CHOLINE KINASE AND CHOLINE PHOSPHOTRANSFERASE IN DEVELOPING FETAL RAT LUNG

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SUMMARY: Determinations of lung choline kinase and choline phosphotransferase activities in developing rat fetuses have revealed that both enzymes increase three days before birth at 19 days gestation. This change is accompanied by a significant elevation in *de novo* lecithin synthesis through the choline incorporation pathway.

INTRODUCTION: Maintenance of alveolar stability and prevention of the Respiratory Distress Syndrome (Hyaline Membrane Disease) requires the presence of adequate amounts of surface active phospholipids (surfactants) (1). The principal component of lung surfactant is choline phosphoglyceride (lecithin) (2). In order to avoid neonatal atelectasis, the basis of Hyaline Membrane Disease, it is essential for the fetal lung in late gestation to increase its capability for lecithin synthesis and elevate the concentration of intracellularly stored phospholipids (3,4). Thus, prior to birth, lecithin synthesis in fetal lung must be carefully controlled such that acceleration is initiated at the proper time in gestation.

Two pathways exist in lung and other tissues for *de novo* lecithin synthesis, *viz.*, the choline incorporation pathway and phosphatidyl ethanolamine

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methylation; studies by ourselves and others suggest that the former is predominant (3). In addition, we have shown (5) in fetal rabbit lung that the terminal enzyme of the choline pathway, choline phosphotransferase (CPT, CDP choline: 1,2-diglyceride choline phosphotransferase, EC 2.7.8.2), is induced by corticosteroids resulting in an augmented pathway rate and earlier enhancement of lecithin production. In the present study, in normally developing rat fetuses, lung choline kinase (CK, ATP: choline phosphotransferase, EC 2.7.1.32), the first enzyme of the choline pathway, and CPT activities have been measured along with the rate of lecithin synthesis from choline.

MATERIALS AND METHODS: Pregnant Sprague-Dawley rats of timed gestation, obtained from Taconic Farms (Germantown, N.Y.), were anesthetized with ether for laparotomy. The fetuses were delivered by Caesarean section into chilled saline and were weighed in order to accurately determine gestational age (3). Fetal lungs were removed and either placed in chilled saline for a few minutes prior to slicing or frozen on dry ice for later enzyme assays. For measurement of lecithin synthesis *in vitro*, lung samples (pooled from several fetuses in many instances) were sliced on a Stadie-Riggs microtome and then placed in a flask containing 4 ml of Krebs-Ringer bicarbonate solution at pH 7.4. Following a 5 min pre-incubation, 2 μCi of ^{14}C-choline (Amersham/Searle, methyl-^{14}C-choline chloride, 60 mCi/mmole) were added and tissues were incubated for 1 hr at 37^{o} under 95% O_2/5% CO_2. Subsequently, lipids were extracted by the technique of Folch, *et al.* (6) and incorporation of isotope into lecithin was determined (5). Determinations of CK and CPT activities were performed on lungs frozen at -30^{o} for 1-2 weeks and homogenized in 4 volumes of 0.05 M Tris-HCl, pH 8.0 containing 5 mM EDTA and 10 mM dithiothreitol. Choline kinase activity was determined in lung homogenates by the technique of McCaman, *et al.* (7) with minor modifications. CPT was measured essentially as described elsewhere (5,8). Preliminary studies demonstrated that for both CK and CPT: a) product formation was linear with time up to 10 min, b) initial velocities were proportional to protein concentration, and c) substrate concentrations

employed were saturating. Lung protein concentrations were measured in homogenates by the method of Hartree (9) and were found to be virtually constant throughout gestation in agreement with the findings of others (10).

RESULTS: Measurement of ^{14}C-choline incorporation into lecithin with lung slices revealed a relatively constant rate from 16 to 19 days gestation. After 20 days, however, the rate of ^{14}C-lecithin production began to increase and, as shown in Table I, was twofold greater ($p<.001$) in term fetuses (22 days) as compared with those of 16-20 days gestation. Figures 1 and 2, based on

TABLE I

LECITHIN SYNTHESIS FROM CHOLINE

IN SLICES OF FETAL RAT LUNG

Gestational Age (days)	No.	^{14}C-Lecithin* (CPM/mg lung/hr)
16-20	8	1900 ± 111
22**	9	3908 ± 444

*Table includes mean ± SEM.

**Term fetuses.

analyses of over 600 fetal lungs, represent specific activities of lung choline kinase and choline phosphotransferase as a function of gestational age. It was found that CK activity rises ($p<.001$) from an average of 740 pmole/min/mg protein at 16-17 days gestation to 1440 at 19-20 days (the -3 to -2 day interval of Fig. 1). The specific activity then declines to a mean of 930 at term, and further to 500 in one-day-old newborn rats. Choline phosphotransferase (Fig. 2) increases from 39 pmole/min/mg protein six days before birth to 54 at 19-20 days gestation ($p<.02$). After reaching a peak mean figure of 60 at 20-21 days ($p<.001$), lung CPT declines as the fetus approaches term but does not continue to fall in the neonate.

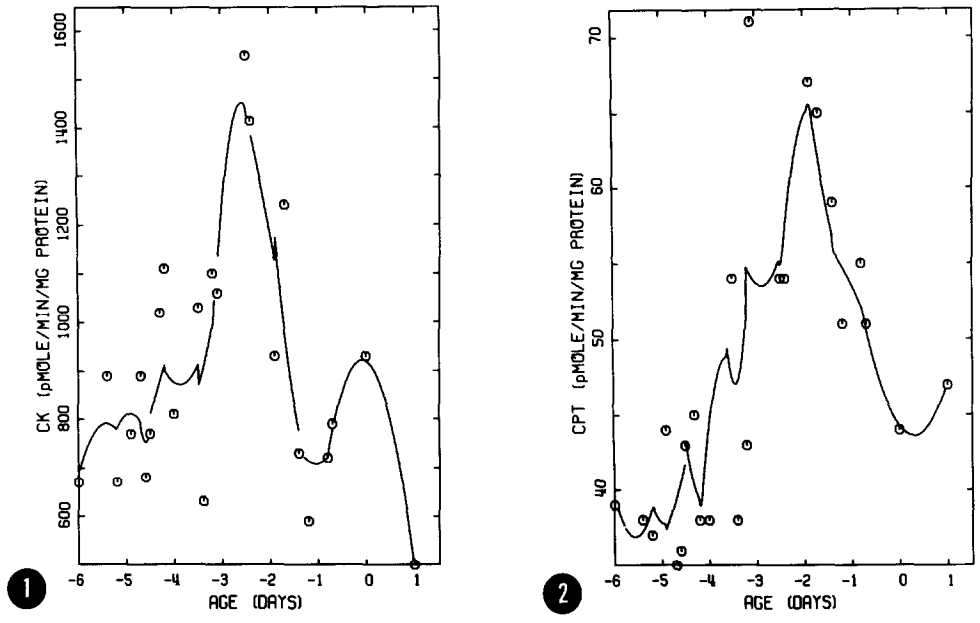

Fig. 1. Choline kinase in developing rat lung. Components of the 0.3 ml reaction mixture were 33 mM Tris-HCl, pH 9.0, 10 mM ATP, 11 mM $MgCl_2$ and 5 mM choline (1.7 μCi/μmole of ^{14}C-choline). Following a 6 min incubation at 37°, unreacted choline was removed as the reineckate salt by 2 ammonium reineckate precipitations and ^{14}C-phosphoryl-choline was determined by scintillation counting in Aquasol. Term gestation (22 days) is represented by day zero, and -6 corresponds to 16 days gestation. Curve was computer drawn for best fit with each point assigned a weight based on total number of determinations.

Fig. 2. Choline phosphotransferase activity in developing rat lung. Components of the 0.5 ml reaction mixture were 30 mM Tris-HCl, pH 8.0, 15 mM $MgCl_2$, 0.002% Triton X-100, 1 mM 1,2-dipalmitoyl glycerol, and 2 mM CDP-choline (0.6 μCi/μmole cytidine diphosphocholine, methyl-^{14}C). After 5 min incubation at 37° lipids were extracted with $CHCl_3$, washed twice with 2 M KCl, and ^{14}C-lecithin was determined by scintillation counting in toluene-PPO-POPOP. (See Fig. 1 legend for discussion of graphical methods.)

DISCUSSION: In order to account for increased pulmonary lecithin concentration found in late gestation with many species (3,4) a change from the steady state of lung lecithin synthesis, in early gestation, to a considerably higher level seems likely. The lung slice data presented herein are consistent with an enhanced rate of lecithin synthesis via the choline incorporation pathway in the last 10% of gestation (20-22 days). This, plus the fact that phospha-

tidyl ethanolamine methylation is relatively inactive in fetal lung (3), suggests that the former pathway accounts for increased pulmonary lecithin.

Conversion of choline to the phosphoglyceride involves three enzymatic reactions: 1) phosphorylation of choline, 2) activation of phosphorylcholine to the CDP-choline intermediate, and 3) incorporation of phosphorylcholine into 1,2-diacylglycerol to form lecithin (11). We postulated that the rate of one or more of these reactions increases to elevate the flux of choline in developing fetal lung (3). Since modulation of reaction rates is achieved mainly by control of enzymes catalyzing key reactions (12), we have examined this hypothesis in the present study by initially determining choline kinase and choline phosphotransferase activities. The results shown in Fig. 1 and 2 indicate that CK and CPT specific activities, presumably reflecting enzyme concentration, increase significantly after 19 days gestation. The peak for CK is reached one day before the CPT peak and both enzymes decline as the fetus approaches term.

Weinhold, *et al.* (13) recently reported similar elevations at approximately the same gestational age for CK and CPT of fetal rat liver. For fetal lung, however, they observed relatively constant activities throughout gestation (10). The discrepancy between the latter findings and those reported herein may be attributable to differences in methodology since Weinhold and associates: 1) employed different tissue preparations (a microsome-free system for CK and microsomes for CPT), and 2) did not measure maximal velocities, the reactions being monitored at non-optimal pH with apparently non-saturating concentrations of choline and CDP-choline.

The two enzymes measured in this study exhibit many of the features proposed by Weber (14) as characteristic of key catalysts in a metabolic sequence (3). Our results suggest that choline kinase and choline phosphotransferase may play an important role in the initiation of enhanced lecithin synthesis through the choline pathway of fetal lung.

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