.[23] For the qualitative and quantitative analysis of fatty acids in phosphatidylcholine, the scraped spots were methanolized with BF_3-methanol as reported by MORRISON et al.[27] and analyzed by gas-liquid chromatography under the following conditions : column temperature, 185°C ; N_2 flow rate, 35 ml/min; 10% DEGS on chromosorb W, 60-80 mesh packed into a 2m×3mm glass column. Fatty acid peaks on chromatograms were identified quantitatively by comparing peak areas after correction, as described by ETTRE et al.[14] In all comparisons, Student's t test was used to determine the significance of the differences between the two groups.

Surface tention-area diagram. Surfactant isolated by the density gradient method was suspended in physiological saline at a concentration of 1 ml/g, and 3 ml of this solution was gently placed on the surface of 50 ml of physiological saline in a Teflon bath equipped with a movable barrier which could compress the surface area from 100% to 20% with approximate 3 min/cycle at room temperature. Five, 15, 30 60 and 120 minutes after start, surface tension-area diagram were drawn on a chart with an X-Y recorder and the γ min, γ max and stability index were calculated from the results under the assumption that the surface tension of saline is 72 dyn/cm[30].

Pressure-volume curve. Rats were anesthetized with sodium pentobarbital and their lungs were removed immediately. The lung was forced to expand with positive air pressure which ranged from 0 mm H_2O to 30 mm H_2O, since higher pressures often caused the lung was rupture. Pressure and volume, which were measured with a potentiometer connected to a volumetric syringe, were monitored electrically and recorded with an X-Y recorder after amplification. In addition, lungs filled with saline were maintained in physiological saline and connected to a manometer and resorvoir. The pressure-volume relationship was determined by plotting at pressure increments of 1 cm H_2O.

Analysis of fatty acid composition in total lipids of diet.

Diets were ground to a powder, and the lipids were extracted by the method of FOLCH et al. The lipids extracted were hydrolysed with 4% KOH ethanol solution at 60°C in a water bath for 30 minutes in a slight modification of BLOMHOFF's method[6]. Then the solution was acidified to pH 1.0 with 0.1N H_2SO_4, and the fatty acids were extracted with ethyl ether. As described above, fatty acids were analyzed by gas-liquid chromatography after methanolysis.

Measurement of elastin and collagen in the lung.

The lung was removed and weighed and minced finely with scissors until each fragment was approximately 1-2 mm in size. The minced lung was dehydrated in an ethnol-ether (3:1) solution for two hours and then with acetone three times for 30 minutes each time. The elastin content of the dehydrated and defatted lung tissue was measured gravimetrically according to the method reported by LANSING et al.[26] after digestion with 0.1N NaOH. The collagen content of the lung tissue in the solution was measured colorimetrically as hydroxyproline according to the method reported by WOESSNER[46] after hydrolization with 6N HCl at 105-110°C for 48 hours as reported by WOLINSKY[47]. The collagen content was calculated by multiplication of a coefficient deduced from the number of hydroxyproline residues in a collagen molecule.

Results

Fatty acid analysis of control and EFA deficient diets.

The results were shown in Table 1. In the control diet nearly 50% of the total fatty

Table 1. Fatty Abid Component of Total Lipids in Experimental and Control Diet

Fatty Acid Moiety	Control Diet (mol %)	Exp. Diet (mol %)
C10 : 0	n. d.	2.89
C12 : 0	0.12	4.68
C14 : 0	0.79	15.26
C14 : 1	n. d.	2.13
C14 : 2	n. d.	1.51
C16 : 0	16.56	33.26
C16 : 1	1.24	2.44
C16 : 2	n. d.	0.85
C18 : 0	1.79	9.82
C18 : 1	21.42	23.88
C18 : 2	48.87	2.25
C18 : 3	3.22	0.63
C20 : 0	n. d.	0.41
C20 : 1	1.52	n. d.
C20 : 4	2.04	n. d.
C22 : 1	0.82	n. d.
C22 : 6	1.63	n. d.

* n. d. not detected.

acids were linoleic and arachidonic acids, and in the EFA deficient diet only 2% of the total fatty acids were linoleic acid.

Effect of essential fatty acid deficient diet.

Rats fed the EFA deficient diet developed the following within two months : scaliness of the skin and dandruff and ridged tail or scaly tail, as already reported by BURR and BURR[8)9)] in 1929. As shown in Figure 1, the body weight of rats fed the FEA deficient

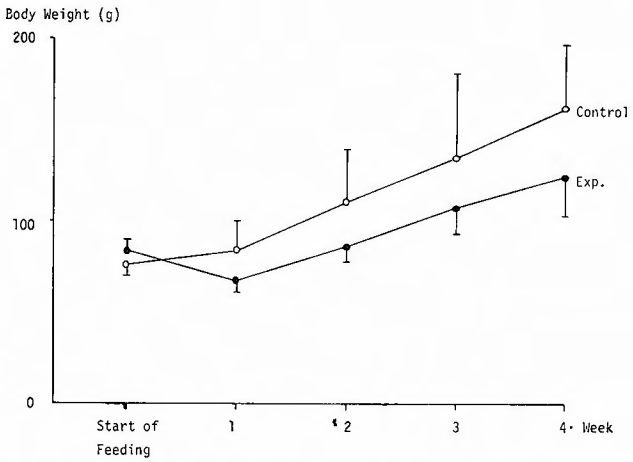


Fig. 1. Body Weight Change during Feeding of Experimental and Control Diets.
* Each value is calculated from 5 animals as mean±standard deviation.

diet decreased during the first week and then gradually increased, but the increment was far smaller than that of the conrol group. These findings and the time course of the change in body weight of the EFA deficient group showed that the diet used in this experiment was a true EFA deficient diet. This diet was used since it seemed more realistic to discuss the influence of an EFA deficient diet than that of an EFA absent diet, since one rarely eats a totally EFA deficient diet in daily life. The organ weights of rats fed the EFA deficient diet and their ratio to body weight are summarized in Table 2. The influence of the diet appeared to be different from organ to organ. The lung was affected by the diet in both sexes. Interestingly there was a sexual difference in the effect of the EFA deficient diet on some organs, such as the gonads.

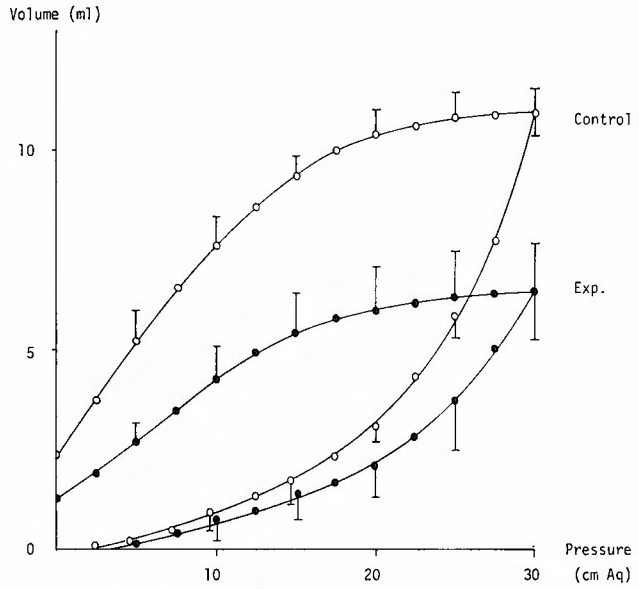
Effect on pressure-volume relationship of the lung.

The pressure-volume relationship in the air-filled lungs was examined ; the results are shown in Figure 2. The lungs of the EFA deficient group were more rigid or more difficult to expand than those of control group. Stability indices of the lung in the pressure-volume relationship, as calculated by the method of GLUENWARD[19)], were 0.818 in the control and 0.758 in the EFA deficient group. When the lung was inflated with saline instead of air, no difference in pressure-volume relationships was noted between the two groups, as shown in Figure 3.

Table 2. Organ Weights of Rats Fed Experimental Diet for 2 Months.

	Female		Male	
	Control Group	Exp. Group	Control Group	Exp. Group
Body Weight (g)	189.0±29.7	148.0±15.4	245.0±47.7	220.0±32.1
Heart (g)	0.78±0.14 (4.12±0.09)×10^{-3}	0.69±0.10 (4.71±0.76)×10^{-3}	0.95±0.10 (3.94±0.58)×10^{-3}	0.84±0.02 (3.83±0.09)×10^{-3}
Kidney (g)	1.75±0.28 (9.26±0.04)10^{-3}	1.32±0.17 (8.93±1.20)×10^{-3}	2.57±0.32 (10.65±1.90)×10^{-3}	1.78±0.12 (8.09±0.55)×10^{-3}
Lung (g)	2.31±0.91 (11.97±2.95)×10^{-3}	1.12±0.17 (7.66±1.63)×10^{-3}	2.67±0.53 (11.03±2.54)×10^{-4}	1.32±0.07 (6.02±0.32)×10^{-3}
Liver (g)	7.55±1.34 (39.90±0.85)×10^{-3}	7.31±1.07 (47.21±2.33)×10^{-3}	9.33±1.61 (38.43±6.04)×10^{-3}	8.13±0.49 (36.97±2.24)×10^{-3}
Spleen (g)	0.63±0.16 (3.31±0.30)×10^{-3}	0.33±0.06 (2.24±0.18)×10^{-3}	0.63±0.17 (2.53±0.19)×10^{-3}	0.37±0.05 (1.70±0.23)×10^{-3}
Hypophysis (mg)	9.25±1.06 (49.10±2.12)×10^{-6}	5.50±1.50 (36.80±6.52)×10^{-6}	9.00±0.86 (37.35±5.76)×10^{-6}	6.83±1.04 (31.06±4.73)×10^{-6}
Gonad (g)	70.52±0.19 (373.9±10.1)×10^{-3}	38.99±8.89 (268.8±80.6)×10^{-3}	2.73±0.24 (11.27±1.11)×10^{-3}	2.77±0.07 (12.62±0.34)×10^{-3}
Thymus (g)	0.22±0.10 (1.23±0.71)×10^{-3}	0.26±0.01 (1.87±0.18)×10^{-3}	0.33±0.14 (1.35±0.65)×10^{-3}	0.27±0.04 (1.21±0.18)×10^{-3}
Adrenal Gland (mg)	74.51±7.78 (395.9±21.0)×10^{-6}	42.33±4.19 (286.6±22.8)×10^{-6}	63.32±5.77 (261.7±24.8)×10^{-6}	37.17±2.88 (169.0±13.1)×10^{-6}

1) Each value calculated from 3 or 4 rats (mean ± standard deviation).
2) Lower row shows ratio to body weight and standard deviation.



Gruenwald Index
Control Group : 0.818 Exp. Group : 0.758

Fig. 2. Air Pressure-Volume Diagram of the Lung in Experimental and Control Rats.
* Each value is calculated from 5 animals as mean±standard deviation.

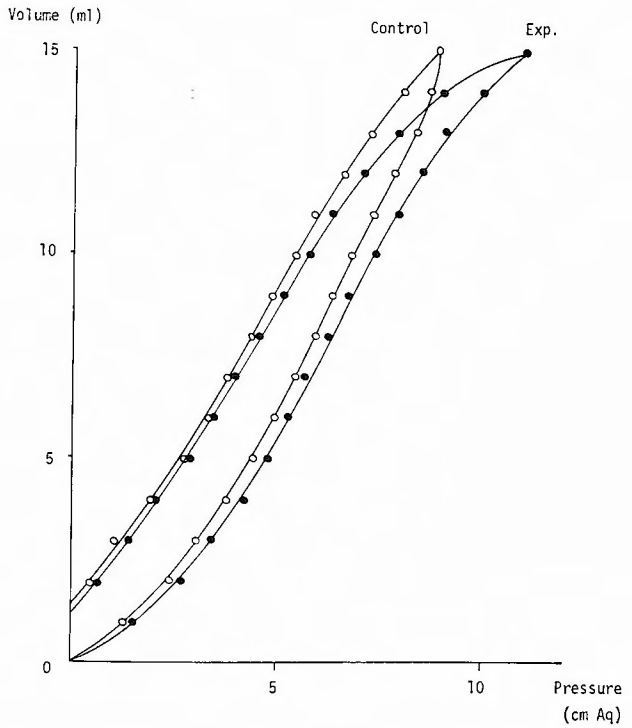


Fig. 3. Saline Pressure-Volume Diagram of the Lung in Experimental and Control Rats.
* Each value is calculated from 5 animals.

Collagen and elastin content of the lung.

There were no significant differences in dry lung weight, or relative content of collagen and elastin per gram of dry weight between the two groups, as shown in Table 3.

Table 3. Collagen and Elastin in Lungs of Experimental and Control Rats.

	Control Group	Exp. Group
Dry Lung Weight (mg)	257.8±45.0	230.3±21.5
Elastin Content (mg/g dry weight of lung)	120.0±20.9	117.7±13.2
Collagen Content (mg/g dry weight of lung)	16.9±2.4	17.3±1.5
Ratio of Elastin/Collagen	6.64±1.28	6.80±0.52

1) Values are given as mean ± standard deviation.
2) Each group contains 7 or 8 animals.
3) No statistically significant difference between the two groups ($P<0.05$).

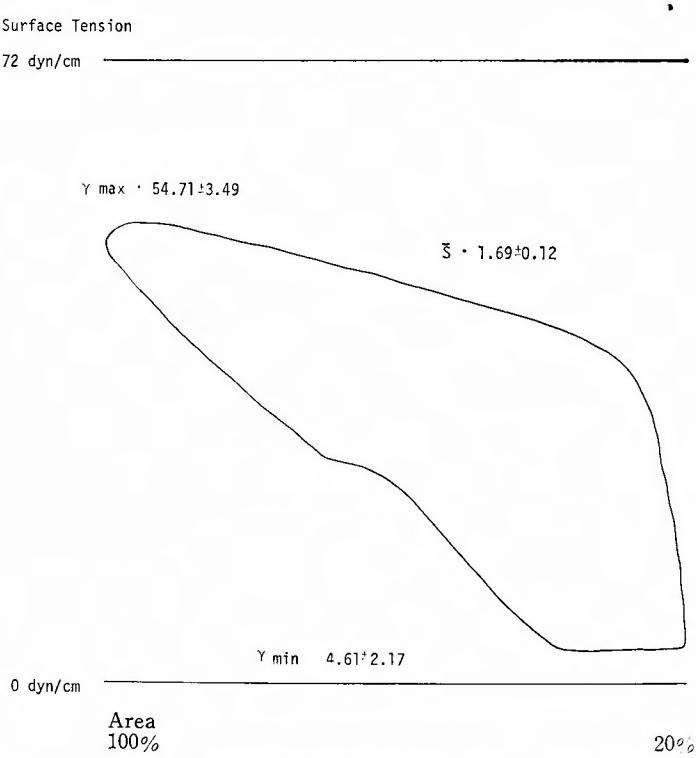


Fig. 4. Surface Tension-Area Diagram of Alveolar Surfactant of Control Rats.

1) Each value is calculated from 5 rats as mean±standard deviation.
2) Typical diagram is shown in this figure.
3) $\bar{S}$: Stability Ratio.
4) γ max : Maximam Surface Tension.
5) γ min : Minimum Surface Tension.

Surface tention-area diagram of alveolar surfacetant.

Figures 4 and 5 show surface tension-area diagrams of conrol and EFA deficient groups. A comparison of these two diagrams shows that the decline in surface tension in both groups was similar, as the surface areas decreased, until it reached a plateau at about 24 dyn/cm. Further compression of surface area in the EFA deficient group, however, did not produce an additional decrease in surface tension.

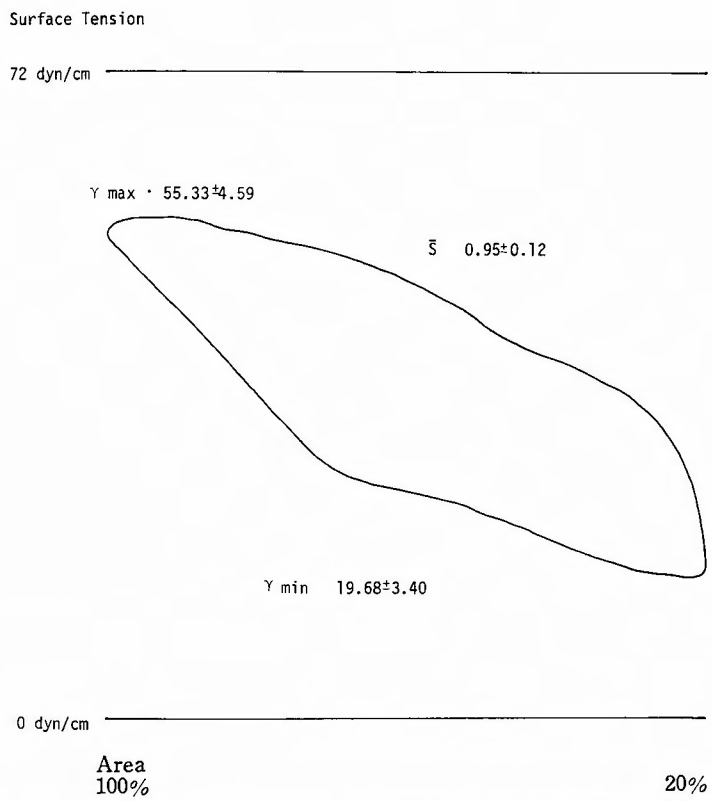


Fig. 5. Surface Tension-Area Diagram of Alveolar Surfactant of Experimental Rats.

1) Each value is calculated from 5 rats as mean±standard deviation.
2) Typical diagram is shown in this figure.
3) $\bar{S}$: Stability Ratio.
4) γ max : Maximam Surface Tension.
5) γ min : Minimum Surface Tension.

Lipid analysis of the lung and liver.

Table 4 shows the results of phospholipid analysis: sphingomyelin, phosphatidylcholine, phosphatidylethanolamine and an unkown component, PX. There were no quantitatively significant differences between the control and EFA deficient groups. Table 5 shows the results of the analysis of fatty acids in phosphatidylcholine of alveolar surfactant and in lung tissue by gas-liquid chromatography. A marked difference in the fatty acid components in phosphatidylcholine was found between the control and EFA deficient groups.

Table 4. Phospholipid Content of Alveolar Surfactant and Cellular Phospholipid in Rat Lung.

Cellular Phospholpids

	Control Group	Exp. Group
Sphyngomyelin	1.63±0.35	1.85±0.12
Phosphatidylcholine	7.31±1.13	7.29±0.53
PX	0.29±0.07	0.32±0.03
Phosphatidylethanolamine	3.02±0.70	3.13±0.26

Alveolar Phospholipids

	Control Group	Exp. Group
Sphyngomyelin	0.02±0.01	0.05±0.01
Phosphatidylcholine	0.93±0.19	0.97±0.29
PX	0.11±0.05	0.10±0.03
Phosphatidylethanolamine	0.12±0.04	0.07±0.02

1) Values are given as mean±standard deviation and μ mol/g wet weight of lung.
2) Each group contains 5 or 6 animals.
3) PX · unidentified phospholipid component.
4) No statistically significant difference between the two groups ($P<0.05$).

Table 5. Fatty Acid Components in Cellular and Alveolar Phosphatidylcholine of Rat Lung.

	Cellular PC		Alveolar PC	
Fatty Acid Moiety	Control Group (mol %)	Exp. Group (mol %)	Control Group (mol %)	Exp. Group (mol %)
C14 : 0	3.75±0.51	6.05±0.13	5.58±0.37	8.49±1.36
C16 : 0	58.40±1.71	51.42±1.90	73.58±1.77	62.82±2.75
C16 : 1	6.97±0.86	8.58±0.38	9.04±1.61	15.01±1.41
C18 : 0 *	7.55±0.72	8.80±0.56	1.46±0.43	1,80±0.37
C18 : 1	10.65±0.36	18.19±1.15	4.13±0.57	8.99±1.36
C18 : 2	7.29±1.38	2.31±0.33	4.18±0.84	1.01±0.19
C20 : 4 *	5.40±0.78	4.63±3.13	2.03±0.39	1.52±0.86

1) Values are given as mean±standard deviation.
2) Each value is calculated from 5 animals.
3) * no statistically significant difference between the two groups ($p<0.05$).

Palmitic acid was the major component of the fatty acids in both alveolar and cellular phosphatidylcholine.

There was less palmitic, linoleic and arachidonic acids in both alveolar and cellular phosphatidylcholine and more myristic, palmitoleic and oleic acids in the EFA deficient group than in the control group. These differences, however, were more marked in alveolar

Table 6. Fatty Acid Components in Phosphatidylcholine of Rat Liver.

Fatty Acid Moiety	Control Group (mol %)	Exp. Group (mol %)
C16 : 0 *	25.82±1.38	24.37±1.65
C16 : 1	0.78±0.21	2.37±0.11
C18 : 0	22.96±1.29	29.36±2.17
C18 : 1	5.15±0.55	14.73±1.13
C18 : 2	16.87±3.18	6.94±0.64
C20 : 3 *	1.67±0.39	3.10±1.40
C20 : 4 *	21.10±3.45	14.61±3.40
C20 : 5	Trace	2.51±0.61
C22 : 6	5.66±0.92	2.02±0.46

1) Values are given as mean±standard deviation.
2) Each value is calculated from 5 animals.
3) * : no statistically significant difference between the two groups ($p<0.05$).

surfactant phosphatidylcholine than in cellular phosphatidylcholine. Table 6 shows the results of the analysis of fatty acids in liver phosphatidylcholine. The pattern of the fatty acid components of phosphatidylcholine in both groups was similar to that of the lung except for palmitic acid.

In the liver, palmitic acid comprised only one fourth of the total fatty acids of phosphatidylcholine, and there were no significant difference in the palmitic acid content between the two groups. This finding was quite different from the fatty acid composition of alveolar and cellular phosphatidylcholine in the lung, especially that of alveolar surfactant.

Discussion

It was demonstrated in this study that an abnormality in the mechanical properties of the lung can be induced by feeding rats an EFA deficient diet, as shown in Fig 2. This abnormality was reflected in the lung pressure-volume relationship which showed that the lungs of EFA deficient rats could not retain air without more pressure to keep open the alveolae than in the control group. This abnormal mechanical property of the lung might be due to one of two factors : a) the lung itself increases in rigidity due to a rise in its elastin or collagen content or by a change in the character of these components[10)22)36)] ; or b) the function of alveolar surfactant is damaged. To distinguish between these two possibilities, the content of collagen and elastin, known to be responsibel for lung rigidity[32)], was determined, as shown in Table 3, and no difference was demonstrable between the two groups. In addition, there was no difference between the control and the EFA deficient groups in the pressure-volume relationship of lungs with alveoli and air ducts filled with saline instead of air in order to exclude the influence of alveolar surfactant, as shown in Fig 3. These results indicate that the difference in the pressure-volume relationship between the two groups was due to neither the difference of collagen and elastin content nor the

change of character of these components. Therefore, it is proposed that the lung instability induced by the EFA deficient diet is caused by alteration of alveolar surfactant.

Since the presence of surfactant in the lung was suggested by VON NEERGAARD[44] in 1929, surfactant has been considered an indispensable factor for the normal function of the lung, protecting alveoli from collapse at end expiration. So the surface tension of surfactants extracted from the lungs of EFA deficient and control animals was compared. There was a significant increase in the minimum surface tension (Fig. 4 and 5) of surfactant extracted from EFA deficient rats. This suggests that the difference on the pressure-volume curve was caused by increased minimum surface tension at the alveolar air-liquid interface when the alveolar surface was reduced. The stability ratios[13] shown in these figures indicate that surfactant from the EFA deficient group acts at the air-liquid interface to a lesser extent than does that from the control group.

As is well known, pulmonary surfactant is a lipoprotein[34] and alveolar surfactant contains abundant phospholipids, among which phosphatidylcholine is the most responsible for surface activity at the air-liquid interface. The phospholipid content of the lung and liver, which actively synthesizes lipids, was analyzed. It was found that the lipids consisted mainly of phosphatidylcholine, especially dipalmitoyl phosphatidylcholine[17], in the lung, as described above. On the basis of the average number of molecules in a unit surface area, dipalmitoyl phosphatidylcholine is theoretically most suitable to act as surfactant at the air-liquid interface in the alveoli[35], and in fact it does have the greatest surface tension reducing activity when surface tension is measured with a modified Wilhelmy balance[13]. Although no significant quantitative difference was noted between the EFA deficient and control groups in the total phosphatidylcholine content of surfactant, as shown in Table 4, the palmitic acid content of phosphatidylcholine was significantly lower in the EFA deficient group. These results indicate that a qualitative change in the fatty acid composition of phosphatidylcholine is caused by EFA deficiency. It is supposed that the decrease of dipalmitoyl phosphatidylcholine in surfactant reflects the decrease of palmitic acid in phosphatidylcholine and that it can not effectively reduce minimum surface tension as surfactant at the air-liquid interface of the lung. The palmitic acid content in phosphatidylcholine differed in the lungs and the livers, as shown in Tables 5 and 6, in response to the EFA deficient diet. Palmitic acid is not an EFA and can be synthesized in mammaliam spesies. In the present study, the amount of palmitic acid in liver phosphatidylcholine was not influenced by EFA deficiency. However in the lung, the palmitic acid content of surfactant phosphatidylcholine was decreased. It is still unknown what mechanism changes the constitution of phosphatidylcholine. The results obtained are difficult to explain by the simple biochemical processes of lipid metabolism. There seem to be different pathways of lipid metabolism in phosphatidylcholine synthesis between the lung and liver from the biochemical point of view. According to the recent observation of AKINO et al.[1], there was no significant difference in the enzyme activity involved in phosphatidylcholine synthesis between EFA deficient and control groups; they suggested that more complicated mecha-

nisms might be involved in this phenomenon. Further investigations on the different phosphatidylcholine responses between the lung and liver in EFA deficiency will provide further understanding of the mechanism of change of the fatty acid pattern in phosphatidylcholine.

It was reported from this laboratory that EFA deficiency was initiating factor of cholesterol stone by changing bile acids composition in the liver[21] and it decreased steroid hormones formation in the adrenals[40], although EFA are not part of the composition of bile acid synthesis in the liver nor of steroid hormone synthesis in the adrenal glands. This report shows that EFA deficiency increased the minimum surface tension of alveolar surfactant by decreasing the amount of dipalmitoyl phosphatidylcholine in surfactant, although it is not itself a component of surfactant phosphatidylcholine synthesis. These experimental results suggest that EFA play an important role in biochemical functions. WENE et al.[45] reported that in two adults given fat-free intravenous infusions for two weeks, the EFA content of serum phospholipids decreased from 21.2% to 3.2%, triglycerides from 14.1% to 2.6%, free fatty acids from 9.6% to 2.0% and cholesterol esters from 48.8% to 9.8 %. It may be that EFA deficiency occurs in infants with severe congenitial heart disease who can not take sufficient milk containing abundant EFA. From the clinical stand point, the low respiratory efficiency induced by EFA deficiency in infants might have a lethal effect on respiratory function after operation, because their reserve capacity of respiratory function is lower than that of adults. It can be concluded that supplements of EFA before operation will improve not only surgical condition during hypothermia but also post-operative respiratory function following radical corrective surgery of congenital heart disease in infants.

Summary

In this study of the influence on respiratory function of essential fatty acid (EFA) deficiency, the mechanical properties and biochemical changes of the lung in rats fed an EFA deficient diet were compared with those in control rats. It was found that the lungs of the EFA deficient group retained less air than those of the control group. This was caused not by an increaeses in the rigidity of the lung itself but by a change in the property of surfactant ; the minimum surface tension in surfactant extracted from EFA deficient rats was found to be increased when plotted in surface tension-area diagrams, so this surfactant was less able to maintain the stable state of the lungs. The total amount of phosphatidylcholine in lung surfactant was not significantly different, but the palmitic acid content of the lung phosphatidylcholine in surfactant was decreased in the EFA deficient group. Phosphatidylcholine, especially dipalmitoyl phosphatidylcholine, is considered to be an important factor which decreases surface tension at the air-liquid interface in the lung and alveolae open at end-expiration. The decrease of dipalmitoyl phosphatidylcholine, as reflected by a decrease in palmitic acid in phosphatidylcholine, might change the mechanical properties of the lung. Palmitic acid in liver phosphatidylcholine of EFA deficient diet

rats was not decreased, although EFA was lowered. Thus these appear to be different lipid metabolic pathways between lung and liver phosphatidylcholine. The correction of EFA deficiency by supplmenting these lipids before operation should be useful in maintaining good respiratory function after operation.

Acknowledgement

The author is grateful to Prof. Dr. YORINORI HIKASA, The Second Department of Surgry, Faculty of Medicine, Kyoto University for overall instruction and to Dr. RYUSUKE MURAOKA, The Second Departmnt of Surgery, Faculty of Medicine, Kyoto Univesity for his helpful guidance and cooperation. The author also thanks Prof. Dr. KIMIO YASUHIRA and Dr. YASUHIRO SUZUKI, Department of Pathology, Chest Disease Research Institute, Kyoto University for their constant collaboration and helpful discussion in this investigation.

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和文抄録

肺表面活性物質に対する必須脂肪酸欠乏の影響についての実験的研究

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必須脂肪酸欠乏状態が呼吸機能に及ぼす影響について，ラットを使用して実験的研究を行った．2ケ月間にわたって必須脂肪酸欠乏食で飼育したラットは，空気を使用した肺の圧量曲線による検討では，対照群に比較して，肺胞の拡張困難がみられた．その原因としては，肺組織自身の弾性成分の量的，質的変化による可能性，あるいは肺表面活性物質の変化を介しての影響が推測された．しかしながら空気に代って生理的食塩水による肺の圧量曲線及び肺組織のコラーゲン，及びエラスチン含有量の測定では，必須脂肪酸欠乏群と対照群では差がみられず，従って肺組織の弾性成分の変化に原因するものではないことが判明した．そこで肺表面活性物質を抽出して modified Wilhelmy balance により表面張力を測定した結果，最小表面張力は欠乏食群で異常に高い値を示した．この原因を解明するため表面活性物質の主成分であるレシチンの生化学的分析を行ない，両群の間にレシチンの量的な差はないが，レシチンの構成脂肪酸の内，肺において特異的に多いパルミチン酸の含有率が欠乏食群で有意に減少している事が判明した．欠乏食群の肺表面活性物質の最小表面張力が異常に高い原因は，必須脂肪酸欠乏による脂質代謝の影響を介してのレシチン中のパルミチン酸の減少による表面活性物質としての効率の低下によるものと考えられ，必須脂肪酸は正常な呼吸機能を維持するために必須な因子であると結論された．