

In vivo studies on pathways for the biosynthesis of lecithin in the rat

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ABSTRACT The in vivo biosynthesis of lecithin in rats has been studied with the precursors choline-1,2-¹⁴C, ethanolamine-1,2-¹⁴C and methionine-CH₃-¹⁴C or -CH₃-³H. Lecithin synthesis from choline is rapid in all organs. No sex difference was observed in this pathway. The biosynthesis of lecithin by methylation of phosphatidyl ethanolamine is of quantitative significance in the liver, but not in extrahepatic tissues. More lecithin is synthesized by this pathway in female rats. In liver the lecithin synthesized via both pathways enters a common pool which is in rapid equilibrium with lecithin of blood plasma. A sex difference in the utilization of radioactive ethanolamine for the formation of phosphatidyl ethanolamine was observed (greater utilization in the female). Incorporation of ethanolamine into phospholipids of extrahepatic tissues was slow in both sexes. With labeled methionine as precursor the liver cytidine diphosphate (CDP) choline had a specific activity identical with that of liver lecithin after 20 min, while the specific activity of phosphoryl choline remained low. With labeled choline as precursor the phosphoryl choline reached a specific activity 50 times that of lecithin after 20 min, while the specific activity of CDP choline was only four times that of lecithin. These findings indicate that the reaction:

$$\text{CDP choline} + \text{diglyceride} \rightleftharpoons \text{phosphatidyl choline} + \text{CMP}$$
is freely reversible in vivo.

KEY WORDS lecithin · biosynthesis · alternative pathways · liver · extrahepatic tissues · sex differences · rat · transmethylation · phosphatidyl ethanolamine · intermediates · cytidine diphosphate choline · phosphoryl choline · methyl-³H methionine · choline-1,2-¹⁴C · ethanolamine-1,2-¹⁴C

TWO PATHWAYS ARE KNOWN for the de novo biosynthesis of phosphatidyl choline (lecithin):

(a) Phosphatidyl ethanolamine can be converted to lecithin by a stepwise methylation using the methyl groups from adenosyl methionine (1, 2). This pathway

also represents a mechanism for the de novo biosynthesis of choline. A sex difference in the utilization of methionine methyl groups in lecithin formation has been reported (3).

(b) Preformed, free choline can be incorporated into lecithin via phosphoryl choline and cytidine diphosphate choline (CDP choline) (4).

In addition, it has been reported that free choline can be incorporated into lecithin in vitro by a calcium-stimulated pathway which bypasses phosphoryl choline and CDP choline (5). Most probably this incorporation takes place via an exchange reaction between free choline and preformed lecithin; if so, it does not represent a de novo synthesis of lecithin. It is not known whether the mechanism is active in vivo.

Furthermore, lecithin can be formed by acylation of lysolecithin by means of acyl CoA (6). Since lysolecithin is probably formed by hydrolysis of lecithin, this pathway does not represent a de novo synthesis of lecithin.

The different pathways for the biosynthesis of lecithin have been extensively studied in vitro (1-7), but their relative importance in different organs in vivo remains to be assessed. It is also unknown whether lecithins formed by the different mechanisms have different physiological functions.

In the present work we have studied the incorporation of ethanolamine, choline, and the methyl group of methionine into phospholipids in different organs and into the phospholipid precursors phosphoryl choline and CDP choline in the liver.

MATERIALS AND METHODS

L-Methionine-CH₃-³H (specific activity 230 mc/mole) was purchased from the Radiochemical Center, Amersham, England; L-methionine-CH₃-¹⁴C (specific activity 2.25 mc/mole) and choline-1,2-¹⁴C (specific activity 2.75 mc/mole) from Phillips-Duphar, Amsterdam,

Netherlands; and ethanolamine-1,2-¹⁴C (specific activity 1 mc/mole) from Volk Radiochemical Co., Chicago, Ill.

Rats of mixed strain weighing 170–250 g were used in all experiments except those in which water-soluble intermediates were isolated. In these experiments male rats weighing 300–350 g were used. The animals had free access to food and water until the experiments were started. All radioactive precursors were administered intraperitoneally (except where otherwise stated) in 1 ml of 0.9% NaCl solution.

The animals were killed by decapitation. The organs were immediately homogenized in a small volume of ice-cold water and extracted with chloroform–methanol according to Folch, Lees, and Sloane Stanley (8). In several experiments the chloroform layer was subjected to thin-layer chromatography using Silica Gel G and chloroform–methanol–water 65:25:4, as described by Habermann, Bandtlow, and Krusche (9). Both the ¹⁴C-activity from injected choline and ³H-activity from injected methionine were found almost exclusively in the lecithin spot. In routine practice, therefore, the chloroform extracts were directly evaporated to dryness and subjected to hydrolysis to assess the lecithin-choline specific activity. The lipids were hydrolyzed in 4 N HCl at 100° for 14 hr under reflux. The liberated fatty acids were removed by filtration and the total and specific activity of choline was determined, after measuring the concentration of choline in the filtrate (10). For the determination of radioactivity, choline was isolated as the Reinecke salt from the diluted filtrates after addition of carrier choline. The choline was reisolated by decomposing the choline reineckate according to Bremer and Greenberg (11) with hydrochloric acid and methyl ethyl ketone.

In experiments in which phosphoryl choline, phosphoryl ethanolamine, CDP choline, and CDP ethanolamine were isolated, the liver was homogenized in 150 ml of 67% ethanol in a Waring Blendor. The precipitated proteins were removed by centrifugation and extracted once more with 50 ml of 67% ethanol. Diethyl ether (250 ml) and water were added to the combined extracts until two phases were obtained. The water phase, combined with water washings of the ethanol–ether phase, was filtered and used directly for column chromatography on Dowex 1-X4 in the formate form as previously described (1). The ethanol–ether phase was used to complete the extraction of lipids from the precipitated proteins.

Phosphoryl choline and phosphoryl ethanolamine were not completely separated on the Dowex-formate column. These compounds therefore had to be further purified by means of paper chromatography. The column fractions (containing radioactivity) were evaporated to

dryness and chromatographed on Whatman No. 4 paper with propanol–formic acid–water 60:24:16 (1). Narrow strips were cut from the chromatograms. Phosphoryl choline was located with iodine vapor, phosphoryl ethanolamine with ninhydrin. Subsequently the compounds were eluted from the rest of the chromatograms with water. Phosphate (12) and radioactivity were determined in the eluates.

CDP choline and CDP ethanolamine were located in the fractions eluted from the column by measuring the absorbancy at 280 m μ (1). As the fractions were contaminated with other nucleotides, these compounds also had to be further purified on paper to obtain reliable specific activity measurements. The appropriate column fractions were evaporated to dryness at room temperature under a stream of air and subsequently chromatographed on Whatman No. 4 paper with ethanol–0.04 N acetic acid 6:4 (4). The CDP choline and CDP ethanolamine spots were located in UV light and eluted with water. The eluates showed the typical cytidine absorbancy spectra under both acid and alkaline conditions. The specific radioactivity was calculated from the measured radioactivity and the absorbancy at 280 m μ of the eluates ($E_{280} = 13 \times 10^3$ for cytidine nucleotides).

All radioactivity measurements were made in a Packard Tri-Carb liquid scintillation spectrometer. For the simultaneous determination of ³H- and ¹⁴C-activity in experiments where both isotopes were used simultaneously, the samples were evaporated to dryness and dissolved in 0.3 ml of methanol. To this solution was added 10 ml of a scintillator solution containing 5 g of 2,5-diphenyloxazole (PPO) and 0.3 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) per liter. Split channel counting was used with a high voltage setting of 6 (approximately 1200 volts). The window settings were: channel 1, 10–50, channel 2, 100– ∞ . The ³H-activity was found exclusively in channel 1. The activity in channel 2 thus represented ¹⁴C-activity only. Corrections for ¹⁴C-activity in channel 1 and for quenching were made by adding internal standards both of ¹⁴C and ³H to each sample. With these settings the counting efficiencies were about 8% for ³H and about 20% for ¹⁴C.

In experiments with one isotope only, 10 ml of a solution of scintillators (5 g of PPO, 0.05 g of POPOP, 80 g of naphthalene, 385 ml of dioxane, 385 ml of xylene, and 230 ml of ethanol) was added to 0.5 ml samples of the aqueous or methanolic solutions.

RESULTS

Lecithin Formation in Liver

Figure 1 shows the time course for the incorporation of choline and for the methyl group of methionine into liver

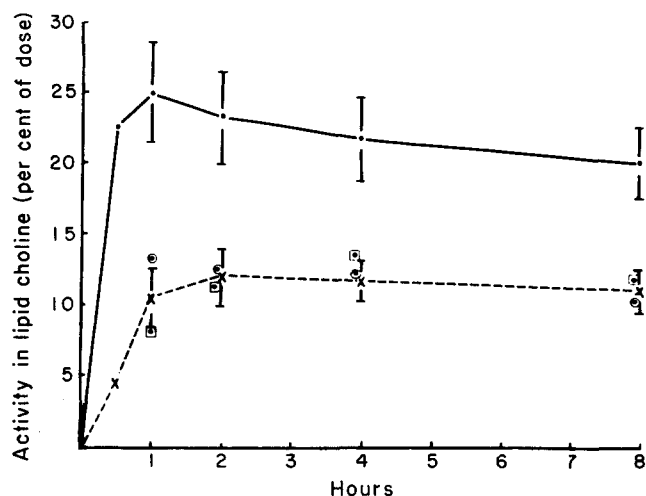


FIG. 1. Labeling of liver lipid choline of rats after intraperitoneal injection of methionine- $\text{CH}_3\text{-}^3\text{H}$ and choline- $1,2\text{-}^{14}\text{C}$. Points with bars represent the average from five animals with standard deviation of the mean. Points without bars represent single animals. ●—● ^3H -activity in female rats. ×---× ^{14}C -activity in female rats. ○ ^3H -activity in male rats. □ ^{14}C -activity in male rats.

lecithin. The methyl group of methionine is rapidly incorporated; maximum specific activity occurred less than 1 hr after the injection. At this time an average of 25% of the injected tracer dose is recovered in liver lecithin in female rats. As previously shown by Natori (3), only about half as much is incorporated into the liver lecithin in male rats.

The incorporation of choline was somewhat slower than that of the methyl groups of methionine. The highest incorporation occurred 1–2 hr after the injection. No significant sex difference in choline incorporation was observed.

The sex difference in the incorporation of methionine methyl groups into choline has been ascribed to a higher activity of the methionine adenosyltransferase (ATP:L-methionine S-adenosyltransferase, EC 2.4.2.13) in female rats. This sex difference in methyl group incorporation might also be due to a greater endogenous synthesis and oxidation of methyl groups in male rats, which thus would dilute the injected labeled methyl groups to a greater extent than female rats. This would also explain the lower incorporation of methyl-labeled methionine into the liver proteins in male rats (3).

To test this possibility male and female rats were injected with ethanolamine- $1,2\text{-}^{14}\text{C}$. A similar rate of conversion of ethanolamine to choline in both sexes would indicate a more rapid synthesis of methyl groups in male rats. Figure 2 shows the results of this experiment. Interpretation of the results was complicated by the unexpected finding that the female rats incorporated about twice as much of the labeled ethanolamine as male rats into their liver lipids. Thin-layer chromatography showed that the incorporated radioactivity was recovered in the

cephalin and lecithin fractions in both sexes. As the labeled lecithin in this experiment evidently had been formed by methylation of labeled phosphatidyl ethanolamine (1), these results show that there is a sex difference in the biosynthesis of phosphatidyl ethanolamine from free ethanolamine.

Figure 2B indicates that the female rats synthesize lecithin by the methylating pathway more rapidly than do male rats, since a higher percentage of the labeled phosphatidyl ethanolamine was converted to lecithin in their livers. This conclusion is based on the assumption that the liver pool of phosphatidyl ethanolamine is about equal in the two sexes. Our results thus support the hypothesis that the greater activity of the methionine-activating enzyme in female rats diverts a higher percentage of methionine methyl groups to lecithin synthesis (3).

Lecithin Formation in Extrahepatic Tissues

Figure 3 shows the incorporation of choline- $1,2\text{-}^{14}\text{C}$, methionine- $\text{CH}_3\text{-}^3\text{H}$, and ethanolamine- $1,2\text{-}^{14}\text{C}$ into lecithin of different organs of female rats. The results are expressed as the ratio (specific activity of lipid choline in each organ):(specific activity in lipid choline in liver). It is evident that choline is efficiently incorporated in all organs tested, although rather more slowly than in the liver. Except for the heart, nearly maximum incorporation is reached in 2 hr. In comparison, methionine methyl groups seem to be poorly utilized for lecithin formation in all organs except the liver. These results were obtained after intraperitoneal injection of the precursors. We have also measured the incorporation of radioactive choline and of radioactive methionine into the lipids of the different organs after *subcutaneous* injection. We found that the

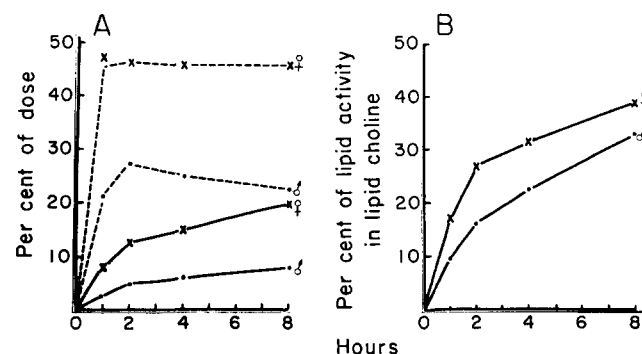


FIG. 2A. Incorporation of intraperitoneally injected ethanolamine- $1,2\text{-}^{14}\text{C}$ into liver lipids and its conversion to lipid choline in male and female rats. Each point represents single animals. ●---● and ×---×, total radioactivity recovered in the liver lipids of male and female rats, respectively. ●—● and ×—×, radioactivity recovered in liver lipid choline in male and female rats.

FIG. 2B. Choline data of Fig. 2A plotted to show the percentage of total liver lipid activity recovered as choline in male and female rats.

incorporation of choline in the liver was approximately halved while it increased up to three times in extrahepatic tissues. In contrast, the organ distribution of methionine activity was almost identical for the two administration routes.

The low specific activity of lecithin in extrahepatic tissues after the injection of methyl-labeled methionine is in agreement with previous *in vitro* results which indicated that the methylating pathway is localized mainly in the liver (11). It is nevertheless striking that the lecithin specific activity of extrahepatic tissues increased almost linearly for 8 hr in the methionine-injected animals (Fig. 3), while maximum activity in the liver was reached after 1 hr (Fig. 1).

There are several possible explanations for this phenomenon: (a) Liver lecithin synthesized by transmethylation is rapidly hydrolyzed, making a rapid transfer of free choline possible. (b) Methionine in extrahepatic tissues turns over relatively slowly. (c) Extrahepatic tissues do not synthesize lecithin by transmethylation, but receive it intact from the liver.

The first possibility was excluded by the experiments

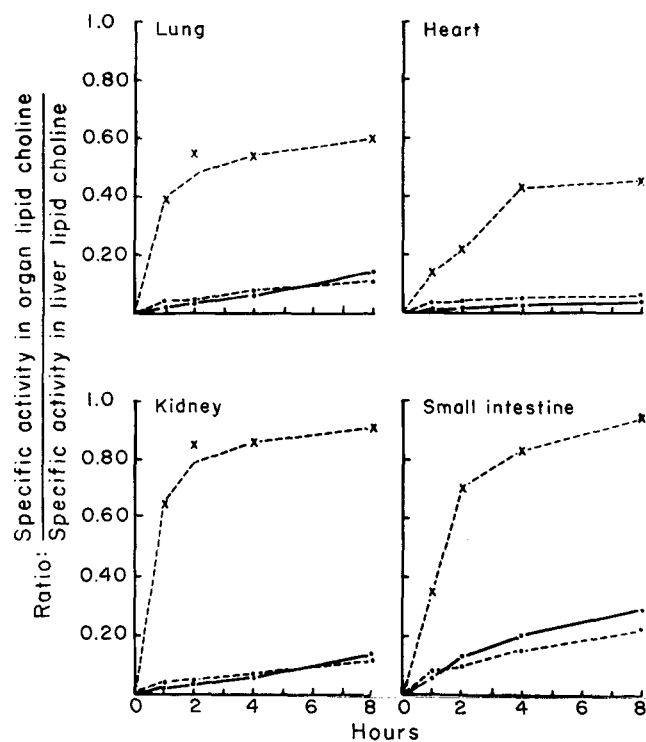


FIG. 3. Labeling of lipid choline in different organs of female rats injected intraperitoneally with methionine- $\text{CH}_3\text{-}^3\text{H}$, choline- $\text{I},2\text{-}^{14}\text{C}$, or ethanolamine- $\text{I},2\text{-}^{14}\text{C}$, relative to liver as a standard (1.0).

●—● ^3H -activity. Each point is the average for five animals. X—X ^{14}C -activity in animals injected with ^{14}C -labeled choline. Each point is the average for 5 animals. ●---● ^{14}C -activity in animals injected with ^{14}C -labeled ethanolamine. Each point represents a single animal.

TABLE 1 EFFECT OF UNLABELED CHOLINE ON THE INCORPORATION OF CHOLINE- ^{14}C AND OF METHIONINE- ^3H INTO LIPID CHOLINE OF DIFFERENT ORGANS IN THE FEMALE RAT

Organ	Specific Radioactivity			
	^{14}C		^3H	
	Rat 1	Rat 2 (control)	Rat 1	Rat 2 (control)
	<i>cpm/mg choline</i>			
Liver	3,500	13,700	42,300	40,200
Plasma	2,940	13,400	24,900	29,300
Kidney	2,500	9,200	1,020	1,880
Small intestine	1,820	10,300	5,300	6,000
Lung	1,430	6,300	1,620	2,000
Heart	920	2,500	830	660

Each rat received $2,8 \times 10^6$ cpm of choline- $\text{I},2\text{-}^{14}\text{C}$ and $4,4 \times 10^6$ cpm of L-methionine- $\text{CH}_3\text{-}^3\text{H}$. Rat 1 was given 30 mg of unlabeled choline with the labeled precursors intraperitoneally, and subsequently 30 mg of choline every half hour until killed after 4 hr. Rat 2 received injections of 0.9% sodium chloride instead of unlabeled choline.

shown in Table 1. When relatively large amounts of unlabeled choline were given along with the radioactive choline and methionine, the incorporation of labeled choline was reduced to about one-fourth in all organs, while that of methionine was scarcely or not at all affected either in the liver or in extrahepatic tissues. A transfer in the form of phosphoryl choline is also unlikely, as the specific activity of liver phosphoryl choline increases only slowly after the injection of methyl-labeled methionine (Table 3).

Our results do not permit a definite distinction between the alternatives (b) and (c) mentioned above. The turn-over time of methionine in different tissues is unknown. The possibility of lecithin transfer must be given serious consideration, especially as a rapid equilibration of liver and plasma lecithin takes place (Fig. 4). A transfer of lecithin via plasma seems to take place in the laying hen, as it has been shown that egg lecithin is synthesized in the liver (13).

Source of Plasma Lecithin

Figure 4 shows that a rapid equilibration of liver and plasma lecithin takes place. This is in agreement with results obtained with radioactive phosphate (14). There seems to be no significant difference between lecithin synthesized by the methylating pathway and lecithin formed from free choline. The slightly more rapid appearance of methyl-labeled lecithin in plasma is probably explained by the observation that liver lecithin reaches its maximum specific activity earlier after the injection of methyl-labeled methionine than after the injection of labeled choline (Fig. 1). Lecithins synthesized by the two pathways thus seem to enter a common metabolic pool.

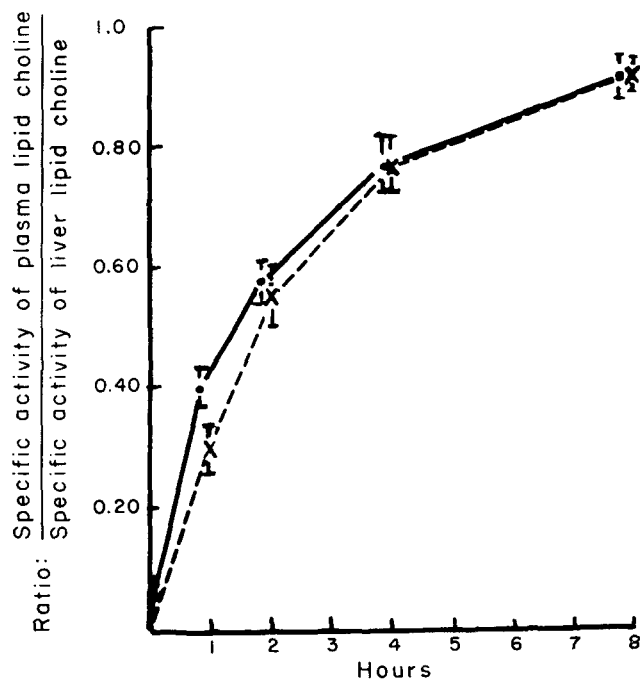


FIG. 4. Equilibration of plasma lecithin with liver lecithin in rats after intraperitoneal injection of methionine- $\text{CH}_3\text{-}^3\text{H}$ and choline-1,2- ^{14}C . Each point represents average values from five animals. Bars show the standard deviation of the mean.

●—● Ratio (^3H -specific activity of lipid choline in plasma): (^3H -specific activity in lipid choline in liver). X--X Ratio (^{14}C specific activity of lipid choline in plasma): (^{14}C specific activity of lipid choline in liver).

Table 2 shows the specific activities of phospholipid choline in different liver subcellular fractions at various time intervals after injection of the two lecithin precursors. The highest specific activity was found in the microsomes, both from labeled methionine and from labeled choline. The mitochondria had the lowest specific activity, while choline isolated from the phospholipids in the particle-free supernatant fraction showed an intermediate degree of activity. These results are in accordance with *in vitro* studies which have shown that both the methylating pathway (15) and the pathway utilizing preformed choline (16) are localized mainly in the microsome fraction of the liver cell.

Turnover Studies on Phosphoryl Choline and CDP Choline

The turnover of rat liver lecithin and of rat liver phosphoryl choline have been studied by Dawson (17) with radioactive inorganic phosphate as precursor. His studies indicated that the pathway via phosphoryl choline is not the only mechanism for lecithin biosynthesis. Whereas the methylating pathway represents one alternative, the calcium-stimulated incorporation of free choline into lecithin reported by Dils and Hübscher (5) may represent a third mechanism for lecithin formation. We found it of

interest, therefore, to study the incorporation of free choline into phosphoryl choline, CDP choline, and the phospholipids. We had found lecithin to reach its maximum activity in little more than 1 hr when labeled choline was injected (Fig. 1). If choline is incorporated mainly via phosphoryl choline and CDP choline, the turnover time of phosphoryl choline should be similar to the time required to obtain maximum incorporation of labeled choline into lecithin. We therefore determined the specific activity of phosphoryl choline, CDP choline, and lecithin in the liver of single animals at different time intervals after the injection of labeled choline and methionine.

Table 3 shows that choline is rapidly incorporated both into phosphoryl choline and into lecithin, and these results are compatible with the assumption that phosphoryl choline represents an obligatory intermediate in the biosynthesis of lecithin from free choline (17). The specific activity of CDP choline was, however, surprisingly low, although its specific activity was higher than that of lecithin in all the animals injected with choline- ^{14}C . In the animal killed after 80 min, a time at which maximum incorporation of choline into the lecithin usually is reached (Fig. 1), the total radioactivity in lecithin was 1.4×10^6 cpm. The specific activity of phosphoryl choline was 59,000 cpm/ μmole , only slightly lower than the specific activity of phosphoryl choline in the animal killed after 20 min. Thus, if phosphoryl choline is an obligatory intermediate in lecithin biosynthesis from choline, a total of 15–20 μmoles of phosphoryl choline must have been incorporated into lecithin in 80 min. The metabolic pool of CDP choline in rat liver is approximately 0.5 μmole (18). If this compound is an obligatory

TABLE 2 SPECIFIC ACTIVITY OF LIPID CHOLINE IN LIVER SUBCELLULAR FRACTIONS OF FEMALE RATS INJECTED INTRAPERITONEALLY WITH CHOLINE- ^{14}C AND METHIONINE- ^3H

Time after Injection	Isotope Injected	Specific Activity		
		Microsomes	Supernatant Fraction	Mitochondria
		<i>cpm/mg choline</i>		
10	^{14}C	1,200	800	500
	^3H	5,300	3,600	2,700
30	^{14}C	7,600	6,550	4,380
	^3H	17,200	13,550	11,700
60	^{14}C	16,000	11,000	8,400
	^3H	20,500	11,300	11,100
120	^{14}C	14,800	13,000	10,900
	^3H	18,300	13,400	10,100

4.4×10^6 cpm of L-methionine- $\text{CH}_3\text{-}^3\text{H}$ and 2.8×10^6 cpm of choline-1,2- ^{14}C were injected into each animal. The liver was homogenized in 10 volumes of 10% sucrose. Mitochondria were sedimented at $18,000 \times g$ for 10 min, resuspended and then sedimented at $8,000 \times g$ for 10 min. Microsomes were obtained by centrifugation at $100,000 \times g$ for 45 min. Total activities were not determined.

TABLE 3 INCORPORATION OF ETHANOLAMINE-¹⁴C, CHOLINE-¹⁴C, AND METHIONINE-³H INTO LIVER PHOSPHOLIPIDS AND PHOSPHOLIPID PRECURSORS IN MALE RATS

Compound Injected	Time	Total Radioactivity			Specific Radioactivity		
		Phosphoryl Choline	Lipids	Phospholipid Choline	Phosphoryl Choline	CDP Choline	Phospholipid Choline
	<i>min</i>		<i>cpm</i>			<i>cpm/μmole</i>	
11.6 × 10 ⁶ cpm choline-1,2- ¹⁴ C	20	1,200,000 (10.4%)	550,000 (4.8%)	552,000 (4.8%)	80,000	6,400	1,520
13.75 × 10 ⁶ cpm choline-1,2- ¹⁴ C	80	750,000 (5.4%)	1,370,000 (10%)	1,450,000 (10.5%)	59,000	6,900	4,020
13.75 × 10 ⁶ cpm choline-1,2- ¹⁴ C	270	250,000 (1.8%)	2,220,000 (16.1%)	2,185,000 (15.9%)	16,500	10,200	8,770
20 × 10 ⁶ cpm L-methionine-CH ₃ - ¹⁴ C	20	9,000 (0.05%)	1,840,000 (9.2%)	1,690,000 (8.5%)	530	6,500	6,400
20 × 10 ⁶ cpm L-methionine-CH ₃ - ¹⁴ C	80	34,000 (0.17%)	3,410,000 (17%)	3,205,000 (16%)	2,100	15,500	13,300
26 × 10 ⁶ cpm ethanolamine-1,2- ¹⁴ C	20	Phosphoryl Ethanolamine 4,150,000 (16%)	4,400,000 (16.9%)	—	Phosphoryl Ethanolamine 300,000	CDP Ethanolamine 400,000	—

Body weight of rats: 300–350 g.

intermediate between phosphoryl choline and lecithin in rat liver, the CDP choline must have turned over 30–40 times in the same time interval. In spite of this, the specific activity of CDP choline was found to be only one-eighth of the specific activity of phosphoryl choline.

There are two possible explanations of this observation: (a) choline and (or) phosphoryl choline is incorporated into lecithin by mechanism(s) bypassing CDP choline. As stated in the introduction, *in vitro* experiments have shown that there indeed exists a mechanism for the incorporation of free choline into lecithin bypassing both phosphoryl choline and CDP choline (5). The significance of this mechanism *in vivo* is unknown.

(b) The specific activity of CDP choline may be kept low by a rapid equilibration of CDP choline with preformed lecithin.

As calculated above, the one-way turnover rate of CDP choline must be relatively rapid to explain the incorporation of choline and of phosphoryl choline into lecithin. To explain the relatively low specific activity of CDP choline observed, therefore, the equilibration of this compound with lecithin must be extremely rapid.

To distinguish between these possible explanations, phosphoryl choline and CDP choline were isolated from livers of rats injected with methyl-labeled methionine. The specific activity of liver CDP choline from these animals was found to be nearly identical with that of liver lecithin, while the specific activity of phosphoryl choline was much lower. These results do indeed show that a rapid equilibration of CDP choline with liver lecithin takes place. Thus, the reaction of CDP choline with diglyceride must be freely reversible *in vivo*. In this connection it is of interest that the CDP choline level in the livers of choline-deficient animals is not reduced (18).

For comparison we also determined the specific activity of phosphoryl ethanolamine and CDP ethanolamine in the liver of a rat injected with labeled ethanolamine (Table 3). The results in this case were different from those obtained with labeled choline. The CDP ethanolamine was found to have a specific activity even higher than that of phosphoryl ethanolamine. The specific activity of phosphatidyl ethanolamine was not determined in our experiments, but considering the pool size of this phospholipid in the liver, 50–60 μmoles (1), it can be concluded with certainty that its specific activity was far below that of CDP ethanolamine. Thus, there is a distinct, unexplained difference between CDP choline and CDP ethanolamine as intermediates in phospholipid metabolism.

DISCUSSION

Equilibration of Lecithin Metabolic Pools

The results reported in the present communication indicate that lecithin synthesized by the different pathways in liver microsomes enters a common metabolic pool which is in rapid equilibrium with the plasma and possibly also with the lecithin in the cytoplasmic and mitochondrial compartments of the cell. A rapid equilibration of the phospholipids of different cell compartments has also been observed after the administration of labeled fatty acids (19). Phospholipid synthesized in the microsomes is also the most likely source of bile lecithin. It has been observed that after the injection of labeled phosphate, the bile phospholipids have a much higher initial specific activity than the average specific activity of the liver phospholipids (20).

The observation that plasma lecithin rapidly acquires the specific activity of liver lecithin after administration of methyl-labeled methionine, while the specific activity of lecithin in other tissues remains at a low level, confirms that the liver is the main source of plasma lecithin (14). As stated under Results, this does not exclude the possibility that a transfer of liver lecithin to extrahepatic tissues via the plasma takes place.

The synthesis of lecithin by transmethylation probably represents the only mechanism for choline synthesis in the animal organism (2). Our results strongly suggest that the liver dominates quantitatively in choline biosynthesis.

Cellular Localization of Lecithin Biosynthesis

There are several reports in the literature which suggest that lecithin can be synthesized in the mitochondria as well as in microsomes. Isolated mitochondria can incorporate fatty acids into lecithin (21), but it is doubtful whether this incorporation represents *de novo* synthesis as opposed to reacylation of lysolecithin formed by hydrolysis. It has been reported that phosphoryl choline can be incorporated into lecithin by mitochondrial preparations (22), but a possible contamination with microsomes was not excluded. Both phosphoryl choline, glycerophosphoryl choline and glycerophosphate have been found to stimulate respiration in isolated liver mitochondria, but the mechanism involved in these effects is unknown (23). It seems likely that phospholipid reactions are involved. On the other hand, the enzyme transferring phosphoryl choline to diglyceride (CDP choline:1,2-diglyceride cholinephosphotransferase, EC 2.7.8.2) has been found to be localized only in the microsomes (16). Our results on the incorporation of choline and of methionine methyl groups into the lecithin of cell subfractions (Table 2) are in agreement with the *in vitro* observations that lecithin is synthesized mainly in the microsomes, but they do not exclude the possibility of lecithin synthesis from these precursors in the mitochondria. The rate of incorporation of both precursors into the lipids of the mitochondria seems to be relatively rapid; if the incorporation were the result of extramitochondrial synthesis followed by transfer to the mitochondria, one would expect a more gradual rise in the specific radioactivity of the mitochondrial lecithin, as compared to the microsomal lecithin. One possible explanation of these results might be that newly synthesized lecithin enters a small extramitochondrial pool which is subsequently distributed to the different cell compartments. So far, no indications of the existence of such a pool have appeared. The question of possible lecithin biosynthesis in the mitochondria therefore must still be considered unsolved.

Quantitative Importance of the Methylating Pathway

An accurate estimation of the relative quantitative importance of the methylating pathway in lecithin biosynthesis is not possible from our data, but a tentative calculation can be done. In the Results section we have calculated that 15–20 μ moles of phosphoryl choline may have been incorporated into the liver lecithin of a rat (300 g body weight) in 80 min, i.e., 12–15 μ moles of lecithin are formed per hour. This calculation is based on the assumption that incorporation into lecithin is the only metabolic fate of phosphoryl choline and that choline is not incorporated by any mechanism bypassing phosphoryl choline. This estimate is, therefore, a maximum estimate. The liver of a rat weighing 300 g contains approximately 300 μ moles of lecithin (1). The turnover time of liver lecithin (estimated with radioactive phosphate) has been estimated to be approximately 20 hr (24). Our estimate thus agrees fairly well with this observation.

The formation of lecithin by methylation may similarly be calculated from the conversion of ethanolamine to choline. If we consider that the incorporation of labeled ethanolamine into phosphatidyl ethanolamine is instantaneous, that this labeled phosphatidyl ethanolamine is confined to the microsomal fraction of the cell, and that the phosphatidyl ethanolamine in this cell subfraction is metabolically homogeneous, we can make the following calculation from the data in Fig. 2. The total liver pool of phosphatidyl ethanolamine in a rat weighing 200 g is approximately 50 μ moles (1), half of which we assume to be in the microsomes. After 2 hr, approximately 30% of the ethanolamine incorporated into the liver lipids of the female rat had been converted to choline, i.e. 7–8 or 3–4 μ moles/hr (Fig. 2B). A similar calculation for the male rat will give about half this amount per hour. These may be minimum values, as some time is needed before maximum specific activity of the phosphatidyl ethanolamine is reached after the administration of labeled ethanolamine. Also, some of the labeled ethanolamine will be confined to the mitochondria where no (or less) methylation takes place.

Altogether, it seems justifiable to conclude that the methylating pathway for lecithin biosynthesis is of considerable quantitative importance in the liver. In comparison, it seems to be a pathway of minor importance in extrahepatic tissues.

CDP Choline and CDP Ethanolamine Turnover

The studies on the labeling of CDP choline and CDP ethanolamine revealed a peculiar difference in the behavior of these intermediates. As stated above, the low specific activity of CDP choline may be due to its rapid equilibration with lecithin. On the other hand, no cor-

responding phenomenon was observed with CDP ethanolamine. This may be due in part to the much smaller pool of phosphatidyl ethanolamine in the liver. As phosphoryl ethanolamine seems to be incorporated into the phospholipids at least as rapidly as phosphoryl choline (Table 3), the specific activity of the phosphatidyl ethanolamine pool will be relatively much higher than that of lecithin. If the equilibration of CDP ethanolamine with phosphatidyl ethanolamine in addition is relatively slow, the difference in behavior may be accounted for. However, it must be considered whether this phenomenon indicates the existence of alternative mechanisms for the incorporation of choline and (or) phosphoryl choline into lecithin.

The finding that administration of methyl-labeled methionine gives a rapid and relatively selective labeling of the liver lecithin pool, which subsequently is equilibrated with plasma lecithin, can be utilized to study the regulation of plasma lipoprotein synthesis.

This work was supported by grants from The Norwegian Council of Cardiovascular Diseases.

Manuscript received 1 June 1965; accepted 13 August 1965.

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