Perinatal Rat Lung Retinol (Vitamin A) and Retinyl Palmitate

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ABSTRACT. The potential role for retinol (vitamin A alcohol) in the differentiation of the developing lung prompted this study in the perinatal rat. High performance liquid chromatography was used to separate, detect, and quantitate retinol and retinyl palmitate in lipid extracts of tissue and serum. Fetal and maternal blood showed the presence of retinol, whereas no retinyl palmitate was detected. On the other hand, fetal and postnatal lungs contained retinyl palmitate as well as retinol. Considerable changes in the content of lung retinyl palmitate were found during lung development. Fetal lungs (17–21 days of gestation) contained 2.3 ± 0.36 µg/g wet weight (mean $\pm$ SD) of retinyl palmitate and 0.14 ± 0.05 µg/g of retinol. Lungs of pups (1–10 days old) contained much less retinyl palmitate, 0.63 ± 0.20 µg/g, whereas the amount of retinol was the same as in fetal lungs. The surprisingly high content of retinyl palmitate in fetal lung and its depletion after birth may be functionally related to retinol action in the developing lung. (*Pediatr Res* 18:1297–1299, 1984)

Abbreviation

HPLC, high performance liquid chromatography

Retinol (vitamin A) in lung was demonstrated over 50 years (15) ago, but the exact function of this nutrient in pulmonary metabolism and its potential relationship to neonatal lung development is only of recent interest.

Retinol is essential for differentiation and integrity of respiratory epithelial cells of upper airways (1, 25). Lung cytosol contains intracellular retinol and retinoic acid-binding proteins (17, 18); the quantity of the latter changes postnatally in the rat (17). Since one-fourth of the cells in the alveolar region of lung are epithelial (6), clinical studies on neonatal retinol status (3, 13, 19, 21) and retinol's possible relationship to neonatal lung disease (10, 20) are being assessed.

The ability of rat lung to accumulate retinol after large doses (15) poses questions concerning the existence of a lung storage form of vitamin A, its chemical character, and function. Adult rat lungs contained retinyl esters after intravenous injections of chylomicra labeled with radioactive retinyl esters. The major ester was retinyl palmitate (9). Recently, HPLC identified retinyl palmitate and smaller amounts of other retinyl esters in adult rat lung (2). Although it is well established that retinyl esters (mainly

palmitate) are storage forms of retinol in the liver which are depleted during vitamin A deficiency (for review, see Ref. 8), no information is available thus far about the presence and possible function of retinyl palmitate in the fetal lung.

The objective here was to study whether fetal lungs contain retinyl palmitate and to determine whether the amounts of retinol and retinyl palmitate change during perinatal development of rat lung.

MATERIALS AND METHODS

Chemicals. High purity spectral grade solvents of Burdick and Jackson Laboratories, Inc. (Muskegan, MI) were used. Synthetic all-*trans*-retinol, retinyl acetate, and retinyl palmitate were from Sigma Chemical Co. (St. Louis, MO).

High performance liquid chromatography. Retinol and retinyl palmitate were determined by HPLC using a programmable liquid chromatographic system (Spectra Physics; San Jose, CA). The UV spectrophotometer system was a ISCO-V⁴ variable detector (Lincoln, NE). The HPLC column was reversed-phase µBondapak C_{18} (10-µm particle size) stainless steel column, 3.9 mm i.d. $\times$ 30 cm (Waters Associates, Inc., Milford, MA). A modification of the system previously described was used (2). In our system, 100% methanol was used as the mobile phase to separate retinol, retinol acetate (added to some extracts as an internal standard), and retinyl palmitate. The flow rate was adjusted so retinol was eluted in the 5th min and retinyl palmitate in the 11th min. The flow rates were as follows: 0–5 min, 1.0 ml/min; 6 min, 1.5 ml/min; 7 min, 2.0 ml/min; 12 min, 1.5 ml/min; and 13 min, back to 1.0 ml/min.

Standard curves of peak heights at identical sensitivities of the instrument against known amounts of retinol and retinyl palmitate were used. The standard solutions of retinol and retinyl palmitate were prepared in 100% ethanol and quantitated using the extinction coefficient of $E^{1\%}_{1cm}$ for retinol of 1780 at 325 nm and for retinyl palmitate of 940 at 325 nm (5, 12).

Tissue source and extraction method. Disease-free pregnant albino rats were obtained from SASCO (St. Louis, MO). At various times, pregnant mothers were anesthetized with ether and individual fetuses were removed for dissection. The live fetuses were quickly decapitated, and the lungs were removed, placed on dry ice, and analyzed within 30–60 min.

Postnatal lungs were obtained by decapitation, quick dissection, and placing on dry ice. Occasional hemorrhagic lungs, which were obvious from their appearance and increased weight, were not used. While still alive under anesthesia, some maternal rats received a tracheostomy and were then placed on a rodent respirator (Model 680, Harvard Apparatus Co., Millis, MA). The chest cavity was then opened, the pulmonary artery was catheterized, and the lung was perfused with 10–12 ml of warm saline simultaneously with ventilation over a 2-min period. This resulted in white, virtually blood-free adult lungs.

Tissue was processed according to established methods for lipid extraction (7). The samples (approximately 100 mg wet weight) were homogenized by Polytron P-10 (Kinematika, Switzerland) for 1 min with 20 volumes of a mixture of $CHCl_3$:CH_3OH, 2:1, containing 50 μg/ml of butylated hydroxytoluene, and then filtered. One ml of the filtrate was added to 0.2 ml of 0.37% KCl, mixed, and then centrifuged to separate the layers. The top layer was removed. The bottom layer was evaporated under nitrogen. The residue was dissolved in 25 μl $CHCl_3$ and 25 μl MeOH, and an aliquot of not more than 25 μl was injected onto the HPLC column. The sample was protected from direct light by covering iced containers with aluminum foil. A typical HPLC chromatogram of retinol, retinyl acetate, and retinyl palmitate shows clear separation of standards (Fig. 1*A*). Serum was mixed with an equal volume 100% ethanol and extracted twice with 4 volumes of HPLC-grade hexane.

DNA determination of two to four lung homogenates at four different perinatal ages (in duplicate) was done by the diphenylamine method (4).

RESULTS

First, we have established that fetal lung contains, in addition to retinol, a considerable amount of material which eluted from the HPLC in the position of retinyl palmitate (Fig. 1*B*). No retinyl palmitate was detected in samples of fetal or maternal sera.

The amount of retinol and retinyl palmitate in extracts of lungs of rats of various perinatal ages was then determined. The pattern of retinol and retinyl palmitate content in prenatal and postnatal lung is shown in Figure 2. There was no apparent change in retinol content in lung from 17-day-old fetuses to 10 days after birth. The mean value was 0.14 ± 0.05 μg/g wet weight for 24 fetal lungs and 0.11 ± 0.02 μg/g for 17 neonatal lungs. The data points from three litters at 17-day gestation are only suggestive that the amount of retinol was lower (0.09 μg/g). The amount of lung retinyl palmitate changes abruptly after birth. In the 24 fetal lung samples of 17–20.5 days gestation, the mean
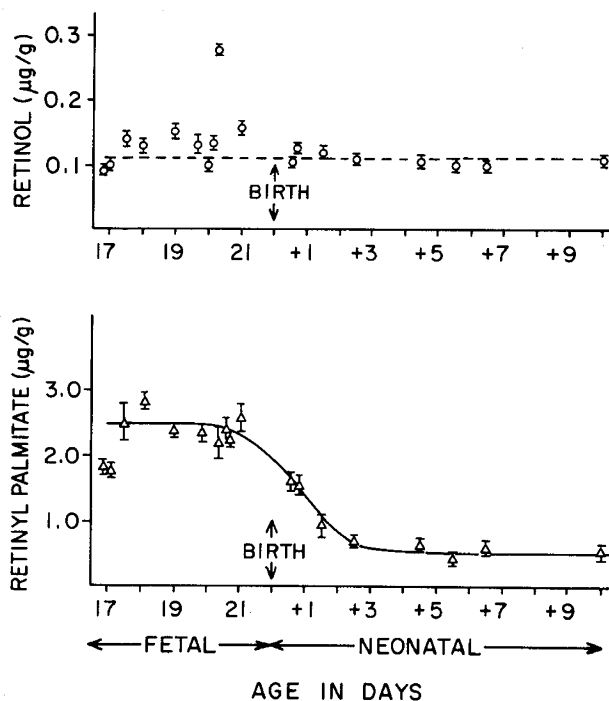
Fig. 2. Retinol and retinyl palmitate in developing lung. A total of 11 fetal (two of three 17-day values are identical) and eight postnatal litters were analyzed. The *points* show mean ± SD of three separate littermates. After 20 days gestation and postnatally, only one lung was needed from each fetus. At 18–20 days, 1.5–3 lungs were pooled. At 17 days gestation, 7–9 fetal lungs of the same litter were required, and the *point* is a mean of two separate determinations.

Table 1. *Lung retinol and retinyl palmitate content based on lung DNA*

Day	Retinol	Retinyl palmitate
Fetal	(μg/mg DNA)*	
18–19	0.06	1.0
20	0.07	1.2
Postnatal		
1.5–4	0.06	0.29
10	0.07	0.35

* Mean values of 1 litter at each age.

was 2.3 ± 0.36 μg/g in comparison to 0.63 ± 0.20 μg/g in 17 lungs 1.5–10 days after birth ($p < 0.001$). This difference in retinyl palmitate content is as striking when calculated per mg lung DNA (Table 1).

DISCUSSION

Since this is the first report on the presence of retinyl palmitate in fetal lung, the quantitation by HPLC used here must be compared with data on adult lungs. Our method used on blood-free adult rat lung gave retinyl palmitate (2.5–4.0 μg/g) values that compare favorably with those reported earlier for total lung retinol (1.5 μg) (15) and the recent report (8 μg/g) using HPLC (2). Our interest was only in perinatal retinyl palmitate. Therefore, longer retention times were not used that would assay retinyl stearate, an ester that accounts for about one-quarter of adult rat lung retinyl ester (2, 9). The HPLC method used here was clearly reproducible. Analysis of four separate lungs from the same litter or analysis of six lungs from two separate but identical gestation litters on the same day gave a standard deviation of within 12–14% of the mean. Finally, analysis of fetal livers using this method showed retinyl palmitate at 17 days gestation of 6.5 μg/
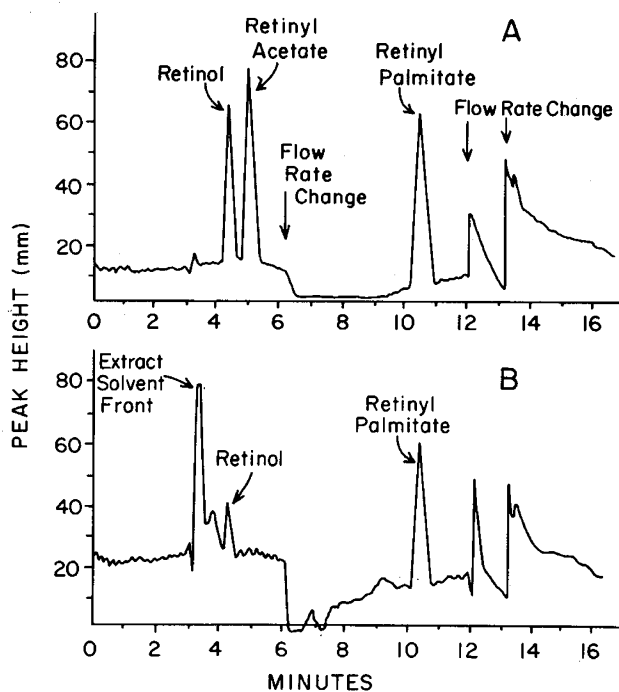

Fig. 1. HPLC chromatograms *A*, separation of standards used for the determination of rat lung retinol and retinyl palmitate (retinyl acetate was added as an external standard). *B*, separation of retinol and retinyl palmitate in a fetal lung extract prepared as described in "Materials and Methods."

g, at 20 days gestation it was 11 μg/g, and in liver of 1.5-day old pups it was 24 μg/g. Over 95% of the total liver retinol extracted was present as retinyl palmitate. These values are in close agreement with the recent values of Ismadi and Olson (11) on total retinol in the livers of the 20-day rat fetus of 5–7 μg/g, and the suckling 1.5-day rat pup of 20μg/g.

Our results show that fetal rat lung contains 15–20 times more retinyl palmitate than free retinol, but the ratio abruptly falls to only 6–8 times as much 1–10 days after birth (Fig. 2). Although the water content of lung would obviously be different in the fetal and postnatal lung, the marked differences in perinatal lung retinyl palmitate in comparison to retinol are additionally documented when based on lung DNA at four separate perinatal ages (Table 1). There are at least two possibilities to explain the presence of such high fetal lung retinyl palmitate and its rapid decrease at birth. Firstly, this could represent general nonspecific storage of retinyl ester derived in some form from the maternal circulation. That source would end with birth. In the presence of limited reserves of retinol, a greater portion of the total body retinol (as retinyl ester) is found in kidney and possibly other organs with lesser amounts in liver (14), and it is probable that limited reserves are present in both fetal mice and rats (16). Little placental transfer of maternal blood retinyl palmitate should occur since the major form of the vitamin in maternal plasma is retinol bound to retinol-binding protein (22). Indeed, we could not detect retinyl palmitate in fetal blood, maternal blood, or in placenta (data not shown). If there were placental esterification of retinol and rapid release of retinyl palmitate, this ester should be detected in fetal blood. This appears not to be the case since we could not demonstrate retinyl palmitate in fetal blood. There also would be no retinyl palmitate expected in fetal blood due to the absence of a postprandial absorptive state in the fetus itself. Although retinol has been noted in human amniotic fluid, no esters were found (24). All these observations suggest that fetal lung esterifies retinol.

A second reason to explain the high fetal lung retinyl palmitate stores is that they are functionally competent. Whether the fetal liver has the ability to release retinol into the blood stream to make it available for other vitamin A-dependent organs is not known. The possibility of a certain autonomy of the lungs which is not dependent on the supply of liver retinol is an attractive but yet unsolved one. If such were the fact, the fetal lung has apparently sufficient local vitamin A stores necessary for providing and maintaining essential lung epithelial differentiation and integrity during the perinatal period in which the alveoli are formed (23). The change in lung retinyl palmitate is remarkably different than what occurs in liver. We have observed that as perinatal age changed from the 17-day fetus to the 1.5-day-old neonate, the lung retinyl palmitate decreased 4-fold (2.3 to 0.6 μg/g), but the liver retinyl palmitate increased 4-fold (6.5 to 24 μg/g). Other reports confirm this rapid rise in perinatal rat liver retinyl esters (11). The rapid decrease in lung retinyl palmitate at birth is also interesting in that it coincides with the postnatal rise in lung cellular retinoic acid-binding protein (17). Meanwhile, there was an obvious steady level of perinatal lung retinol (Fig. 2), paralleling unchanging levels of cellular retinol-binding protein pre- and postnatally (17). Hydrolysis of stored retinyl palmitate to retinol and further oxidation to metabolically active retinoic acid could be important during the perinatal period.

The detection of retinyl palmitate in fetal lungs reported here poses the question of the functional role of the retinyl esters in lungs. Until now, liver has been emphasized as being the primary source of retinol for the retinol-dependent organs (8). A great deal is known about retinol metabolism in vitamin A deficiency, but several questions remain unanswered, including how retinol stores are mobilized in normal vitamin A-sufficient animals. The presence of a considerable amount of fetal lung retinyl palmitate and its depletion during perinatal development suggests that these stores are physiologically functional and the utilization of these stores in lung development might be independent of liver vitamin A.

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