# Fetal Rat Lung Development: Lipids and Surface Tension Properties After Decapitation *in utero*[1] (36572)

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During embryologic development, the mammalian lung undergoes a unique biochemical differentiation which allows certain pneumocytes to rapidly synthesize surface active agents which, when released into the alveolar spaces, reduce the surface tension to levels allowing normal lung dynamics to occur. The reduction of surface tension within the terminal respiratory spaces provides a stability to the lung which is critical for the survival of the newborn. These surface active substances, collectively termed "surfactants," are rich in phospholipids, the most important of which is dipalmityl lecithin (1–4). Recent studies suggest that adrenal corticosteroids and/or thyroid hormones may play fundamental roles in initiating or regulating the synthesis or release of these critically important surface active lipids in lung (5–9).

Most of the evidence, although indirect, suggests that type II pneumocytes are responsible for the synthesis of surfactant. Electron microscope studies of these cells indicate that the phospholipid component is synthesized by the endoplasmic reticulum and ultimately is packaged in homogeneous granules containing components derived from the Golgi apparatus (9–12). Multiple lamellae in tightly packed, highly ordered, geometrical arrangements develop within these granules as they reach maturity, hence the term "osmiophilic lamellar body" (OLB). Ultimately these granules are secreted, presumably by an exocrine process, onto the alveolar surface where the phospholipid component, in conjunction with other substances, forms a complex lining layer. This layer forms a structural interface between inspired air and the surrounding alveolar tissue which prevents collapse of the alveolus during periods of inspiration. At the molecular level, the major pathway for lecithin synthesis in lung involves the random acylation of diglycerides and condensation with cytidine diphosphocholine (13). Characteristically the fatty acids of lecithins with surfactant properties are highly saturated.

The factors which initiate or control the synthesis and release of surfactant are not well defined. Morphologic and biochemical studies suggest that both synthesis and secretion are exocrine phenomena influenced by hormonal and neurohumoral agents (14–16). Goldenberg, Buckingham and Sommers (14) propose that surfactant release may be under a vagal control system. Recent studies imply that in mature lung cortisone and thyroxine influence the rate of surfactant synthesis and release. In immature fetal lungs, cortisone is reported to stimulate surfactant production, thereby promoting the functional maturation of lung (5, 7, 8). These studies have raised the possibility that certain endocrine events may be responsible for either initiating surfactant synthesis or regulating its rate of synthesis or release.

The present experiments were performed to determine the effects of *in utero* decapitation on the biochemical and biophysical properties of developing lung. Decapitation in the rat early in fetal life results in a profound adrenal insufficiency and to a much lesser extent, thyroid deficiency (17–20). These effects are mediated primarily through the

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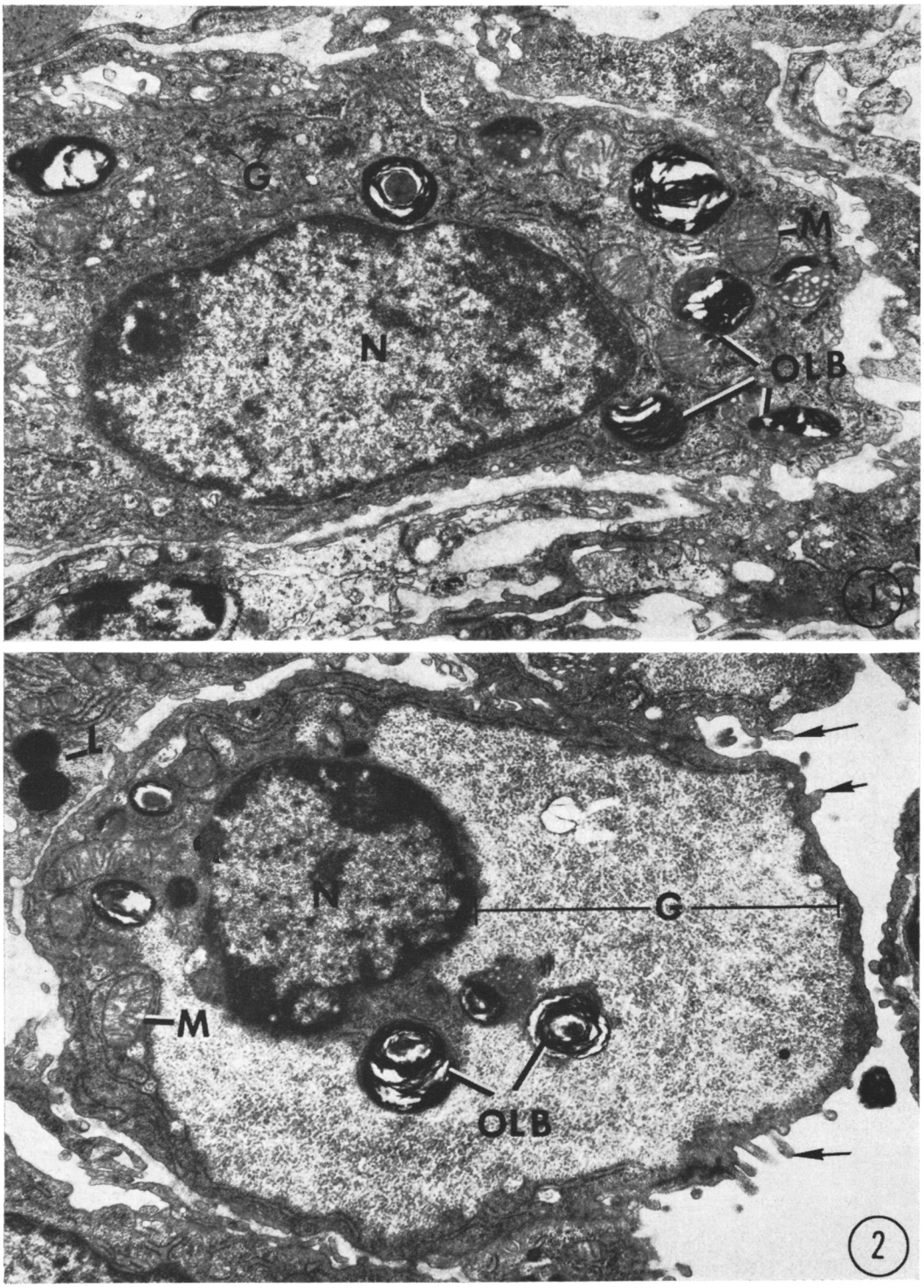


FIG. 1. Type II pneumocyte of full term rat fetus containing numerous osmiophilic lamellar bodies (OLB) in various stages of development. At this stage of cytodifferentiation, little glycogen (G) is present; however, ribosomal and endoplasmic reticular elements are relatively abundant. (N) Nucleus; (M) mitochondria.

FIG. 2. Type II pneumocyte from full term rat fetus decapitated on day 16 *in utero* demonstrating massive glycogen pool (G), few osmiophilic bodies (OLB) and relatively little endoplasmic reticulum. A few small surface microvilli (arrows) are present. (L) Lipid; N, nucleus.

fetal pituitary and are not a result of an absence of the brain or hypothalamus (17, 21, 22). Decapitation does not profoundly affect organogenesis or somatic growth.

*Materials and Methods.* Fetal rats were decapitated *in utero* during day 16 of gestation when the lungs were in the bronchial budding stage. Sham-operated litter mates served as controls. The fetuses were allowed to continue differentiation until term (day 22). The lungs from fully mature live fetuses were processed for light and electron microscopy, lipid composition, and surface tension reducing properties.

Lipids, extracted by the method of Folch, Lees and Sloane-Stanley (23) were dried under nitrogen and resuspended in chloroform–methanol (2:1) to a known volume. Phospholipids were separated from neutral lipids by subjecting a known aliquot from each sample to silicic acid chromatography (24). The phospholipids were dried under nitrogen and resuspended to a known volume in chloroform–methanol mixture. Phosphatidyl choline was separated by thin layer chromatography (25). Following visualization and direct mineralization by sulfuric acid, the phosphorus content was determined for phospholipid quantitation (26). Both total phospholipid and phosphatidyl choline samples were run in triplicate. Phospholipid weights were calculated on the basis of P $\times$ 25. An aliquot from pooled phospholipid extracts was methylated (27) and analyzed for fatty acid composition. Peak areas were measured by computing peak height times the width at half height. The percentage of the saturated fatty acids is taken from acids with chain lengths 14:0, 16:0, 18:0.

Maximum ($\gamma_{max}$) and minimum ($\gamma_{min}$) surface tensions were determined on a modified Wilhelmy balance (28) from lung minces prepared by pooling 2 g whole lung in physiological saline (10 ml/g tissue). The crude lung extract was passed through gauze (to remove tissue debris) into a clean Teflon trough and allowed to age for 10 min. The material was then cyclically compressed and expanded twice from 100 to 15% area; the second cycle was recorded. Duration of one full cycle was 9 min.

*Results.* Histological studies of the lungs of decapitated fetuses revealed an enlargement of the alveolar spaces. The size, shape and number of pneumocytes did not appear to be altered. These findings indicate that decapitation does not greatly influence the histogenesis of lung or that patency of the trachea or swallowing is necessary for alveolar space formation. At the level of the electron microscope (cf. Figs. 1 and 2), type II pneumocytes contained increased quantities of glycogen and relatively few OLB at all stages of differentiation (9). These changes suggest that decapitation results in impaired cytodifferentiation of type II pneumocytes and a decrease in surfactant production. Alterations in quantity or quality of intraalveolar lamellar materials were not observed.

The lipids in lungs of normal and decapitated fetuses at day 22 are shown in Table I. The lungs of decapitates showed a significant reduction in total lipids, phospholipids, and lecithin. The percentage of phospholipid fatty acids (PFA) (Table II) in these lungs were markedly altered with the major differences occurring in the C-16:0 and C-18 family. The degree of saturation of PFA in decapitated lungs was also diminished. The surface

TABLE I. Lipid and Surface Tension Measurements of Decapitated and Normal (Sham-operated) Fetal Lungs.

| | Av $\pm$ SE | |
|---|---|---|
| | Normal | Decapitated |
| Total lipid ($N = 7$) (mg/g wet wt) | 27.2 $\pm$0.935 | 15.3[b] $\pm$2.55 |
| Phospholipid ($N = 6$) (mg/mg lipid) | 0.451 $\pm$0.032 | 0.332[b] $\pm$0.011 |
| Lecithin ($N = 6$) (mg/mg phospholipid) | 0.580 $\pm$0.012 | 0.479[b] $\pm$0.018 |
| Surface tension | | |
| min $\gamma$ (dynes/cm) ($N = 6$) | 13.7 $\pm$1.18 | 19.5[a] $\pm$0.72 |
| max $\gamma$ (dynes/cm) ($N = 6$) | 29.9 $\pm$0.353 | 39.4[a] $\pm$1.43 |

[a] $p < .05$; [b] $p < .01$.

TABLE II. Percentage of Phospholipid Fatty Acids.[a]

| | 14:0 | 15:0 | 16:0 | 16:1 | 17:0 | 18:0 | 18:1 | 18:2 | % Sat. |
|---|---|---|---|---|---|---|---|---|---|
| Normal | 2.1 | 2.5 | 63.7 | 7.7 | 0.8 | 8.2 | 11.4 | 3.6 | 74.0 |
| Decapitated | 0.9 | 3.5 | 45.1 | 5.5 | 1.8 | 15.2 | 22.3 | 5.2 | 61.8 |

[a] First number of fatty acid designation is chain length; second is number of double bonds.

activity of lung minces from these animals failed to reduce surface tension to that of the normal values (Table I).

The results of these integrated morphologic, biochemical and biophysical studies indicate that the obliteration of the fetal pituitary early in lung differentiation does not greatly impair histogenesis. At the level of the electron microscope, however, type II pneumocytes appear immature, containing large quantitites of glycogen and relatively few OLB. This immaturity is further reflected by deficiencies in pulmonary lipids, especially phospholipids, and the inability of these lungs to effectively initiate and promote surface active properties.

Previous studies have shown that *in utero* decapitation of the rat results in a deficiency of ACTH, TSH and in turn, the hormones of the adrenal cortex and thyroid. A deficiency or absence of these hormones in the fetus leads to altered lipid metabolism characterized by an increase in the lipids of liver, blood and subcutaneous tissues (29–32). These effects are due primarily to adrenal corticosteroids and not thyroxine (33). Although growth hormone is also known to affect lipid metabolism, most studies suggest that a deficiency in this hormone does not occur in the decapitated rat fetus (17, 29–32). Collectively these data strongly imply that the alterations in lipid metabolism accompanying *in utero* decapitation are mediated through the pituitary–adrenal axis.

The observation that lung phospholipids are depressed in the decapitated fetus indicates that lipid metabolism in lung is unique and is largely independent of the mechanisms regulating lipid metabolism in the liver and possibly other organs. Other workers, using different model systems, have reached similar conclusions (34, 35).

Few studies of the influences of hormones on phospholipid metabolism have been reported, and these have concerned primarily the effects of pituitary hormones on target receptor cells (17–20, 36, 37). The action of these agents on phospholipid metabolism in lung has not been reported. Studies of the effects of thyroxine and cortisone on lung compliance and on the ability of lung minces to reduce surface tension indirectly imply that these hormones increase surfactant synthesis, its release, or half-life (5, 7, 8). Our data show that phospholipid synthesis may be initiated in the lung in the presence of adrenal and thyroid deficiency, but that these phospholipids do not accumulate to normal levels by the time of birth.

*Summary.* Decapitation *in utero* produces hormonal deficiencies which do not influence lung organogenesis but retards pneumocyte differentiation at the organelle and molecular levels. Such lungs contain decreased quantities of phospholipid and functionally are impaired in their ability to reduce surface tension to normal levels. The mechanisms by which these alterations are produced are most likely related to hormonal deficiencies during fetal development. These deficiencies may specifically alter lung phospholipid metabolism. Retardation of phospholipid metabolism may, however, be secondary to a more generalized retardation of pneumocyte differentiation. We favor the latter hypothesis in so far as pituitary failure occurring in man and animals with fully mature lungs does not lead to clinical respiratory distress (33, 38). Further inquiry into the manner in which hormones influence lung metabolism will require evaluation of the effect of specific hormonal replacements on *in utero* decapitated or hypophysectomized fetuses and on lung explants differentiating *in vitro*.

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