SPECIALIA

Pulmonary di-and-triacylglycerols during the perinatal development of the rat

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Summary. Diacylglycerol (DG) and triacylglycerol (TG) levels in rat lung tissue were determined from day 17 of gestation to day 10 post partum and studied in parallel with ultrastructural differentiation. The DG level, although rather low at all measured stages, rose significantly between days 17 and 19 and at birth. TG level increased steadily during the whole studied period and especially between days 17 and 19 and at birth. In DG as well as in TG, saturated fatty acids were predominant. The rising of TG levels paralleled the appearance and accumulation of lipid vacuoles in mesodermal cells lying in contact with type II cells. The possible role of these cells is discussed.

Lung surfactant is characterized by a very high phospholipid content and, therefore, the pathways of phospholipid synthesis have been extensively investigated both in adult and in fetal lung[1].

Similarly, the surfactant synthesizing cells, the type II pneumocytes, have been thoroughly studied in a number of species[2]. By contrast, very little attention has been given so far to neutral lipids of the lung and to the cells where these compounds are stored. These lipofibroblasts are interstitial cells, filled with lipid vacuoles[3-7] and lying between blood capillaries and alveolar epithelium.

In the present work, we examined the evolution of diacylglycerol and triacylglycerol levels in perinatal rat lung as well as their fatty acid composition; the data were studied in parallel with the morphological evolution of lipofibroblasts.

Sprague-Dawley female rats were mated overnight and the next morning was considered as day 0 of gestation. 17-, 19-, 20- and 21-day-old fetuses, newly born rats and 1-, 5- and 10-day-old pups were killed by decapitation. Their lungs were dissected and processed either for biochemical analysis or for electron microscopy. Total lipids were extracted with chloroform-methanol[8]. DG and TG were separated by TLC on activated silica-gel plates using petroleum ether-ether-acetic acid (75-24-1 v/v) as developing solvent. DG and TG areas were identified by comparison with check samples. Concentrations were measured enzymatically[9] using di/tri palmitin and di/tri olein for standard curves. Fatty acid determinations were performed on DG and TG aliquots by GLC as described previously[10]. Electron microscopic observations were always performed on the middle part of the right lung fixed according to Hirsch and Fedorko[11].

Electron microscopic observation showed that on day 17 of gestation the lung tissue was still completely immature: epithelial as well as mesodermal cells were undifferentiated (figure 1), and lipid vacuoles were very seldom found in the latter. On day 19 of gestation, whereas lamellar bodies appeared in some epithelial cells, lipid vacuoles became more frequent in the mesodermal cells lying in the vicinity. From day 19 and especially from birth on, the number and volume of lipid vacuoles in each individual lipofibroblast considerably increased (figure 2).

Biochemical results showed that the DG levels remained rather low (figure 3); they represented 0.4–1.40% of the total lipid pool, according to the stage. As DG represent a key precursor in the synthesis of most phospholipids as well as TG, they might not accumulate in large quantities[12]. Nevertheless, significant increases occurred between days 17 and 19 ($p < 0.01$) and at birth ($p < 0.01$). As also shown on figure 3, the TG level measured on day 17 was comparatively low, then it increased rapidly, especially between days 17 and 19 (3-fold) and at birth (2-fold). As seen from the table, fatty acid composition of both DG and TG was characterized by a high saturated fatty acid content, palmitic acid being the major one. This composition was different from that of acylglycerols of other fetal rat tissues such as

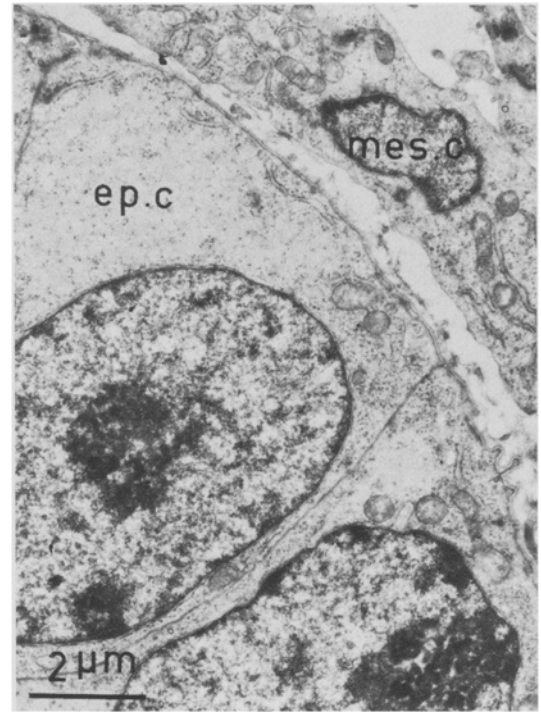

Fig. 1. Lung tissue of a 17-day-old rat. Epithelial as well as mesodermal cells still look immature. In epithelial cells, no lamellar bodies are found and glycogen is accumulating (the latter appears as irregularly bright granules, following treatment with Hirsle and Fedorko's fixative). In mesodermal cells, lipidic vacuoles are still very seldom found. ep.c, epithelial cell; mes.c, mesodermal cell. × 7500.

Fatty acid composition of diacylglycerols and triacylglycerols expressed as percentage of total fatty acids. All values are given as mean ± SE

Age in days	Number of litters	Fatty acids: Myristic acid C_{14}:0 DG	TG	Palmitic acid C_{16}:0 DG	TG	Stearic acid C_{18}:0 DG	TG	Oleic acid C_{18}:1 DG	TG	Total saturated fatty acids DG	TG
17	5	10.5±0.1	5.3±0.2	46.7±1.0	48.7±0.4	20.8±0.9	19.8±1.0	16.9±1.1	20.0±1.2	78 ±0.4	74 ±1.4
19	6	3.8±0.5	2.1±0.3	37.6±1.1	35.7±1.3	14.6±0.8	12.1±0.7	28.5±2.2	32.6±0.8	56.1±1.1	50 ±1.9
20	7	4.1±0.9	2.3±0.3	39.8±4.1	45.4±2.6	16.6±0.7	12.2±1.3	25.2±0.4	27.8±2.5	60.5±2.6	59.9±2.3
21	5	5.7±0.6	4.3±0.6	46.0±1.2	46.2±0.7	16.3±0.9	10.8±0.3	21.9±1.0	28.3±0.5	68 ±0.9	61.3±1.0
Birth	7	2.5±0.9	2.0±0.3	38.0±3.0	44.0±0.7	15.2±1.2	12.7±0.9	20.2±2.1	28 ±0.3	55.7±2.9	58.7±1.7
1	6	2.8±0.2	3.6±0.5	47.2±3.3	44.1±1.7	16.8±1.2	14.7±0.3	22.7±1.7	32.2±2.1	66.8±3.2	62.4±2.5

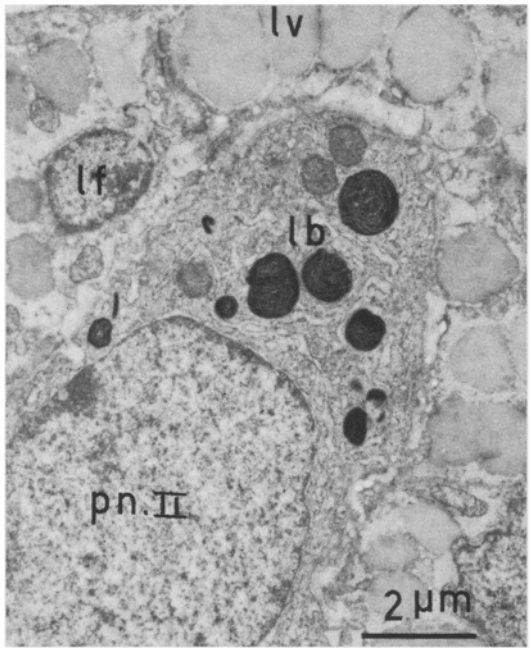

Fig. 2. Lung tissue of 5-day-old young rat. Mesodermal cells (lipofibroblasts) lying in contact with type II cells are loaded with lipidic vacuoles. lb, lamellar body; lb, lipofibroblast; lv, lipidic vacuole; pn. II: type II pneumocyte. × 7500.

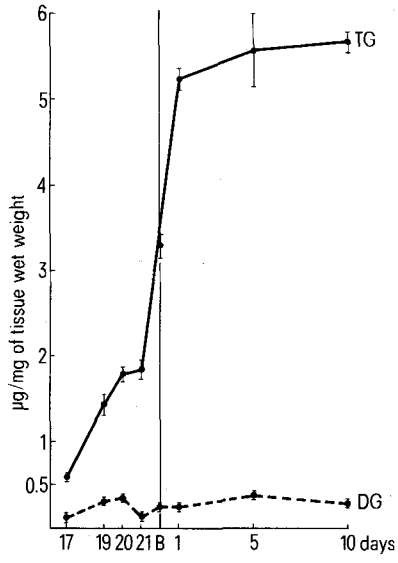

Fig. 3. Age related changes in the concentration of DG and TG in rat lung homogenates. The means and standard errors are presented. Each point represents at least 5 litters, or, in the case of suckling pups, at least 10 animals.

liver and plasma[13] and therefore appeared to be specific for lung acylglycerols. In addition, the DG and TG total saturated fatty acid levels decreased sharply between days 17 and 19 of gestation: in particular, palmitic acid which on day 17 accounted for about 46% of the total fatty acids, dropped to 37% and 35% on day 19 of gestation ($p < 0.01$). It is interesting to note that these changes correspond exactly to the onset of surfactant synthesis in type II pneumocytes on day 19.

In conclusion, these data show that the evolution of DG and, especially, TG levels parallels the gradual accumulation of lipid vacuoles in the lipofibroblasts. As indeed in postnatal lung the content of these vacuoles consists mainly of neutral lipids[6,7], lipofibroblasts represent a storage site of acylglycerols. For the time being, one can only speculate about the role of these cells and the significance of their lipid load. Among the various functions that lipofibroblasts might perform, some are most probably to their very specific localization; they very often lie in contact with capillary endothelium on one side and with type II pneumocytes on the other. Therefore, these cells may be regarded as possible mediators transmitting various precursors from the blood vessels to the type II pneumocytes. In particular, they might take up the free fatty acids and glycerol which are released from plasmatic chylomicrons and lipoproteins under the action of endothelial lipoprotein lipases[14,15]; then they might either transfer them to the type II pneumocytes, or store them intracellularly in newly synthesized TG. Thus the lipofibroblasts might participate in the regulation of surfactant metabolism by modulating the supply of phospholipid precursors from the blood stream to the type II pneumocytes.

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