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Metabolic Fate of Lysolecithin Injected into Rats

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Akino, T., Yamazaki, I. and Abe, M. *Metabolic Fate of Lysolecithin Injected into Rats.* Tohoku J. exp. Med., 1972, **108** (2), 133–139 —— The incorporation of labeled lysolecithin into various rat organs was studied. Lysolecithin injected was rapidly taken up by various organs and it was found that the conversion into lecithin proceeded more rapidly in the liver and intestine than in other organs. The mode of formation of lecithin from lysolecithin in various organs was also studied *in vivo*. Using lysolecithin doubly labeled with the glycerol and fatty acid portions, it was shown that lysolecithin injected was preferentially converted to tetraenoic lecithin in all organs examined by Lands' pathway, while the saturated+monoenoic species of the lung and brain was formed with $^{14}C/^{3}H$ of approximately 2.0 by Marrinetti's pathway. These results suggest that the lysolecithin-lecithin cycle between liver and plasma may serve to retain polyunsaturated fatty acids, and the saturated lecithin in the lung known as a surfactant may only in some parts be derived from exogenous lysolecithin. ———— plasma lysolecithin; dipalmitoylecithin

Lysolecithin has been shown to be a normal constituent of plasma (Marinetti *et al.* 1959). Several investigations have suggested that lysolecithin in the circulating plasma may partly be derived from lecithin by lecithin cholesterol acyltransferase (LCAT) present in plasma *in vitro* (Glomset, 1962, 1968, Shah *et al.* 1964). Stein and Stein (1966) also found that injected lysolecithin is rapidly taken up and converted to lecithin mainly by Lands' pathway in rat organs. On the other hand, Kanoh (1969) found that lysolecithin is mainly incorporated into tetraenoic lecithin in rat liver slices. Further, we reported in our previous paper (Akino *et al.* 1971) that saturated species of lecithin in the lung may be formed by transacylation of the two molecules of lysolecithin from the incorporation patterns of lysolecithin labeled with (^{3}H)-glycerol and (^{14}C)-palmitate.

The present work was undertaken to elucidate the metabolic fate of injected lysolecithin and we have obtained additional data concerning the preferential conversion of lysolecithin to specific molecular species of lecithin in various rat organs.

Materials and Methods

(2-^{3}H) glycerol (specific activity, 400 mCi/mmole), (9, 10-^{3}H) palmitate (specific activity, 500 mCi/mmole) and (1-^{14}C) palmitate (specific activity, 57.7 mCi/mmole) were purchased from the Radiochemical Centre (Amersham, Great Britain).

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Lysolecithin labeled with (^{3}H) glycerol was biosynthetically prepared as described by Kanoh (1969). Lysolecithin labeled with (^{3}H) or (^{14}C) palmitate was prepared in the same manner as reported previously (Akino *et al.* 1971), using 10 mCi of (^{3}H) or 500 μCi of (^{14}C) of palmitate. The specific activity of lysolecithin thus obtained was 20.4×10^4 cpm/μmole for (^{3}H) glycerol, 56.8×10^4 cpm/μmole for (^{3}H) palmitate and 14.6×10^4 cpm/μmole for (^{14}C) palmitate respectively. About 10 μmoles of lysolecithin were mixed with 1 ml of 7% fat-free bovine serum albumin in saline, in which the molar ratio of lysolecithin to albumin was about 1 : 10. The amount of lysolecithin used in each experiment ranged in 9.9–12.6 μmoles.

Male Wistar strain rats weighing 220–250 g were used. Under light ether anesthesia the animals were carefully injected *via* the femoral vein with one ml of the lysolecithin-albumin solution. At various time intervals following injection, the rats were sacrificed by puncturing the abdominal aorta and the organs were washed with warm saline injected through the portal vein. The organs were removed, weighed, minced and homogenized in chloroform-methanol (2 : 1 by vol.). The lipid extract was washed by the procedure of Folch *et al.* (1957). The fractionation of lipids was performed in the manner described previously (Akino *et al.* 1970), using a combination of DEAE-cellulose column chromatography and preparative thin-layer chromatography. The purified lecithin was further subfractionated into species of different degrees of unsaturation by silica gel H thin-layer chromatography with a two-step solvent system described in our previous paper (Akino *et al.* 1971). The saturated and monoenoic fractions which could not be separated from each other in this procedure were not further classified.

Phosphorus was determined by the method of Bartlett (1959). Radioactivity determinations were made with a Horiba liquid scintilation counter, using a Toluene scintilator prepared as described by Snyder (1964). The ^{3}H and ^{14}C activities were calculated according to the channel ratio method. All solvents used here were flushed with N_2 gas and contained 0.02% of 4-methyl-2, 6-di-tert.-butylphenol to avoid oxidation of unsaturated lipids.

Results and Discussion

Table 1 shows the results of two experiments on the incorporation of lysolecithin into different organs. It was noted that intravenously injected lysolecithin was taken up rapidly by various rat organs including the brain; the highest concentration of radioactivity was found in the liver. As early as 5 min after injection considerable parts of lysolecithin were converted to lecithin. The conversion ratio seen in Table 1 gives an indicator of the different rates at which the lysolecithin was converted to lecithin. In the liver and intestine, significantly higher conversion ratios were observed than in the other organs examined, where this reaction proceeded at slower rates. The patterns of tissue uptake between glycerol and fatty acid portions of lysolecithin were not essentially different from each other. These findings were in good agreement with the results reported by Stein and Stein (1966).

Table 2 shows the percentage distribution of total radioactivity in different lipid classes after injection of (^{3}H) palmitoyl-lysolecithin. A considerable incorporation of radioactivity into triglyceride in the liver and intestine was clearly demonstrated. In contrast, the incorporation patterns into each neutral lipid class in other organs were almost the same as those in the lung.

Table 3 shows the molecular species composition of lecithin from different rat organs. It was noted that the lung and brain contained remarkable amounts of

TABLE 1. *Uptake of lysolecithin labeled with (^3H)glycerol (Exp. 1) or (^3H)palmitate (Exp. 2) and conversion to lecithin by different rat organs*

Organ	Exp. Number	Time after injection (min)	Tissue uptake (% of injected dose)	Radioactivity in lecithin* (%)	Conversion ratio†
Liver	1	5	13.7	35.1	0.54
		30	24.9	85.1	10.1
	2	30	15.3	68.8	4.02
		90	23.5	81.6	25.1
Lung	1	5	2.4	22.7	0.30
		30	3.5	53.8	1.25
	2	30	1.5	40.3	0.73
		90	1.7	67.8	2.58
Intestine	1	5	3.7	45.5	0.83
		30	6.1	66.5	2.83
	2	30	4.1	74.7	8.9
		90	5.1	64.6	9.1
Kidney	1	5	0.7	29.2	0.43
		30	1.8	40.4	0.70
	2	30	0.9	39.3	0.79
		90	0.9	50.4	1.50
Heart	1	5	1.3	38.5	0.63
		30	0.4	30.9	0.46
	2	30	0.4	46.0	0.98
		90	0.2	46.7	1.15
Spleen	1	30	0.7	31.7	0.48
	2	30	0.8	39.0	0.74
Brain	2	30	0.2	34.0	0.52

* % of radioactivity in each organ recovered in the lecithin fraction.
† The ratio is given as the value of radioactivities of lecithin to those of lysolecithin.

TABLE 2. *Distribution of radioactivity in lipid classes after intravenous injection of (^3H)palmitoyl-lysolecithin into various rat organs*

Organ	Time after injection (min)	TG	DG	FFA	MG	PE	PC	Sph	Lys-PC
Liver	30	8.7	1.5	0.1	0.1	2.9	68.8	0.8	17.1
	90	9.9	1.3	0.1	0.1	2.8	81.6	0.9	3.3
Lung	30	0.7	1.8	0.1	—	1.5	40.3	0.5	55.1
	90	3.2	1.4	0.1	—	1.0	67.8	0.3	26.2
Intestine	30	10.9	3.8	0.8	—	1.0	74.7	0.4	8.4
	90	19.2	6.4	0.8	0.2	1.3	64.6	0.4	7.1

TG, triglyceride; DG, diglyceride; FFA, free fatty acid; MG, monoglyceride; PE, phosphatidylethanolamine; PC, lecithin; Sph, sphingomyelin; Lys-PC, lysolecithin.

saturated+monoenoic species and relatively small amounts of tetraenoic species. The patterns of lecithin subspecies in other organs were essentially similar except for relatively large amounts of saturated+monoenoic species in the spleen.

TABLE 3. *Molecular species composition of lecithin from various rat organs*

Content of lecithin (μmoles/g tissue)	Liver	Lung	Intestine	Kidney	Heart	Spleen	Brain
	21.2±1.2	11.8±0.9	11.4±1.1	16.8±2.1	10.7±1.9	8.4±1.3	22.6±1.4
Subfraction (% of total)							
Saturated +monoenoic	18.3±1.8	55.9±3.9	26.8±1.8	18.3±1.8	18.3±2.1	32.0±1.8	63.1±5.6
Dienoic	27.4±3.0	19.3±2.1	31.6±6.3	24.0±3.0	27.3±3.2	22.2±2.1	7.3±0.8
Tetraenoic	34.2±4.0	15.9±5.4	22.8±2.2	42.1±5.4	30.8±3.0	21.8±1.9	16.1±3.0
Hexaenoic	18.4±3.2	8.9±1.9	14.0±1.1	15.6±0.8	23.6±1.9	24.0±3.1	13.5±1.9

The values represent mean±S.D. from four rats.
Intestine lecithin contained 4.8% of trienoic species.

In order to determine the mode of formation of lecithin from lysolecithin in different organs, lysolecithin doubly-labeled with (^{3}H) glycerol and (^{14}C) palmitate was injected into rats and lecithin from each organ was further fractionated into molecular species. As seen in Table 4, it was noted that glycerol labeled lysolecithin injected was preferentially converted into tetraenoic species in all organs examined even in organs already containing small amounts of tetraenoic lecithin such as the lung and brain (Table 3). The ratio of ^{14}C to ^{3}H in unfractionated lecithin was close to 1.0 in all organs. In organs other than the lung and brain, the ratios in subspecies were also approximately 1.0, except for the slightly higher values in saturated+monoenoic fraction. The results indicate that *in vivo* injected lysolecithin taken up by various organs is mainly converted to tetraenoic lecithin by the acylation reaction as described *in vitro* by Lands (1960). However, the lung and brain are known to contain considerable amounts of saturated lecithin (Brown 1964, Renkonen 1966). Namely, a relatively high incorporation of ^{14}C activity into saturated+monoenoic species was observed, while the incorporation of ^{3}H activity was lowest among species, and the ratios in the fraction were approximately 2.0. The results suggest that the saturated + monoenoic species (presumably saturated) converted from lysolecithin in both organs may not be formed *via* the acylation reaction, but mainly *via* Marinetti's pathway (Erbland

TABLE 4. *Incorporation of lysolecithin labeled with (^{3}H)glycerol and*

	Liver		Lung		Intestine	
	^{3}H	^{14}C/^{3}H†	^{3}H	^{14}C/^{3}H	^{3}H	^{14}C/^{3}H
Specific activity* of lecithin						
Unfractionated	4.19	1.04	1.23	1.29	1.86	0.82
Saturated+monoenoic	1.09	1.39	0.81	1.91	1.63	0.98
Dienoic	3.02	1.19	1.54	0.87	1.35	0.63
Tetraenoic	8.59	1.00	2.47	0.78	3.30	0.83
Hexaenoic	3.28	0.87	0.96	0.67	1.19	0.91

* Specific activity is given as 10^3 cpm per μmoles-P. † The ratio of ^{14}C/^{3}H

and Marinetti 1965) in which two molecules of lysolecithin react to form each one molecule of lecithin and glycerylphosphorylcholine.

Recently evidence was obtained from the studies on the metabolic heterogeneity of lecithin that tetraenoic lecithin in the liver (Kanoh 1969) and lung (Akino *et al.* 1971) was introduced *via* Lands' pathway and the saturated one in the lung *via* Marinetti's pathway (Akino *et al.* 1971). The present work has added further evidence that *in vivo* injected lysolecithin was preferentially converted to tetraenoic lecithin in all organs *via* the acylation reaction with endogenous arachidonic acid. Balint *et al.* (1967) found that the tetraenoic lecithin synthesized in the liver was secreted into plasma at a higher rate than into bile. Holub and Kuksis (1971) recently reported that the subsequent gradual release of labeled liver arachidonoyl lecithin into plasma was accompanied by a steady rise in the radioactivity of plasma cholesterol arachidonate. Sugano (1971) also reported that the lecithin-cholesterol acyltransferase of plasma from rats starved overnight preferentially formed cholesterol arachidonate. The lysolecithin-lecithin cycle presented by Stein and Stein (1966) may be assumed to serve to make putting out polyunsaturated fatty acids to cholesterol in plasma.

The saturated species (dipalmitoyl lecithin) of the lung is known as a surfactant maintaining alveolar functions (Brown 1964). In spite of the fact that the species could be formed from exogenous lysolecithin, it is doubtful from the studies on turn-over rate of lung lecithin whether the major parts of dipalmitoyl lecithin in the lung is derived from plasma lysolecithin. The turn-over time of total plasma phospholipids is calculated to be about 68 min on the basis of their half lives (47 min) found by Stein and Stein (1966). Namely, approximately 1/68 of plasma phospholipids will disappear from plasma and be taken up by all tissues in one minute. Accordingly, the value of plasma phospholipids incorporated into tissues in one minute is calculated to be about 0.46 μmole in a rat weighing 250 g. When 2% of exogenous lysolecithin are taken up by the lung as seen in Table 1, only about 0.002 μmole per g of lysolecithin will be incorporated into the lung in one minute. On the other hand, the turn-over rate of dipalmitoyl lecithin is calculated as 0.01 μmole per minute per g from the half life time found by Tierney

(^{14}C)palmitate into molecular species of lecithin at 30 min after injection

Kidney		Heart		Spleen		Brain	
^{3}H	$^{14}C/^{3}H$	^{3}H	$^{14}C/^{3}H$	^{3}H	$^{14}C/^{3}H$	^{3}H	$^{14}C/^{3}H$
1.12	0.89	0 42	1.06	0.85	0.96	0.06	1.17
0.53	1.20	0.39	1.19	0.91	0.98	0.03	1.75
1.30	0.91	0.35	1.11	0.57	0.81	0.05	1.04
1.62	0.89	0.76	1.09	1.70	1.06	0.25	0.44
1.05	0.57	0.29	0.96	0.28	0.54	0.02	0.63

is calculated as unity of $^{14}C/^{3}H$ of lysolecithin injected.

et al. (1967) and the lung lecithin pool size seen in Table 3. Hence, the contribution of lysolecithin originating from plasma to total lecithin synthesis in the lung seems to be relatively small, amounting to about 1/5 and exogenous lysolecithin does not seem to be the main origin of lecithin present in the lung.

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