1718 CULTURE OF TYPE 2 CELLS FROM FETAL RABBIT LUNGS. Gary L. Sanford, Suck K. Lee, Stephen F. Ryan, Michael F. Frosolono and Robert S. Bienkowski (Spon. by Emile M. Scarpelli). Pediatric Pulmonary Div., Albert Einstein College of Medicine and Department of Pathology, St. Lukes-Roosevelt Hospital Medical Center, New York, New York.

Type 2 alveolar epithelial cells synthesize a surfactant which is important for maintaining lung stability. To isolate these cells, fetal rabbit lungs (27-29 days gestation) were digested enzymatically, and differential adhesion to plastic substrate was used to obtain a population enriched in epithelial cells. Cells were cultured using selective medium (with D-valine instead of L-valine) to suppress growth of contaminating fibroblasts; after 4-5 days, the cultures were confluent. Cells were examined at electron microscope level; in a typical culture from 29 day fetuses, approx. 10-40% were identified as "definite" type 2 (cuboidal or elliptical, surface microvilli, cytoplasmic lamellar bodies), and 50-60% were identified as "probable" type 2 (same criteria as for "definite", except no lamellar bodies). The cells incorporated (14C)-choline into disaturated phosphatidylcholine (dspc), a major component of lung surfactant. Specific activities of the acyl transferase system were compared using palmitoyl-CoA and oleyl-CoA as donor substrates, and 1-sat-2-lyso-pc as receptor substrate. At 27 days gestation, the ratio (palmitoyl-CoA/oleyl-CoA) was 0.39, while at 29 days the ratio was 3.5. These data suggest that changes in acyl transferase donor specificity are important in regulating synthesis of dspc. We conclude that this culture system is a useful model for studying metabolism of lung surfactant.

● 1719 HUMAN SURFACTANT: A THERAPEUTIC TRIAL IN PREMATURE RABBITS. Howard Schneider, Mikko Hallman, Kurt Benirschke, Louis Gluck. Univ. of Calif., San Diego, Depts. of Pathol. & Pediatrics, La Jolla, California.

The ability of human surfactant (HS) isolated by differential & density gradient centrifugation from amniotic fluid to alter pulmonary pressure-volume (P/V) loops in 27-day surfactant deficient fetal rabbits was evaluated. Fetuses were delivered & allowed to breathe spontaneously. Either 2 μmoles of HS in .1cc 0.6% NaCl, or similarly prepared rabbit surfactant (RS), or saline (controls) was applied endotracheally by tracheostomy. Fetuses were mechanically ventilated 15 min. sacrificed, & lungs degassed 15 min. at -20 cmH_2O. Static, first cycle pulmonary P/V loops were measured by syringe & H_2O manometer. Inflation & deflation volumes were recorded at 0,2,5,10,15,20,25, & 30 cmH_2O:

	Volume In cc/kg Body Weight					
Pressure	20	25	30	20	10	0
Controls(n=13)	5±02	6±03	15±08	10±07	6±03	1±01
HS(n=14)	21±22+	48±24*	68±21*	61±20*	49±19*	16±06*
RS(n=12)	18±13++	47±22*	70±18*	64±19*	49±16*	15±06*

+p <.02 ++p <.005 *p <.001 compared to controls

There were no significant differences between HS and RS. Histology revealed good homogeneous aeration in HS and RS fetuses and poor aeration in controls. HS markedly improved P/V loops in 27 day fetal rabbits and was comparable to RS. HS may be effective in treating RDS once its safety is established.

● 1720 VENTILATORY EFFICIENCY OF NEONATES. Karl Schulze, Mark Stefanski and L. Stanley James, Div. of Perin. Med., Dept. of Ped., Coll. of P&S, Columbia Univ., NY.

The relationship of pulmonary minute volume to the prevailing oxygen consumption (V_I/VO_2), the convection requirement (CR) is a useful index of the overall efficiency of ventilation. This study was undertaken to determine the CR of healthy infants and the relationship of CR to the size and age of the infant. Continuous concurrent measurements of V_I(bias flow pneumotachometry)and VO_2 (open circuit indirect calorimetry) were made for 100 to 150 min. in 31 healthy infants, wt. 650 to 3380 grams, postconceptual age (PA)30 to 42 weeks. Spontaneous changes in V_I and VO_2 followed changes in activity and feeding. Data were divided into 3 min. intervals and linear regression analysis of mean V_I vs mean VO_2 and first differences, (ΔV_I vs ΔVO_2)were performed following time series correction for lags. All infants showed significant positive correlations (V_I vs VO_2 $\bar{r}$=.79, mean slope=46, ΔV_I vs ΔVO_2 $\bar{r}$=.64, mean slope=37.2).

Mean regression analysis of the relationship between the slope of individual V_I vs VO_2 regression lines(S) & the infants BW,age,PA,GA & study wt. demonstrated significant negative correlations between S & several indices of the size & age of the infant. The V_I associated with VO_2=8.5 ml/min.kg for individual infants was also negatively correlated with size and wt.

We conclude that smaller & younger infants are at a relative disadvantage because of high CR,ie.they require a larger V_I for a given VO_2 & relatively greater increases in V_I to meet increases in VO_2. Measurement of CR should prove useful as a pulmonary function test in neonates with & without respiratory diseases.

● 1721 DEVELOPMENTAL PATTERN OF DIAPHRAGM STRENGTH IN INFANCY. Charles B. Scott*, C.W.Sargent*, M.M.Woolley*, A.C.G.Platzker*, A.D.Ramos*, D.Warburton*, and T.G. Keens* (Spon. R.M.McAllister). University of Southern California School of Medicine, Childrens Hospital of Los Angeles, Neonatal-Respiratory Disease Division, Departments of Pediatrics and Surgery, Los Angeles, California.

Diaphragm strength was measured as maximal transdiaphragmatic pressure (Pdi) during airway occlusion in 33 infants aged 11.3 ± 0.6 (SE) months post-conception (mpc), range 8-21 mpc. All infants were asymptomatic at the time of study and required no ventilatory support. 9 infants had previous surgical correction of abdominal wall defects (gastroschisis/omphalocele), 9 infants had previous surgical correction of congenital diaphragmatic hernia, and 15 infants had no thoracic or abdominal surgery. The mean maximal Pdi for all infants was 69.7 ± 3.5 cm H_2O. There were no significant differences between the 3 groups (P = 0.20). In the entire group, there was a significant correlation between maximal Pdi and age (Pdi (cm H_2O) = Age (mpc) × 3.4 + 30.9; r = 0.598; P = 0.0005). In contrast, maximal Pdi in 3 ventilator dependent infants was less than 30 cm H_2O. Subsequently, Pdi increased to above 40 cm H_2O in these 3 infants following the ability to wean from mechanical ventilatory assistance. We conclude that diaphragm strength increases during the first year of life. Since respiratory failure may be viewed as inadequate ventilatory muscle power to overcome increased work of breathing, young infants may be at increased risk for respiratory failure relative to older infants due to decreased diaphragm strength.

1722 PHOSPHOLIPID PRODUCTION IN FETAL LUNG CELL CULTURE PREPARATIONS. Alex Sevanian, Solomon A. Kaplan and Cynthia T. Barrett, Department of Pediatrics, UCLA Center for the Health Sciences, Los Angeles, California.

Type-II (T-II) pneumocytes from 27 day fetal rabbit lungs were grown in an organotypic system (OS) and used to study phospholipid (PL) synthesis. Organotypic preparations were also used as a means for isolating purified T-II cells as well as lung fibroblasts. The PL synthetic properties of the various culture preparations were studied using palmitate, choline and acetate as precursors. In all cultures, excepting fibroblasts, saturated phosphatidylcholine (SPC) was a major PL product. The molar incorporation rate into PL for the substrates studied was: palmitate > choline > acetate. The proportion of these substrates incorporated into SPC versus total phosphatidylcholine (PC) differed between OS and monolayer cultures. In the OS the order of substrate incorporation into SPC as a percent of total PC formed, was acetate > choline > palmitate, whereas in T-II cultures the order was choline > acetate > palmitate. Among the culture preparations examined, the greatest similarity was found between the OS and the mixed fibroblast/T-II preparations. In this regard it was observed that a mixed culture of fibroblasts and T-II cells produced larger proportions of SPC and other surface-active PL than isolated T-II cells. From these observations it appears that the OS is a useful model for examining surfactant PL synthesis of T-II cells, and furthermore, may serve as an effective system for the isolation and study of T-II pneumocytes.

1723 THE BETA-ADRENERGIC RECEPTOR OF THE TYPE II PNEUMOCYTE Donald L. Shapiro and Jacob N. Finkelstein.U.of Roch. School of Med., Strong Mem Hosp., Dept.of Peds.,Roch, NY

The type II pneumocyte secretes surfactant phospholipid in response to beta-adrenergic stimulation. These studies identify the beta-adrenergic cell membrane receptors which mediate this response. The receptors were studied using radiolabeled (^3H) dihydroalprenolol (DHA), a beta-adrenergic antagonist with high affinity for receptor sites. Assays were performed on intact type II pneumocytes freshly isolated from adult rabbits. The reaction was performed in .154M Krebs Ringers Phosphate containing 0.2% bovine serum albumin and 200μM Na-metabisulfite. The assay reaction required inclusion of 100μM phentolamine to minimize nonspecific binding. In the presence of phentolamine, specific receptor binding was indicated by displacement of DHA by 6μM propanolol. The receptor displayed stereospecificity for the (-) isomer of propanolol. The receptor assay reached equilibrium within five minutes and remained stable for at least 20 minutes. Receptor sites were quantitatively related to the number of cells per reaction. Saturation kinetics was demonstrated. Scatchard plot analysis of data revealed only one class of beta-adrenergic receptors and approximately 10^5 receptors per cell. These studies have established conditions for analysis of the beta-adrenergic receptors of intact type II pneumocytes and provided a preliminary characterization. Further study of these beta-adrenergic receptors will help elucidate regulation of the pulmonary surfactant system.