Cortisol Metabolism by the Lung

Stuart M. Brooks, M.D., F.C.C.P.,* **Leon J. Skolton, M.D.,† James J. Wessel, B.S.; and Brandon Taylor, B.S.

Glucocorticoids have been recognized to play an important role in lung development in utero. The identification of specific receptors for cortisol in both the cytoplasm and nuclear fraction of fetal lung homogenates supports the concept that the lung is an important target tissue for this steroid. Administration of cortisol to rabbit fetuses at appropriate stages of gestation accelerates morphologic development of lungs and causes precocious appearance of pulmonary surfactant. In fetal rabbit lungs, the specific cortisol cytosol receptors reach a maximum concentration of 28-30 days, just prior to term, when surfactant can be identified in alveolar washings. The concentration of these specific cortisol binding sites in nuclear fractions have been shown to be greater in lung than in liver.

While cortisol is the predominant active glucocorticoid in human plasma, cortisone, its 11-keto oxidized metabolite is much less active. It undergoes reduction to cortisol by the enzyme 11-beta dehydrogenase for development of steroid activity. It has been reported that the blood of human newborns contains 2.5 times more cortisone and 2.5-3 less cortisol than maternal blood. In fact, the ratio of cortisone to cortisol in the blood may be up to 18 times greater in newborns than in mothers. These studies suggest that cortisone may also play a role in human lung maturation. The present investigation was designed to study cortisol metabolism in adult and fetal rabbit lungs.

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**Associate Professor of Environmental Health and Medicine.
†Professor of Medicine; Chief, Endocrinology and Metabolism Section.

Report requests: Dr. Brooks, Kettering Laboratory, 3323 Eden Avenue, Cincinnati 45227

Material and Methods

Lungs averaging approximately 25 grams each were used in 5 lung perfusion studies. Perfusate consisted of Krebs-Ringer bicarbonate, pH 7.4 mixed in 6.5% bovine serum albumin. Following an initial 10-15 minutes of perfusion 1.8 \( \times \) 10^{-5} molar of C^14 cortisol was added to the perfusate. Serial perfusate samples were collected at 30, 60, 90 and 120 minutes and analyzed chromatographically for cortisol and metabolites. At the completion of 120 minutes of perfusion, aliquots of lung tissue were also analyzed. During perfusion lung weight gain was less than 9%.

Inhalation studies were conducted on aliquots of homogenates of lung and liver tissue from adult male New Zealand white rabbits. Samples were incubated for 2 hours at 37°C in 95% oxygen-5% CO2 with 1.82 \( \times \) 10^{-5} molar of C^14 cortisol, freshly prepared Krebs-Ringer bicarbonate buffer, pH 7.4, 10^{-4} Molar NADP, and 10^{-5} Molar glucose-phosphate. Similar studies were performed on fetal lung tissue of rabbits at 19, 24 and 30 days gestation.

Following extraction for less polar compounds, samples were chromatographed on Whatman No. 3 paper for 7 hours in a solution of toluene:methanol:water (18:3:1). Known amounts of tritiated labeled cortisol, cortisone, tetrahydrocortisone and tetrahydrocorticosterone were added to tissue or perfusate aliquots as recovery standards. Identification of cortisol and metabolites were made on the basis of the relative mobility of non-radioactive standards as determined by blue tetrazolium staining. Fractions of the chromatograms were eluted and radioactivity was counted in a liquid scintillation counter. Quantitation of cortisol and metabolites were reported as percentage of total radioactivity recovered.

Results

Figure 1 shows a typical reconstructed chromatogram of a sample of lung tissue following 2 hours' incubation. Four major peaks identified represent: cortisol (F), cortisone (E), tetrahydrocortisone (THF) and tetrahydrocorticosterone (THE). Subsequent studies revealed that these 4 compounds were the only ones that could be identified in lung tissue or perfusate. No hydroxylated or conjugated metabolites were found. Specific identification of F, E, THF and THE were performed by recrystallization to constant H-C^14 ratio, thus proving the exact iden-

![Figure 1. Reconstructed chromatogram of sample of lung tissue following 2 hours of incubation. Four peaks are identified: cortisol (F), cortisone (E), tetrahydrocortisone (THF) and tetrahydrocorticosterone (THE).](http://journal.publications.chestnet.org/pdfsaccess.ashx?url=/data/journals/chest/20990/)
Figure 2 shows the metabolic pathways of cortisol in lungs. F can be oxidized to E at the 11-beta hydroxy position. This reaction is reversible. Both F and E undergo ring-A reduction at the delta-4 and 3-keto position to form THF and THE. This reaction is not reversible.

Figure 3 shows the results of the lung perfusion studies in 5 adult rabbits. By 30 minutes, 50 percent of the total radioactivity recovered in the perfusate was F. The percentage of F radioactivity in perfusate continued to fall representing only 25 percent of total radioactivity by 2 hours of perfusion. E radioactivity rapidly increased in the circulation. By 30 minutes it represented 50 percent of the total radioactivity and 84 percent by 2 hours. THF and THE gradually increased, but were never more than 10 percent of the total radioactivity. At the end of 2 hours of perfusion, comparison of lung tissue and perfusate radioactivity indicated that perfusate contained approximately 1/3 F radioactivity and 4/5 radioactivity for THF. In contrast the ratio of radioactivity for both E and THE was more than 3 times greater in perfusate than tissue.

Figure 4 shows results of labeled F incubation experiments of lung and liver tissue from adult rabbits. It demonstrated that lung tissue qualitatively metabolized F differently than liver. Lung tissue showed mainly 11-beta dehydrogenase activity, converting approximately 18 percent of F to E. Seventy-five percent of F remained unmetabolized, approximately 3 percent was converted to THF and THE. In contrast, the liver demonstrated primarily ring-A reduction of F. Approximately 53 percent F was converted to THF and 30 percent THE. At the end of 2 hours' incubation, 10 percent F remained unmetabolized. Only 5 percent F was metabolized to E. The amount of metabolism taking place per gram of tissue was greater in liver than lung. The rate of
conversion of F was 7 times greater in liver than lung. Approximately 24 times more THF and 14 times more THE was generated by liver than lung. However, 3 times more E was generated by lung than liver. Incubation studies using fetal rabbit lung tissue of different gestational ages showed the following results (Fig 5): by 19 days approximately 21 percent of F was metabolized, 7 percent being converted to E and 79 percent remained unmetabolized. A small percentage was converted to THF and THE. By 24 days approximately 35 percent of F was metabolized, 32 percent being converted to E and the remaining 3 percent converted to THF and THE. This was similar to adult rabbit, where approximately 20 percent F was converted to E. By 30 days' gestation, 70 percent of F was metabolized, 60 percent being converted to E and the remainder to THF and THE.

**Discussion**

The lung perfusion and tissue studies suggest that rabbit lung tissue concentrates, stores and metabolizes cortisol. The major metabolic pathway for F metabolism in lung is oxidation at the 11-beta position with the formation of E. The major metabolic pathway in liver is ring A reduction and formation of THF and THE. The fetal lung tissue incubation studies suggest that maturation of fetal lung is associated with induction of 11-beta hydroxysteroid dehydrogenase enzyme with maximum conversion of F to E (or E to F).

The predominant active glucocorticoid in adult human plasma is F which is secreted by the adrenal gland. In the infant, plasma F originates largely from transplacental transfer from the mother. Approximately 20 percent of the circulating E in adults is secreted directly by the adrenal glands. The remaining 80 percent results from conversion of F to E by 11-beta dehydroxysteroid enzymes in extra-adrenal sites. Previous reports have suggested that the kidneys are a major site for extra-adrenal E production. The present investigations suggest that the lung is an important site for E formation. While F is converted to E by adult rabbit lung, the amount of conversion seems to be maximal in fetal lungs at 30 days, just prior to birth. This is at a time when pulmonary surfactant is recovered from alveolar washings and maximal number of specific cortisol receptors are identified in lung tissue. This increased E conversion in fetal rabbit lungs could explain the 2-5 times greater blood concentration of E noted in newborn humans.

The physiologic reason for the greater E production by fetal rabbit lung prior to birth can only be surmised. Greater E production may reflect general increased F activity in lung that is associated with the increased concentration of specific cortisol binding sites. Smith and associates have reported different gestation-dependent effects of F in cultured fetal rabbit lung cells. These
investigators suggested that F increased the pulmonary cellular growth in early gestation at 28 days and thus enhanced maturation, but slowed growth after 28 days. If F conversion to E is greater during these last days of gestation, the growth inhibitory effect of F, which could be undesirable for a developing infant, would be minimized. F is cleared 2 to 20 times slower in newborns than in adults. The conversion to E may enhance plasma clearance and thus prevent adrenal and growth suppressant effects of F. Cortisol binding globulin concentration in infants is approximately 25 percent of adults, resulting in less F transporting capacity. Since E is bound adequately to albumin, this may allow better transporting capabilities. Finally, it is possible that at a critical stage of development, cortisone is important in surfactant release and that maturation of lung requires the induction of 11-beta hydroxy steroid dehydrogenase.

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DISCUSSION
In response to Dr. Gillis's question as to whether prostaglandin metabolism is similar in fetal and adult lungs, Dr. Tyler replied that at a rate of 1 µg/kg/min to the left lung, PGE, was 95 percent inactivated and PGE, about 70 percent inactivated in one passage through the lung in both fetus and neonate, consistent with data reported for adults. Next, Dr. Gillis mentioned that in vitro results indicate that 30-60 minutes required to get measurable conversion of arachidonic acid to PG, yet the in vivo conversion occurs much faster. Dr. Tyler replied that the 30 minute in vitro period had been selected for convenience and was probably longer than required for initial conversion. The time required for conversion of arachidonic acid infused into pulmonary arterial blood by the lung in vivo is on the order of seconds.

In response to Dr. Soubra's question as to how HCO; gets into the alveolus of the fetus, Dr. Strang replied that the real question is how the HCO; concentration gets as low (a 10-fold concentration gradient across the endothelial epithelial barrier). Although there is no specific evidence for an HCO; pump out of the airways (or acid secretion into the airways), Dr. Strang felt that the former was the most likely explanation. Adamson in Melbourne has some evidence that the HCO; "pump" can be blocked by acetazolamide. Strang said there is a lot of carbonic anhydride in lung tissue.

In response to a question as to whether the sympathetic nervous system is involved in the hypoxic pulmonary vascular response in the fetus, Heymann reported that the response is not affected by & or & adrenergic blockade or by parasympathetic blockade. The next discussion focused on possible explanations and mechanisms which might explain the finding of apparent increasing reactivity of the pulmonary vasculature with increasing gestation. Dr. Reeser emphasized that both the systemic and pulmonary vessels begin to become reactive at about 100 days of gestation in sheep; yet, we don't understand what changes occur to produce this reactivity. Levin's report that the amount of vascular smooth muscle is relatively constant in fetal sheep between 80 and 140 days of gestation, is at variance with the findings of Wagenwort and Naeye. Heymann said that Reid, using a different technique from Levin, had suggested findings similar to those reported by Levin. If Levin and Reid are correct that there is no increase in pulmonary vascular smooth muscle, what then explains the onset of reactivity of this vascular bed?

It was then pointed out that the calculated resistance and changes in resistance depend upon the inflow pressure, and increasing inflow pressures with increasing gestation might explain the apparent onset of reactivity of the pulmonary bed. Dr. Heymann said the mean pulmonary artery pressure was 30 mm Hg in young fetuses and 57 mm Hg in older fetuses and this might partially explain the apparent increase in reactivity in the older fetus.