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DEPLETION OF RETINYL ESTERS IN THE LUNGS COINCIDES WITH LUNG PRENATAL MORPHOLOGICAL MATURATION

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SUMMARY: Close to birth rat fetuses have lungs which are depleted in retinyl esters. Glucocorticoids administered to pregnant rats accelerate this process. We have investigated changes in fetal lung levels of retinol and retinyl palmitate and accompanying morphological changes after administration of dexamethasone to pregnant rats on day 18 of pregnancy. Here we show that this depletion temporarily coincides with prenatal morphological maturation of the lungs. The data presented support the idea that the maturational effect of glucocorticoids in the developing lungs is linked to vitamin A metabolism.

The proper supply of maternal retinol (vitamin A) to the fetus is essential for normal development and maintenance of the pregnancy [for review see 1]. During the middle of the last third of gestation, a peak in retinyl ester concentration in the fetal rat lung occurs followed by a decline that continues postnatally [2,3]. Human neonates born prematurely have low serum retinol levels due to lack of transfer of maternal retinol [4] and altered pulmonary differentiation [5] resulting in chronic respiratory distress, chronic lung disease and bronchopulmonary dysplasia [for review see 6]. The effect of early postnatal retinyl palmitate supplementation was found to be beneficial in improving the retinol status and in decreasing morbidity in very low birth weight babies [7]. On the other hand, glucocorticoids administered prenatally accelerate lung maturation [8] and as we found recently also lower lung retinyl esters content [9].

In this study we investigated whether or not changes in fetal lung levels of retinyl palmitate and retinol after the administration of dexamethasone or saline to pregnant rats coincide with morphological changes in the prenatal lung.

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MATERIALS AND METHODS

Experimental Procedures: Disease-free vitamin A-sufficient Sprague-Dawley rats of dated pregnancy were obtained from Sasco Company (St. Louis, MO). Four mothers were randomly selected to receive 4 μg dexamethasone/gram body weight (Dexamethasone Sodium Phosphate, American Regent Labs) by intraperitoneal injection and the other four were given the same volume of normal saline. The mothers were injected on day 18 of pregnancy. Following ether anesthesia and midline celiotomy, fetal lungs were sampled from a pair of mothers, randomly selected, one from each group, starting at 4 hours post-injection on day 18, then on days 19 and 21 of gestation and day 1 after birth. Individual fetuses removed for dissection were weighed, measured and decapitated. All the fetal lungs from a litter were pooled. A dissecting microscope was used for dissection of fetal lung. Fetal lungs were taken for histological staining and the remaining samples were frozen in liquid nitrogen and stored for biochemical analysis.

Tissue Preparation and Light Microscopic Examination: Samples of fetal lung were fixed for 24 hours in Perfix (Fisher Scientific Company), transferred to 80% ethanol, embedded in paraffin and stained with hematoxylin and eosin. Parameters used in examination for maturation include the degree of invasion of mesenchyme with uncommitted airways and the increase in the number and size of saccules [10].

Biochemical Procedures: Retinyl palmitate and retinol concentrations were determined by high performance liquid chromatography as previously described [2]. Each sample (approximately 200 mg wet weight) was homogenized by Polytron PT10 (Kinematica, Luzern) for 1 min with 20 vol of a mixture of chloroform:methanol (2:1), containing 50 μg/ml of butylated hydroxytoluene, and then filtered using a Qualitative #4 filter paper (Whatman International, Maidstone, UK). One milliliter of the filtrate was added to 0.2 ml of 0.37% KCl, mixed, and then centrifuged at 1,000 g for 10 min at 4°C. The centrifugation resulted in separation of the mixture into 2 layers. The top layer was removed and discarded. The bottom layer was evaporated under nitrogen. The residue was dissolved in 50 μl chloroform and 50 μl methanol. A 10 μl aliquot of the resulting solution was injected into the column. All samples were shielded from light at all times.

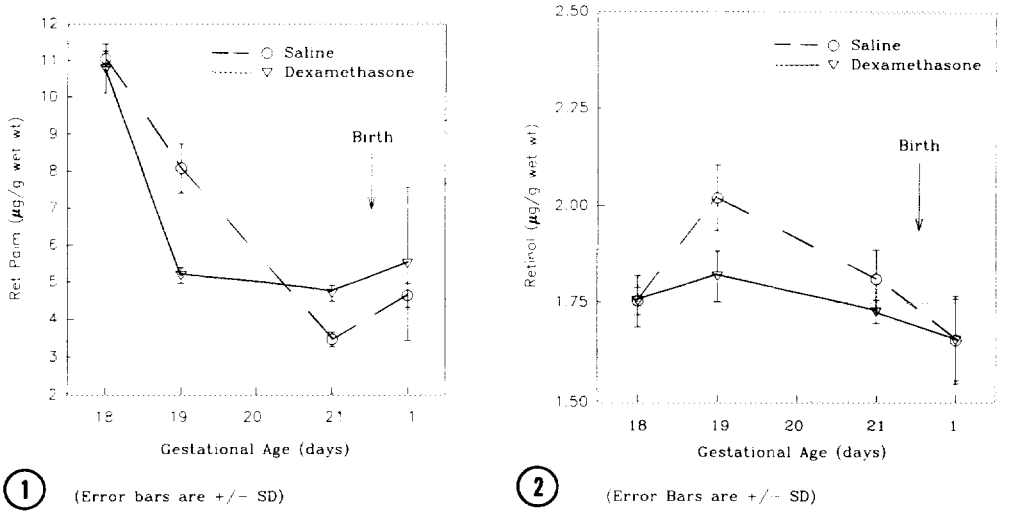
The concentrations of retinol and retinyl palmitate in each sample were determined in triplicate using a programmable liquid chromatographic system (Spectra Physics, San Jose, CA). The spectrophotometer system was a V^4 absorbance detector (ISCO, Lincoln, NE). The HPLC column was reversed-phase μBondapak C_{18} stainless steel column (10 μm particle size, 3.9 mm i.d. X 30 cm) (Waters Associates, Milford, MA). 100% methanol was used as the mobile phase to separate retinol and retinyl palmitate. The flow rate was adjusted to elute retinol in the fourth min and retinyl palmitate in the twelfth min [2].

Standard curves of peak heights at identical sensitivities of the instrument against known amounts of retinol and retinyl palmitate were used for quantitation. The standard solutions of retinol and retinyl palmitate were prepared in 100% ethanol and quantified using the extinction coefficients of E(1%, 1 cm) for retinol of 1789 and for retinyl palmitate of 940, both at 325 nm as previously described [2]. The results are expressed per unit wet weight.

RESULTS

Fetal lung retinyl palmitate levels of saline-treated control rats declined between days 18 and 21 of the gestation as seen in Fig. 1. However, fetal lung retinyl palmitate levels of dexamethasone-treated rats exhibited a more rapid decline between days 18 and 19 (see Fig. 1). On the other hand, there was little change in retinol levels as shown in Fig. 2. At day 18, the retinol levels of both saline- and dexamethasone-treated fetal lungs are not different. During the period between days 18 and 19, retinol levels increased in both groups. However, the retinol level of dexamethasone-treated fetal lung was lower by approximately 0.20 μg retinol/g wet weight. The retinol level remained lower through day 1 postnatally.

Interestingly, the decline in retinyl palmitate levels in the fetal lungs of controls occurring between days 18 and 21 coincides with an increase in invasion of mesenchyme by uncommitted airways and an increase in the number and size of saccules (Fig. 3 A, B). Fetal lungs from dexamethasone-treated mothers revealed similar changes earlier, i.e. between days 18 and 19, which coincided with the rapid decline in retinyl palmitate stores (Fig. 3 C, D).



(1) (Error bars are +/- SD)

(2) (Error Bars are +/- SD)

Fig. 1. Retinyl Palmitate in Perinatal Lungs. Note the decline between days 18 and 19 in fetal lungs after administration of dexamethasone to the mother.

Fig. 2. Retinol in Perinatal Lungs. Note that the retinol level in fetal lungs after administration of dexamethasone to the mother is lower than in saline treated at days 19 and 21.

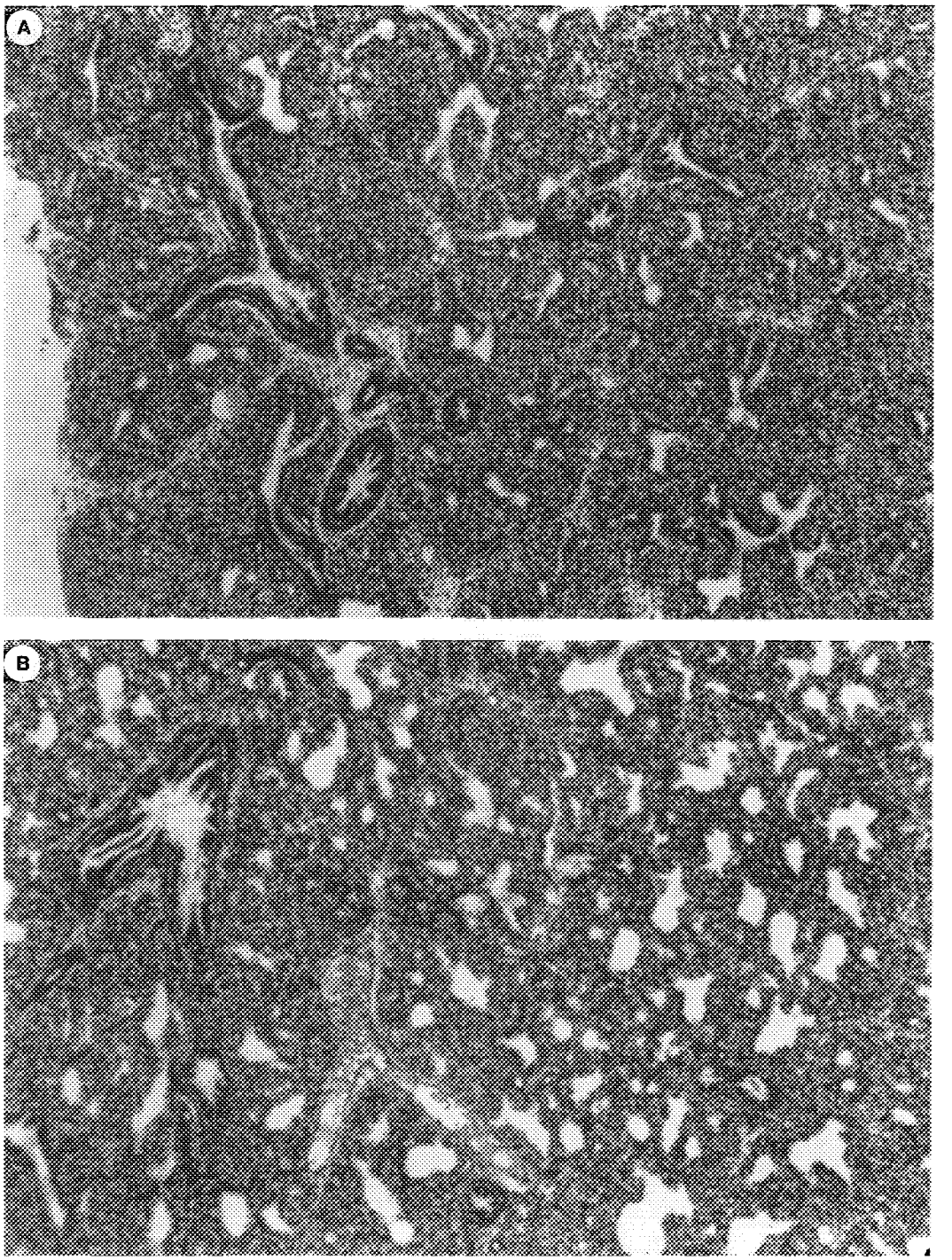


Fig. 3. Fetal Lung Histology. Saline-treated: A. Day 18. B. Day 19. Dexamethasone-treated: C. Day 18. D. Day 19.

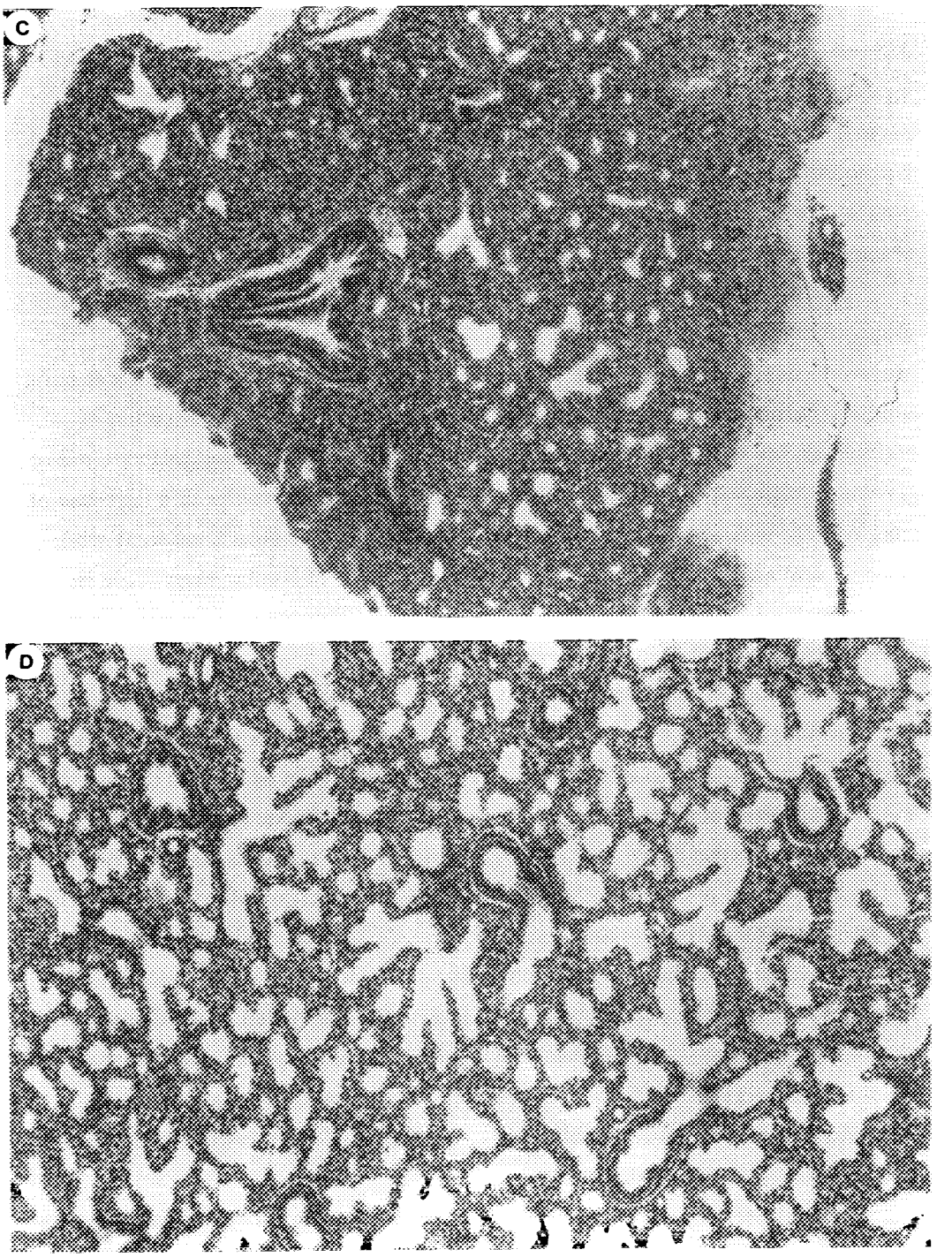


Fig. 3 - Continued

DISCUSSION

Vitamin A (retinol) plays an essential role in the process of cellular differentiation [11]. The respiratory tract is among the first tissues affected by vitamin A deficiency, characterized by a predictable sequence of alterations in normal epithelial morphology from basal cell metaplasia to necrotizing tracheobronchitis to keratinizing squamous cell metaplasia [for review see 1]. Similar histopathological findings may be seen in the tracheobronchial epithelium of very low birth weight [5] human neonates.

During prenatal pulmonary development the lungs are also the target for glucocorticoid action. Indeed, the lungs contain nuclear receptors for both glucocorticoids and retinoic acid [1,12,13]. In addition, the lungs contain a specific binding protein called cellular retinol-binding protein [14]. Administration of retinoic acid to rats results in an increase in lung levels of cellular retinol-binding protein mRNA, whereas administration of dexamethasone results in a decrease of lung cellular retinol-binding protein mRNA [15]. Simultaneous treatment with retinoic acid and dexamethasone results in a decrease of similar magnitude in cellular retinol-binding protein mRNA as well [15]. This suggests some interplay between retinoids and glucocorticoids in the lungs.

The stimulus responsible for the prenatal depletion of retinyl palmitate from the fetal lungs is unknown. The results presented here show that administration of dexamethasone to the mother accelerates this depletion as well as the morphologic development of the lungs. This invites the idea that the release of endogenous glucocorticoids may be responsible for this process. As a consequence of the lowering of retinyl palmitate levels, greater utilization of retinol necessary for lung maturation may occur.

Moreover, it is quite possible that glucocorticoids inhibit the process of esterification where cellular retinol-binding protein is involved [16]. Consequently, the repressive effect of dexamethasone on the expression of the cellular retinol-binding protein gene could be a part of the mechanism inhibiting retinol esterification which in turn would lead to formation of more retinol, and subsequently, its higher utilization.

In summary, we speculate that the effect of dexamethasone on perinatal rat lung maturation is mediated by retinoids. Dexamethasone appears to accelerate the depletion of retinyl palmitate stores and pulmonary maturation between gestational days 18 and 19 in the fetal rat. The lower level of retinol in

dexamethasone-treated fetal lung at day 19 may be the result of increased utilization of retinol into developing tissues.

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REFERENCES

1. Chytil, F. (1992) J. Physiol. L517-L527.
2. Zachman, R.D., Kakkad, B., and Chytil, F. (1984) Pediatr. Res. 18, 1297-1299.
3. Shenai, J.P., and Chytil, F. (1990) Biol. Neonate. 57, 126-132.
4. Shenai, J.P., Chytil, F., Zhaveri, A., and Stahlman, M. (1981) J. Pediatr. 99, 302-305.
5. Northway, W.H. Jr., Rosan, R.O., and Porter, D.Y. (1967) N. Engl. J. Med. 276, 357-368.
6. Stahlman, M.T., and Gray, M.E. (1987) In Prenatal and Perinatal Biology and Medicine (Kretchmer, Quilligan and Johnson, Eds.) pp. 262-310.
7. Shenai, J.P., Kennedy, K.A., Chytil, F., and Stahlman, M.T. (1987) J. Pediatr. 111, 269-277.
8. Liggins, G.C., and Howie, R.N. (1972) Pediatrics 50, 515-525.
9. Shenai, J.P., and Chytil, F. (1994) Int. J. Vit. and Nut. Res. 64, in press.
10. Burri, P.H., Dbaly, J., and Weibel, E.R. (1974) Anat. Rec. 178, 711-730.
11. Wolbach, S.B., and Howe, P.R. (1925) J. Exp. Med. 42, 753-777.
12. Toft, D., and Chytil, F. (1973) Arch. Biochem. Biophys. 157, 464-69.
13. Ballard, P.L. (1977) Fed. Proc. 36, 2660-2665.
14. Bashor, M.M., Toft, D.O., and Chytil, F. (1973) Proc. Natl. Acad. Sci. USA 70, 3483-3487.
15. Rush, M.G., Haq, R., and Chytil, F. (1991) Endocrinology 129, 705-709.
16. Ong, D.E., MacDonald, P.N., and Cubitosi, A. (1988) J. Biol. Chem. 263, 5789-5796.