

## ISOTRETINOIN EFFECTS NEURAL CREST CELLS IN VITRO.

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We have described the clinical manifestations of isotretinoin (ISO) neuroembryopathy (*J Ped*, 105, 595, 1984) and with others have suggested that disturbances in neural crest development may underlie the pathogenesis of this teratogenic disorder (*NEJM* 313, 832, 1985). To investigate this possibility in a controlled experimental situation, cranial neural crest explant cultures were exposed to different concentrations of ISO (2 and 20  $\mu\text{g/ml}$  medium) and cell morphology was monitored at 24 hour intervals for a total of 5 days using inverted phase contrast microscopy. In contrast to the control cultures of confluent flattened cells, ISO treated crest cells became rounded or spindle shaped, separated from their neighbors, and frequently detached from the substrate or clumped together. Similar changes in morphology occurred in trunk neural crest cultures but not in neural tube cells or in cardiac fibroblasts. Exposure to ISO did not appear to change the amount or distribution of antibody binding to cell surface antigens HNK-1 or CSAT. Taken together, these results suggest that ISO selectively effects neural crest cells by decreasing their cell-substratum adhesion and strongly supports the hypothesis that ISO associated birth defects in humans may be caused by abnormalities in neural crest development.

## UPTAKE OF THE MAJOR SURFACTANT APOPROTEIN BY NEONATAL LUNG TISSUE.

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Secreted lung surfactant phospholipid has been shown to be recycled by alveolar type II cells. We hypothesize that the 35 kDa surfactant apoprotein (SAP) participates in surfactant reutilization. SAP was purified from rabbit lung lavage, radio-labeled with [ $^{125}\text{I}$ ], and instilled, along with carrier surfactant, into the lungs of neonatal rabbits. After intervals of 0, 5, 15, 30 or 60 minutes, the rabbits were sacrificed, their lungs lavaged, then the lung tissue homogenized. Uptake of [ $^{125}\text{I}$ ]-SAP by the lung tissue was significant and time-dependent, reaching  $16.9 \pm 1.6\%$  of the amount originally instilled by 1 hour. Less than 1% of the instilled [ $^{125}\text{I}$ ]SAP was detected in blood, liver and kidney. These data are suggestive that instilled [ $^{125}\text{I}$ ]SAP does not cross the alveolar wall. The proportion of trichloroacetic acid-precipitable [ $^{125}\text{I}$ ]SAP in the lung tissue or lavage did not change as a function of time. These data are evidence that the instilled [ $^{125}\text{I}$ ]SAP is not degraded. To control for possible non-specific uptake of protein, lungs of neonatal rabbits were instilled with [ $^{125}\text{I}$ ]-rabbit serum albumin, a protein abundant in alveolar fluid. Less than 3% of the [ $^{125}\text{I}$ ] albumin was taken up by the lung tissue after 1 hr. We have shown that the surfactant apoprotein is taken up by the lung, and thus may be involved in the process of surfactant reutilization.

## SEX DIFFERENCES IN ANTIOXIDANT ENZYME DEVELOPMENT: MALE ADVANTAGE IN THE RABBIT.

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The surfactant system has been the focus of investigation for sex differences in lung maturation. Whereas many studies have demonstrated advanced surfactant maturation in female fetuses, others have found no sex differences or even indications of female disadvantage (*Am. Rev. Resp. Dis.* 124: 435, 1981). The pulmonary antioxidant enzyme (AOE) system, functioning to detoxify  $\text{O}_2$ -free radicals, matures on a time course similar to surfactant development. To explore whether sex differences might be present in AOE system development and to confirm previous findings of sex differences in surfactant development, we examined 2-3 litters of fetal rabbits at each of 4 gestational ages for superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP) activities (U/mg DNA) and for lung DSPC content. \* $p < 0.05$ ; Mean male/mean female values:

Gestl.	SOD	CAT	GP	DSPC(mg/mg prot.)
24d	19.4/18.7	82/87	1.51/1.70	0.032/0.033
26d	24.0/22.5	305/252	1.94/1.68	0.028/0.032
28d	24.9/19.6*	412/288*	3.20/2.52*	0.061/0.041*
30d	27.0/28.6	680/625	3.32/3.31	0.063/0.064

Male fetuses had significantly increased SOD, CAT and GP activities (and DSPC content) suggesting a male advantage in AOE maturation at 28 days. This is the first demonstration that the lung AOE system, important in preparing an organism for the increased oxidant conditions present at birth, may be maturing earlier in the male rather than female animal.

## AUTOCRINE EFFECT OF OXYGEN ON CELL PROLIFERATION.

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the first evidence that oxygen tension regulates the synthesis of a protein with growth-promoting activity in human fibroblasts. Sparse cultures of embryonic fibroblasts were synchronized in the  $G_0$  portion of the cell cycle by serum starvation and then placed under either low (2.5%) or ambient (20%) oxygen for 24 hours. When serum was added to induce proliferation, the number of cells that initiated DNA synthesis was ten-fold greater in 2.5% than in 20% oxygen. Oxygen concentration did not affect the latent period for DNA synthesis. Exposure to 2.5% oxygen during  $G_0$  was sufficient to increase DNA synthesis. Oxygen also affected the mitogenic response to purified growth factors. Placing fibroblasts in 2.5% oxygen during  $G_0$  increased their mitogenic response to epidermal growth factor six-fold. For insulin-like growth factor 1 and platelet-derived growth factor, the increases were two- and six-fold, respectively.

To understand how oxygen regulates cell proliferation at the level of gene expression, we harvested medium conditioned by  $G_0$  fibroblasts under 2.5% oxygen. This conditioned medium increased the mitogenic effect of epidermal growth factor on cells under 20% oxygen. This medium did not stimulate proliferation in the absence of added growth factor. Proteases abolished the activity of this conditioned medium. We conclude that reducing oxygen concentration below that of the ambient air induces fibroblasts to synthesize and secrete a protein with growth-promoting activity. This protein appears to enhance the mitogenic response to growth factors and may act by altering either receptors or intracellular mediators of the growth response.

## ALVEOLAR TYPE II CELL (TII) SYNTHESIS OF SATURATED PHOSPHATIDYLCHOLINE (SPC) IS DELAYED BY ENDOGENOUS AND EXOGENOUS ANDROGENS IN THE FETAL RAT IN VIVO.

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Incorporation of  $^3\text{H}$ -choline into  $^3\text{H}$ -SPC by slices of whole fetal rat lung is lower in males than in females at 20 days gestation (day 0 = mating) (Torday and Dow, 1982), leading to the hypothesis that androgens delay fetal rat lung T II maturation. A marker for T II maturation, de novo synthesis of SPC by purified T IIs, is observed to more than double between days 19 and 21 in the developing fetal rat lung. The rate of increase has been observed to be equal in males and females, but the onset of the increase is delayed in males, such that incorporation of  $^3\text{H}$ -choline into SPC is 30% less in males on day 20 and 21, though not different on day 19. To test the hypothesis that exogenous androgens delay T II maturation, time-mated Sprague-Dawley rats were injected with 1 mg/kg of dihydrotestosterone (DHT) daily from day 14 to sacrifice. Purified T IIs from DHT-treated animals showed decreased  $^3\text{H}$ -choline incorporation into  $^3\text{H}$ -SPC, and lower SPC per T II.

	$^3\text{H}$ -SPC / $10^6$ cells		mcgSPC / $10^6$ cells	
	Control	DHT	Control	DHT
Day 19	5398 $\pm$ 624	3648 $\pm$ 148	47 $\pm$ 12	32 $\pm$ 7
Day 20	8139 $\pm$ 850	5623 $\pm$ 1105		
Day 21	12302 $\pm$ 1123	8992 $\pm$ 865	124 $\pm$ 23	87 $\pm$ 27

(values: mean $\pm$ SEM of 5-10 observations;  $p < 0.05$ , all comparisons) These data suggest endogenous and exogenous androgens delay the onset of fetal rat lung type II cell maturation in vivo. Supported by NIH SCOR HL34616, and a Canadian Lung Assoc. Fellowship.

## FIBROBLASTS REGULATE TYPE II CELL MATURATION VIA BOTH INHIBITION AND STIMULATION OF THE PFF MECHANISM.

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Synchronous production of pulmonary surfactant by the alveolar type II cell is critical for neonatal survival. The timing of this event depends on the maturation of the fibroblast, which produces fibroblast pneumocyte factor (FPF), a low molecular weight protein, in response to various hormones. Fibroblast maturation can be structurally and functionally accelerated (glucocorticoids) or delayed (androgens, insulin). The type II cell subsequently shows augmented or delayed surfactant production in association with the onset of PPF production by the fibroblast. (A) The type II cell is able to respond to exogenous PPF in vivo as early as day 15 in the fetal rat (70% increase,  $p < 0.001$ ), 4 to 5 days before the fibroblast can produce PPF. (B) Incorporation of  $^3\text{H}$ -choline into  $^3\text{H}$ -saturated phosphatidylcholine by type II cells exposed to PPF is increased 50-100% over control ( $p < 0.01$ ), but no increase is noted when conditioned medium from fibroblasts which are unable to produce PPF is mixed with PPF. These data suggest that (A) type II cells are competent to respond to PPF before fibroblasts can make it, and that (B) immature fibroblasts actively inhibit the response of type II cells to PPF. Such a highly labile cell-cell interaction, consisting of both a "brake" and an "accelerator", in which the titer of biologically active PPF is the rate limiting factor, would explain the coordinated quantal increase observed in surfactant synthesis near term. Supported by NIH SCOR grant HL34616