

Human Fetal Lung Type II Pneumonocytes in Monolayer Cell Culture: The Influence of Oxidant Stress, Cortisol Environment, and Soluble Fibroblast Factors

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Summary

A pure culture of human fetal lung type II pneumonocytes has been developed and labeled HFLP. Cells were studied during the period of exponential growth that was observed to occur in this system from day 4 to day 8 after subculture. Correlations with exponential growth were highly significant ($P < 0.001$) in all groups studied. Growth was not greatly different at any of the different oxygen tensions (30, 55, 80, and 145 mm Hg) examined except that growth was slightly more rapid at a PO_2 of 145 mm Hg than at a PO_2 of 80 mm Hg ($P < 0.05$). The uptake of 3H -choline into DSPC at a PO_2 of 30 mm Hg was significantly less than at 55 mm Hg ($P < 0.001$) or 80 mm Hg ($P < 0.001$), whereas the uptake at 145 mm Hg was also significantly less than that at 55 mm Hg ($P < 0.001$) or 80 mm Hg ($P < 0.001$). Uptake at 55 and 90 mm Hg was not significantly different. The response to added cortisol (1 μM) also varied with the oxygen tension, with no effect at a PO_2 of 30 mm Hg while, at a PO_2 of 55 mm Hg the 3H -choline uptake was significantly different on day 5 ($P < 0.001$), day 6 ($P < 0.001$) and day 7 ($P < 0.01$). Similarly, at a PO_2 of 80 mm Hg the difference was significant on day 5 ($P < 0.05$), day 6 ($P < 0.01$) and day 7 ($P < 0.01$). At a PO_2 of 145 mm Hg, the difference was just significant on day 7 ($P < 0.05$). In isolated HFLP cells, an oxidant challenge produced by increasing PO_2 from 30 to 55 mm Hg resulted in a 96% increase in 3H -choline uptake in both the control group ($P < 0.001$) and the steroid-added group ($P < 0.001$). In the combined HFLP and fetal lung fibroblast culture there was a 275% increase over the uptake before oxidant challenge after 48 hr (control group $P < 0.001$, steroid group $P < 0.001$). In the experiment where HFLP cells were grown in the presence of 10% fibroblast-derived medium, the increase after 48 hr was 135% over values before the oxidant challenge (control group $P < 0.001$). In this experiment, the steroid-added group had an increased response compared to the control group 48 hr after the oxidant challenge ($P < 0.02$).

Speculation

It was speculated that the type II pneumocyte requires an unknown cofactor in order to respond to antenatal steroid treatment by increased production of surface active materials. It was further speculated that the change of PO_2 that occurs in pulmonary tissue after delivery acts as an important biologic stimulus to cause increased production of surface active materials, and that this mechanism can be favorably enhanced by pretreatment with steroids, and is mediated in part by soluble factor(s) derived from the fetal lung fibroblast.

Development of the human fetal lung *in utero* occurs at a low oxygen tension (PO_2). The fetal arterial PO_2 is constant throughout

gestation at 20-22 mm Hg, which increases in the neonate after delivery at term to 100 mm Hg by 24 hr of age (17). This acute increase represents an oxidant stress that is well tolerated, without clinical disturbance, in full gestation infants. In the premature infant, however, in whom inadequate production of surface-active materials (SAM) by the type II pneumocyte (7) has resulted in the Respiratory Distress Syndrome (RDS), survival may depend in part upon an adequate increase in intracellular oxygen-protective enzymes in the face of the oxidant challenge of delivery and oxygen therapy (4).

Alterations of PO_2 of this magnitude can influence growth (6,24) and biologic activity (15, 24) in some cell types. The influence of differing oxygen tensions upon growth and incorporation of choline into saturated phosphatidylcholine (DSPC), a major functional component of the SAM in the neonatal lung (20), by isolated type II pneumocytes grown in continuous monolayer cell culture have, therefore, been examined. The effect of pretreatment with cortisol before the oxidant stress has also been examined because this is a recognized form of therapy to enhance fetal lung maturation in premature fetuses likely to develop RDS (11). Steroids can enhance (1) or protect (2) against oxidant stress, though the effect of steroids in neonatal pulmonary oxygen toxicity is uncertain (23).

Lastly, the influence of fibroblasts and soluble fibroblast factors upon the changes in choline incorporation into DSPC by the type II pneumocyte exposed to oxidant stress has been studied.

MATERIALS AND METHODS

The experimental techniques described in this paper are essentially similar to those developed for the study of oxidant stress upon growth and biological activity of the fetal lung fibroblast (24).

Eagle's minimal essential tissue culture medium with Earle's salts (32) was supplemented with 10% fetal calf serum (33) and antibiotics (21). This preparation will subsequently be called "tissue culture medium." The fetal calf serum provides the tissue culture medium with traces (1.4 nm) of cortisol (21). A buffered trypsin-EDTA solution containing 0.5 g trypsin 1:250 and 2.0 g EDTA/liter (32) was used for cell dispersal (21). Plastic tissue culture flasks with a surface area of 75 cm^2 (34) were used to maintain the cultures and flasks with a surface area of 25 cm^2 (34) were used for experimental periods. The tissue culture medium containing cortisol (1 μM) was made up as described previously (19).

The type II pneumocyte line used in these studies was developed in this laboratory, and has been labeled HFLP. Lung tissue was taken at autopsy from a 36-wk-gestation anencephalic fresh stillbirth, and developed into a mixed lung cell culture by established techniques (21). During the course of another experiment

with this culture (19), a dilute suspension of the culture was distributed on a cloning plate to give a theoretical cell count of one cell in each cloning well. On microscopy, some wells were found to contain single cells whereas others contained two or more. From one of the single cell wells was derived the pure fetal lung fibroblast culture (labeled KGH 9L) used in this experiment (24), and from one of the wells containing two cells was developed a mixed cell line which contained only fibroblasts and type II pneumocytes. Because these two cell types separate at different rates from the culture flask surface in the presence of trypsin, it was possible to develop a pure type II cell line from a low density suspension at low oxygen tensions. Chromosomal analysis of HFLP cells shows a diploid configuration and electron-microscopy reveals the characteristic lamellar bodies of the type II pneumocyte. A detailed account of the isolation technique and the cell morphology will be published separately.

Cultures were maintained as described previously (24) under a variety of gases containing 5% carbon dioxide, nitrogen, and various oxygen concentrations. During this equilibration period, the gas and medium were changed daily. The medium used had also been equilibrated in a glass vessel, to prevent gas diffusion, for 2-3 wk with the particular gas being used. For experimental periods, three large flasks had their cells dispersed into a common mixing chamber followed by redistribution of a roughly equal number of cells into 40 small flasks. To half, cortisol (1 μ M) was introduced the day after subculture, and all had the medium and gas changed each day.

Cell numbers were studied during the period of exponential growth that had been observed in preliminary work with this system to occur from day 4 to day 8. Cell numbers before this are affected by losses secondary to dispersal and subculture (27) and may have reduced levels of intracellular oxygen protective enzymes due to contact with trypsin (30). After this time, some flasks showed evidence of confluence that inhibits growth and is associated with alterations of cell metabolism (10). Once metabolic studies had been performed, the cells were trypsinized, diluted, and counted (24). Five flasks were counted on each day for both the steroid-added and the control groups, allowing the calculation of doubling times for each of the oxygen concentrations studied.

Cells in each flask were incubated in the flask for 3 hr at 37°C with 5 ml Earle's balance salt solution (32) containing 1.6 μ Ci/ml [methyl-³H]-choline chloride (New England Nuclear, Lot number 990-207, specific activity 69.5 Ci/mole). The uptake of ³H-choline into DSPC was estimated according to the method of Mason *et al.* (12) as described previously (20). Isotope counting was performed in a Searle Isocap 300 liquid scintillation spectrophotometer and the results are expressed as cpm/10³ cells. Uptake reduced slightly with each successive day of exponential growth, but increased markedly at confluence to 3-4 times that seen during exponential growth. It was preferred, however, to examine the cells during exponential growth because this may mimic the situation *in utero*, and because the metabolic disturbances that occur at confluence could influence results. In the metabolic studies, 1 μ M cortisol was introduced on day 4 after subculture.

Cells were allowed the prolonged equilibration period with the study gases to allow intracellular oxygen protective enzymes to equilibrate with the selected oxygen tensions (18). On days 4 and 7 after subculture, 10 flasks were analyzed for PO₂, PCO₂, and pH immediately before, and 1 hr after, gas change. Analyses were made with a Radiometer BMS Mk 2 blood microsystem. The mean results of these values for each of the gas mixtures used are shown in Table 1.

Having examined cells adapted to the various oxygen concentrations, the effect of acute moderate oxidant stress upon the cells by suddenly increasing the PO₂ from 30.46 \pm 3.7 to 55.10 \pm 5.7 mm Hg was examined. The uptake of ³H-choline into DSPC by HFLP alone was examined; also examined were the uptakes by HFLP with KGH 9L where the original subculture contained equal numbers of both, and by HFLP grown in tissue culture medium of which 10% was cell-free medium derived from cultured KGH 9L fibroblasts. In these experiments, the cortisol-added

medium was not introduced until 24 or 48 hr before the oxidant stress.

Results for cell number and ³H-choline uptake into DSPC were available from 10 flasks each day, of which half contained cortisol (1 μ M). From this data, means and SE for each group were calculated on each day. For the cells equilibrated with the four gases, exponential growth curves could be calculated that gave the doubling time in each gas, and also correlation coefficients for exponential growth in each gas. By using the logarithmic value for each cell count a linear regression for cell growth was derived that could then be compared (25) using Student's *t* test. Values for ³H-choline incorporation in each group were also compared using Student's *t* test.

RESULTS

Doubling times and correlation coefficients for exponential growth of HFLP equilibrated with various oxygen tensions are shown in Table 2. Correlation with exponential growth was highly significant ($P < 0.001$) in all groups. In none of the oxygen tensions examined did the addition of cortisol (1 μ M) significantly affect growth rate, nor was growth greatly different in any of the different oxygen tensions, though growth was slightly more rapid in the control group in a PO₂ of 145 mm Hg than at 80 mm Hg ($t = 2.30$, $df = 38$, $P < 0.05$).

In contrast, however, are the differences observed in ³H-choline incorporation into DSPC by HFLP equilibrated with the various oxygen tensions as shown in Figure 1. At a PO₂ of 30 mm Hg, uptake of ³H-choline is significantly less than at a PO₂ of 55 mm Hg ($t = 4.75$, $df = 38$, $P < 0.001$), or of 80 mm Hg ($t = 6.95$, $df = 38$, $P < 0.001$), whereas the uptake at 145 mm Hg is also significantly less than that at 55 mm Hg ($t = 5.56$, $df = 38$, $P < 0.001$) or 80 mm Hg ($t = 7.65$, $df = 38$, $P < 0.001$). Uptake at 55 and 80 mm Hg is not significantly different ($t = 1.79$, $df = 38$, $P = N.S.$).

The response to added cortisol (1 μ M) also varies with the oxygen tension. There is no effect at a PO₂ of 30 mm Hg ($t = 0.09$, $df = 33$, $P = N.S.$) whereas at a PO₂ of 55 mm Hg ³H-choline uptake is significantly different on day 5 ($t = 5.75$, $df = 8$, $P < 0.001$), day 6 ($t = 6.03$, $df = 8$, $P < 0.001$), and day 7 ($t = 4.41$, $df = 8$, $P < 0.001$).

Table 1. Acid base data: Shown are the mean values for PO₂, PCO₂ and pH obtained in the culture medium during exponential growth of HFLP over four days under gases containing four different oxygen concentrations

Gas	Medium		
	PO ₂ mm Hg (M \pm SD)	PCO ₂ mm Hg (M \pm SD)	pH (M \pm SD)
0.95% O ₂ :5% CO ₂ :Bal.N ₂	30.46 \pm 3.7	45.4 \pm 2.8	7.23 \pm 0.09
4% O ₂ :5% CO ₂ :Bal.N ₂	55.10 \pm 5.7	46.9 \pm 4.3	7.24 \pm 0.09
10% O ₂ :5% CO ₂ :Bal.N ₂	79.98 \pm 4.2	47.3 \pm 3.9	7.22 \pm 0.05
20% O ₂ :5% CO ₂ :Bal.N ₂	145.31 \pm 8.6	46.8 \pm 5.0	7.20 \pm 0.11

Table 2. Cell growth at different oxygen tensions: Doubling times for HFLP grown in four different oxygen tensions, with or without added cortisol

PO ₂ mm Hg (M \pm SD)	Control		+1 μ M cortisol	
	Exponential growth ¹	Doubling time (hr)	Exponential growth	Doubling time (hr)
30.46 \pm 3.7	$r = 0.968$	28.98	$r = 0.971$	28.41
55.10 \pm 5.7	$r = 0.972$	28.50	$r = 0.981$	29.73
79.98 \pm 4.2	$r = 0.970$	31.20	$r = 0.960$	29.95
145.31 \pm 8.6	$r = 0.977$	26.94	$r = 0.920$	28.16

¹ r is the correlation coefficient for exponential growth of each growth curve.

= 8, $P < 0.01$). Similarly, at a PO_2 of 80 mm Hg, the difference is significant on day 5 ($t = 2.67$, $df = 8$, $P < 0.05$), day 6 ($t = 3.79$, $df = 8$, $P < 0.01$), and day 7 ($t = 3.6$, $df = 8$, $P < 0.01$). At a PO_2 of 145 mm Hg, the difference is not significant on day 5 ($t = 1.23$, $df = 8$, $P = N.S.$) or day 6 ($t = 2.18$, $df = 8$, $P = N.S.$) and is just significant on day 7 ($t = 2.84$, $df = 8$, $P < 0.05$).

The results of the acute oxidant stress (PO_2 increased from 30–55 mm Hg) experiments are shown in Figure 2. For the isolated HFLP, the oxidant challenge resulted in a 96% increase in 3H -

choline uptake in both the control group ($t = 5.55$, $df = 8$, $P < 0.001$) and the steroid-added group ($t = 5.66$, $df = 8$, $P < 0.001$). In this experiment, the addition of cortisol ($1 \mu M$) resulted in a fall in uptake ($t = 4.55$, $df = 8$, $P < 0.01$). This experiment has been repeated several times and the cortisol either produces a fall or has no effect, as in the equilibrated gas experiments. In the combined HFLP and KGH 9L culture, there was a significant reduction of activity 24 hr after the oxidant challenge in both groups (control group; $t = 7.67$, $df = 8$, $P < 0.001$); steroid group; $t = 4.15$, $df = 8$, $P < 0.01$) followed at 48 hr by a 275% increase over the uptake before oxidant challenge (control group; $t = 12.23$, $df = 8$, $P < 0.001$; steroid group; $t = 14.39$, $df = 8$, $P < 0.001$). In this mixed cell culture the control group had a greater response than the steroid group ($t = 4.58$, $df = 8$, $P < 0.01$), though this is difficult to interpret because KGH 9L growth rates are affected by the addition of cortisol (24) and one cannot be certain that the proportion of the cells that are HFLP is the same in the two groups. In fact, it is unlikely that they are. Whatever the proportion, it is apparent that the increase of 275% is even greater when expressed in terms of 3H -choline uptake into DSPC/ 10^3 HFLP cells. In the experiment where HFLP cells were grown in the presence of 10% KGH 9L derived medium, a significant reduction in uptake is seen in both groups 24 hr after oxidant challenge (control group; $t = 7.65$, $df = 8$, $P < 0.001$; steroid group $t = 5.43$, $df = 8$, $P < 0.001$) with an increase of 135% over values before the oxidant challenge after 48 hr (control group; $t = 10.46$, $df = 8$, $P < 0.001$; steroid group; $t = 17.72$, $df = 8$, $P < 0.001$). In this experiment, the steroid added group has an increased response compared to the control group 48 hr after the oxidant challenge ($t = 3.10$, $df = 8$, $P < 0.02$).

DISCUSSION

Cell line HFLP offers certain advantages to the study of physiologic and pathologic oxidant stresses upon the fetal lung type II pneumocyte. It is not only a pure human line, but it is derived from a fetal source, an advantage because the young of a species is more resistant to oxygen toxicity than the adult and also primates are the most resistant of mammals (5). Its use *in vitro* provides the opportunity to study type II pneumocyte responses under a variety of controlled conditions and allows its interrelationships with other cells to be better defined. Its other advantage is that it is derived from a fetus of a gestational age at which RDS occurs.

The absence of growth rate differences in different oxygen tensions is contrary to the authors' experience with fetal lung

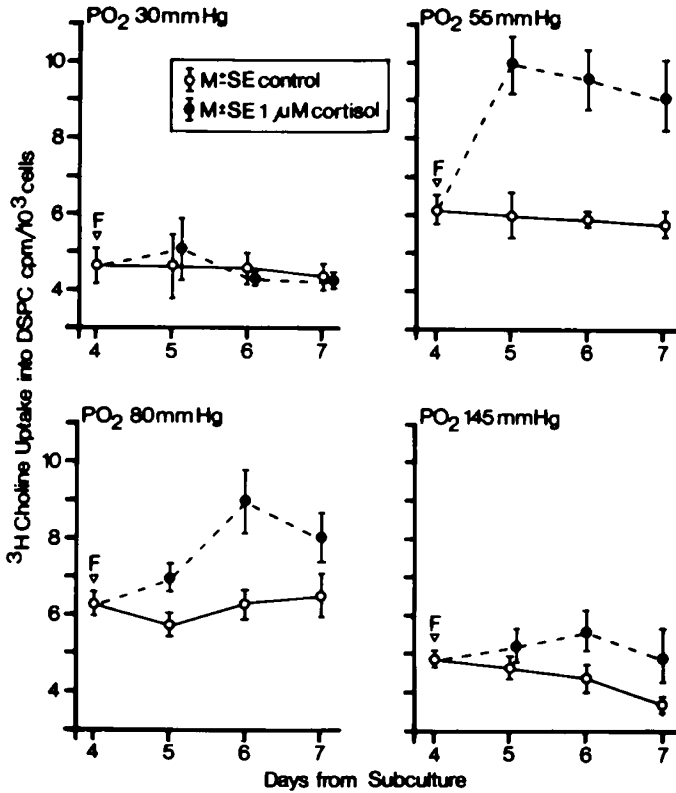


Fig. 1. The influence of cortisol at different oxygen tensions upon 3H -choline incorporation by HFLP is shown. Cell line HFLP adapted to four different oxygen tensions in exponential growth. This figure shows the uptake of 3H -choline into DSPC on days 4–7 from subculture in controls and in cells incubated with $1 \mu M$ cortisol (F) added on day 4. Each point represents the mean of five observations.

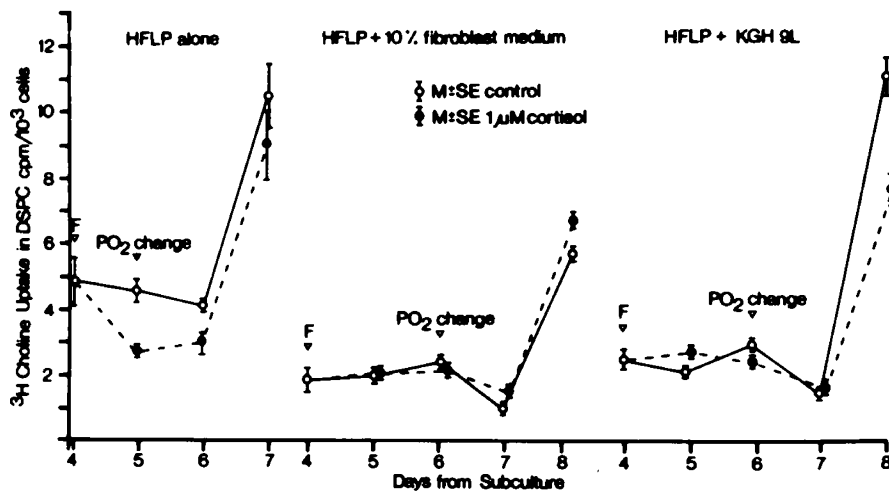


Fig. 2. The influence of oxidant stress, cortisol, and fibroblast factors upon 3H -choline incorporation by HFLP. Cell line HFLP alone, with KGH 9L, or with KGH 9L derived medium in a PO_2 of 30.46 mm Hg exposed to an oxidant stress by a sudden increase of PO_2 to 55.10 mm Hg.

Both control and cortisol-added groups were in exponential growth before the PO_2 change. Cortisol (F) was added at the points indicated by an arrow. Each point represents the mean of 5 observations.

fibroblasts (24), and this difference may be explained by the difference in mitochondrial content of the two cell types which should favor the type II pneumocyte in terms of withstanding oxidant stresses (30). That its growth rate can vary under different oxygen tensions is clear, though these changes reflect the effect of acute alterations of oxygen tension (3). The experimental system described previously does not allow a sufficient time period to study oxidant induced changes of growth rate. These studies will be performed using larger cell culture flasks that give a greater time period before confluence occurs.

Experimental evidence suggests that maturation of the fetal lung in man (14, 22), as in animals (8, 9, 28, 29), is dependent upon its corticosteroid milieu. Glucocorticoids in the human fetus are predominantly in the biologically inactive 11-ketosteroid form. The proportion of cortisol, the major component of the biologically active 11 β -hydroxycorticosteroids, increases with gestation (13), though its origins are ill-defined (26). Exogenous steroids can enhance fetal lung maturation both *in vivo* (11), and *in vitro* (21). It was surprising, therefore, to see no response to steroids at a PO₂ similar to that seen *in utero*, despite the cells' obvious ability to increase choline incorporation into DSPC in response to cortisol at higher oxygen tensions. This suggested that either exogenous steroids do not act directly upon the type II pneumocyte, or more probably that there is some cofactor, missing from the cell culture model, which enhances the type II pneumocytes' response at an intrauterine PO₂.

The observations that an increase in oxygen tension from intrauterine levels results in increased choline incorporation into DSPC suggests that the alteration of intrapulmonary PO₂ that occurs at delivery would act as one stimulus to increase SAM production. This is an attractive hypothesis in biologic terms because the fetus has no need of SAM in its fluid filled lungs, whereas SAM is obligatory with air breathing. It is interesting that the lag period before the oxidant challenge results in increased DSPC production is of the same order as that seen before infants with SAM deficiency (RDS) produce adequate quantities, and, thus, recover from the disease (7). Cortisol and other factors will already have increased SAM content in the airways of the mature fetus, allowing adequate quantities for the first breath and subsequent air breathing.

Whereas the addition of fibroblasts or fibroblast "factors" does not improve the steroid responsiveness of HFLP at a PO₂ of 30 mm Hg, they markedly alter the response to oxidant stress. This supports our previous observations (19) that there are soluble fibroblast-derived factors which can influence the behavior of the type II pneumocyte. The increase in ³H-choline incorporation into DSPC with oxidant challenge is very significant and when enhanced by the addition of fibroblast medium suggests that they may be a fibroblast-derived soluble mediator, and of particular interest is the observation that this enhancement is greater in cells pretreated with cortisol.

REFERENCES AND NOTES

- Allen, J. E., Goodman, B. P., Besarab, A., and Rasmussen, H.: Studies on the biochemical basis of oxygen toxicity. *Biochim. Biophys. Acta*, **320**: 708 (1973).
- Alpert, S. M., Schwartz, B. B., Lee, S. D., and Lewis, T. R.: Alveolar protein accumulation. *Arch. Intern. Med.*, **128**: 69 (1971).
- Bonikos, D. S., Bensch, K. G., Northway, W. H., and Edwards, D. K.: Bronchopulmonary dysplasia. The pulmonary pathological sequel of necrotizing bronchiolitis and pulmonary fibrosis. *Human Pathol.*, **7**: 643 (1976).
- Bonta, B. W., Gawron, E. R., and Warshaw, J. B.: Neonatal red cell superoxide dismutase enzyme levels: Possible role as a cellular defense mechanism against pulmonary oxygen toxicity. *Pediatr. Res.*, **11**: 754 (1977).
- Clark, J. M., and Lambertson, C. J.: Pulmonary oxygen toxicity: A review. *Pharmacol. Rev.*, **23**: 37 (1971).
- Cooper, P. D., Burt, A. M., and Wilson, J. N.: Critical effect of oxygen tension on rate of growth in animal cells in continuous suspended culture. *Nature*, **182**: 1508 (1958).
- Farrell, P. M., and Avery, M. E.: Hyaline membrane disease. *Am. Rev. Respir. Dis.*, **111**: 657 (1975).
- Farrell, P. M., Blackburn, W. R., and Adams, A. J.: Lung phosphatidylcholine synthesis and cholinephosphotransferase in anencephalic rat fetuses with corticosteroid deficiency. *Pediatr. Res.*, **11**: 770 (1977).
- Giannopoulos, G.: Variations in the level of cytoplasmic glucocorticoid receptors in lungs of various species at different developmental stages. *Endocrinology*, **94**: 450 (1974).
- Kilburn, D. G., Lilly, M. D., Self, D. A., and Webb, F. C.: The effect of dissolved oxygen partial pressure on the growth and carbohydrate metabolism of mouse LS cells. *J. Cell Sci.*, **4**: 25 (1969).
- Liggins, G. C., and Howie, R. N.: A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics*, **50**: 515 (1972).
- Mason, R., Nellenbogen, J., and Clements, J. A.: Isolation of disaturated phosphatidylcholine with osmium tetroxide. *J. Lipid Res.*, **17**: 281 (1976).
- Murphy, B. E. P.: Does the human fetal adrenal play a role in parturition? *Am. J. Obstet. Gynecol.*, **115**: 521 (1973).
- Murphy, B. E. P.: Evidence of cortisol deficiency at birth in infants with respiratory distress syndrome. *J. Clin. Endocrinol. Metab.*, **38**: 158 (1974).
- Pawalek, J. M.: Effect of thyroxine and low oxygen tension on chondrogenic expression in cell culture. *Dev. Biol.*, **19**: 52 (1969).
- Rosenbaum, R. M., Wittner, M., and Lenger, M.: Mitochondrial and other ultrastructural changes in great alveolar cells of oxygen-adapted and poisoned rats. *Lab. Invest.*, **20**: 516 (1969).
- Rudolph, A. M.: In: *Congenital Diseases of the Heart*. (Year Book Medical Publishers, Inc., Chicago, 1974).
- Simon, L. M., Liu, J., Theodore, J., and Robin, E. D.: Effect of hyperoxia, hypoxia, and maturation on superoxide dismutase activity in isolated alveolar macrophages. *Am. Rev. Respir. Dis.*, **115**: 279 (1977).
- Smith, B. T.: Fibroblast-pneumocyte factor: Intercellular mediator of glucocorticoid effect on fetal lung. In: *L. Stern: Intensive Care in the Newborn*. (Masson, Boston, pp. 25-32).
- Smith, B. T.: The role of pulmonary corticosteroid 11-reductase activity in lung maturation in the fetal rat. *Pediatr. Res.*, **12**: 12 (1978).
- Smith, B. T., Torday, J. C., and Giroud, C. J. P.: Evidence for different gestation-dependent effects of cortisol on cultured fetal lung cells. *J. Clin. Invest.*, **53**: 1518 (1974).
- Smith, B. T., Worthington, D., and Maloney, A. H. A.: Fetal lung maturation. The amniotic fluid cortisol/cortisone ratio in preterm human delivery and the risk of respiratory distress syndrome. *Obstet. Gynecol.*, **49**: 527 (1977).
- Taesch, H. W.: Glucocorticoid prophylaxis for respiratory distress syndrome. A review of potential toxicity. *J. Pediatr.*, **87**: 617 (1975).
- Tanswell, A. K., and Smith, B. T.: Influence of oxygen tension and cortisol environment upon growth and cortisone conversion to cortisol by cultured human fetal lung fibroblasts. *Biol. Neonat.*, (in press).
- Tanswell, A. K., and Smith, B. T.: The relationship of amniotic membrane 11-oxidoreductase activity to lung maturation in the human fetus. *Pediatr. Res.*, **12**: 957 (1978).
- Tanswell, A. K., Worthington, D., and Smith, B. T.: Human amniotic membrane corticosteroid 11-oxidoreductase activity. *J. Clin. Endocrinol. Metab.*, **45**: 721 (1977).
- Taylor, W. G., Richter, A., Evans, V. J., and Sanford, K. K.: Influence of oxygen and pH on plating efficiency and colony development of WI 38 and Vero cells. *Exptl. Cell Res.*, **86**: 152 (1974).
- Torday, J. S., Smith, B. T., and Giroud, C. J. P.: The rabbit fetal lung as a glucocorticoid target tissue. *Endocrinology*, **96**: 1462 (1975).
- Vidyasagar, D., and Chernick, V.: Effects of metopirone on the synthesis of lung surfactant in does and fetal rabbits. *Biol. Neonat.*, **27**: 1 (1975).
- Yamanaka, N., and Deamer, D.: Superoxide dismutase activity in WI 38 cell cultures: Effects of age, trypsinization, and SV 40 transformation. *Physiol. Chem. Physics*, **6**: 95 (1974).
- The abbreviations used are cortisol 11 β , 17 α , 21-trihydroxy pregn-4-ene-3,20-dione.
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