REDUCED CELL PROLIFERATION IN FETAL LUNG AFTER MATERNAL ADMINISTRATION OF PILOCARPINE. A SCINTILLATION AUTORADIOGRAPHIC STUDY (1)

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ABSTRACT Fetuses were obtained on the 28th gestational day from pregnant New Zealand white rabbits treated daily, on the 24th through the 27th gestational day, with pilocarpine HCl, 5 mg/kg in saline, or saline alone. Lung fragments from these fetuses were incubated for two hours in medium containing ^{3}H-thymidine. Scintillation autoradiography of 1-μm-thick sections of these fetal lungs revealed that the lung tissue from pilocarpine-treated fetuses had significantly lower labelled cell indices for both alveolar epithelial cells and interstitial cells. These results indicate that pilocarpine treatment promotes differentiation of immature cells in the fetal lung at the expense of cell proliferation.

We have recently reported that administration of pilocarpine, a parasympathomimetic drug, to pregnant white rabbits results in increased maturation of fetal lung obtained on the 28th gestational day (Smith et al., '79). Specifically, this pilocarpine treatment caused an increased differentiation of type II alveolar epithelial cells from immature cuboidal cells, and an accelerated maturation of already existing type II cells. The present study was designed to ascertain the effect of this same pilocarpine administration on cell proliferation in these fetal lungs, based on thymidine incorporation and using the rapid method of scintillation autoradiography.

Scintillation autoradiography was pioneered by Panayi and Neill ('72) in a study dealing with cell division in lymphocyte cultures. Durie and Salmon ('75), using tritium with a high specific activity, also studied blood

cell division with this technique. Platkowska ('77) employed stripping film and a scintillation mixture in a study dealing with 6-μm-thick paraffin tissue sections as well as lymphocyte smears. Fischer et al. ('71) mixed scintillator with Epon and obtained electron microscopic results after 14 days of exposure. To our knowledge the present study is the first to apply the method to 1-μm-thick plastic sections. In addition we have simplified the technique somewhat by using a pre-mixed scintillator and limited special handling.

MATERIALS AND METHODS White New Zealand rabbits were mated under direct observation and maintained on a standard diet. The pregnant does received a daily subcutaneous injection of pilocarpine HCl, 5 mg/kg in 1 ml of saline, or saline alone, on day 24 through day 27 of gestation. At the 28th gestational day the does were killed and portions of lung approximately 1 mm x 1 mm x 5 mm were removed from the periphery of the right lower lobe of the most lateral fetus in the left uterine horn. The tissue fragments were placed in culture medium (Gibco MEM, 20% FCS) containing 5μCi/ml ^{3}H-thymidine (Amersham, specific activity 20 mCi/mmol). These were maintained at 37° C for two hours and then removed and fixed in cold 2% phosphate-buffered formaldehyde. The following day the tissues were washed, dehydrated in a graded methacrylate series (Polysciences JB-4) and embedded in the same medium.

Following polymerization, 1-μm-thick sections were obtained, mounted on glass slides and coated with Kodak NTB-3 nuclear track emulsion diluted 1:2 with distilled water. Following drying of the emulsion, the slides were dipped for ten seconds in a scintillation cocktail which was prepared by adding 10 g of Packard Permablend I premixed scintillator (91% PPO, 9% dimethyl POPOP) to 1000 ml of dioxane. The slides were dried, placed in light proof boxes and kept for three days at 4° C. The autoradiographs were

developed for two minutes in Kodak Dektol developer and fixed in Kodak Rapid Fixer for five minutes. Following a 15-minute running water wash, the slides were rinsed through a graded ethanol series to remove the residue of the scintillation mixture and stained lightly with toluidine blue.

The autoradiographs were photographed at 400x magnification and the transparencies were viewed randomly. The numbers of labelled and unlabelled alveolar epithelial cells and interstitial cells in each slide were recorded and analyzed using the Student's t-test.

RESULTS Using scintillation autoradiography, adequate labelling of cells was obtained after only three days of exposure. As shown in figure 1, the labelling was quite distinct and well localized over individual nuclei, while background grain exposure was minimal. Moreover, the use of 1-μm-thick sections enabled the identification of interstitial and alveolar epithelial cells based on position, size and histologic features.

The blind study of the labelled cell index of alveolar epithelium revealed that the number of labelled epithelial cells was decreased significantly ($p<0.001$) in the pilocarpine-treated group when compared with controls (fig. 2). The number of labelled interstitial cells was also significantly lower ($p<0.001$) in the pilocarpine-treated group than in controls (fig.2).

DISCUSSION Conventional autoradiography has been a useful tool in many studies but its application is limited by the fact that it may take weeks to obtain adequate exposure of the emulsion. In scintillation autoradiography the beta particles released from the tritium, in addition to causing direct exposure of emulsion grains, also cause photon release from the scintillator, which further exposes the emulsion. This process allows the exposure time to be markedly reduced, in this study to three days.

Since identification of cell types was required for the present study, plastic-embedded tissue was used rather than paraffin as Platkowska ('77) had used. The combination of 1-μm-thick sections and a thin emulsion layer enabled us to distinguish the distribution of labelling in alveolar epithelial cells as well as in interstitial cells.

It has been established that, following an injurious episode, the type II alveolar epithelium of the adult lung undergoes a proliferative response (Evans et al., '75; Witschi, '76). In addition Balis et al. ('75) demonstrated that injury to the fetal rabbit lung resulted in a similar proliferative response. We have reported elsewhere (Smith et al., in press) that maternal administration of pilocarpine causes an increase in the number of mature type II cells in the fetal lung. That this is not a response to an injurious effect of pilocarpine is confirmed by the findings of the present study which indicate that pilocarpine treatment results in decreased proliferative activity of alveolar epithelial cells.

Cell proliferation has also been demonstrated to be a useful criterion for judging maturation in the fetal lung. Sorokin ('61) demonstrated that the mitotic index in lung decreased with advancing gestational age. In an extensive autoradiographic study, O'Hare and Townes ('70) reported that the

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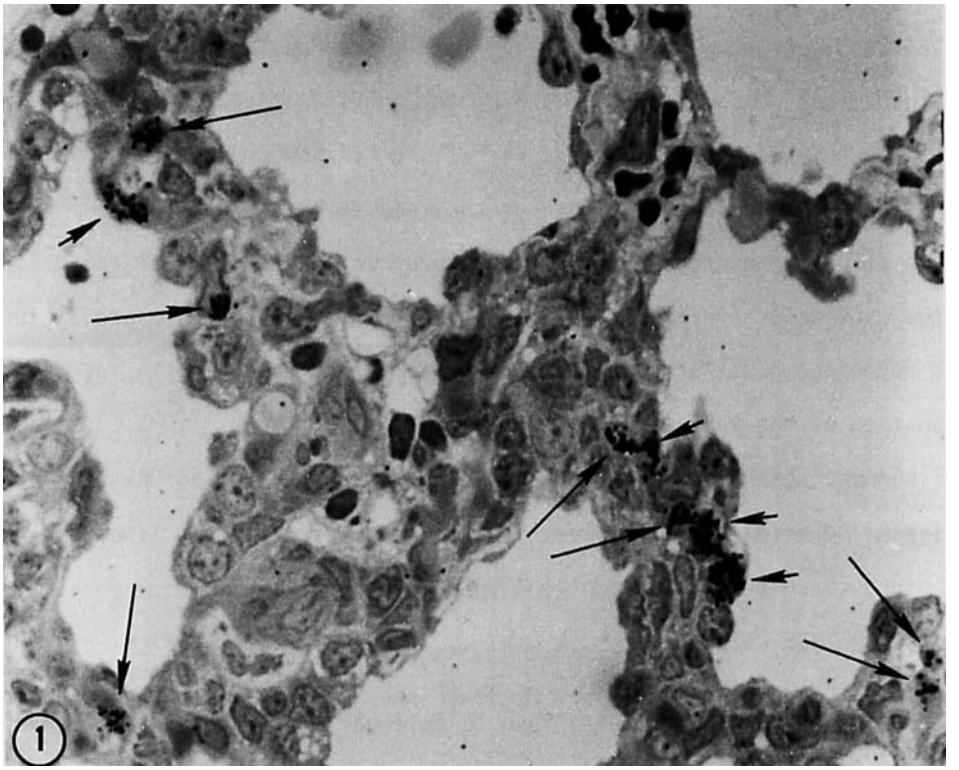
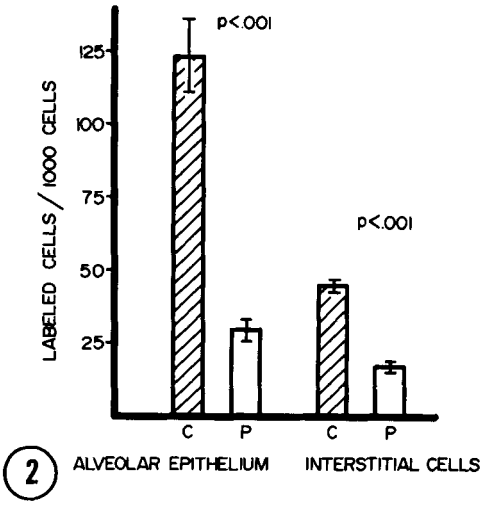
FIGURE LEGENDS

1 Light micrograph of 1-μm-thick plastic section of a control fetal lung illustrating the labelling obtained through scintillation autoradiography. Labelled alveolar epithelial cells (short arrows) and interstitial cells (long arrows) are readily identified. X 1,380.

2 Histogram illustrating the incorporation of ^{3}H-thymidine in the alveolar epithelium and lung interstitium of pilocarpine-treated (P) and control (C) rabbit fetuses. Results are expressed as labelled cells per 1,000 cells and the indicated variation is the standard error of the mean.



1


p<.001
125
100
75
50
25
LABELED CELLS / 1000 CELLS
p<.001
C
P
C
P
ALVEOLAR EPITHELIUM
INTERSTITIAL CELLS

2

labelled cell indices of rat lung alveolar epithelium and interstitium were constant until very late in gestation, when they decreased suddenly. Kauffman ('75) also reported a decrease in cell proliferation late in gestation and concluded that cells were removed from the proliferative "pool" because they were undergoing differentiation. The results of the present study substantiate our previously reported findings and lead to the conclusion that pilocarpine accelerates differentiation and maturation of the alveolar epithelium at the expense of proliferation. These epithelial changes are paralleled by a decreased proliferation of mesenchymal cells, resulting in increased maturation of the lung as a whole.

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