Effects of Epidermal Growth Factor on Lung Maturation in Fetal Lambs

Hakan W. Sundell, MD, Mary E. Gray, PhD, Fredrik S. Serenius, MD, Marilyn B. Escobedo, MD, and Mildred T. Stahlman, MD

The ability of epidermal growth factor (EGF) to induce lung maturation was evaluated in fetal and neonatal lambs. EGF was infused (3-5 days) into one member of 10 fetal twin pairs, one member of 2 term twin pairs, and 2 singleton term lambs. All EGF-treated lambs had evidence of epithelial hyperplasia of the conducting airways typical of the EGF effect. With the exception of the most immature pair, the lungs of treated versus control lambs were judged more mature by morphologic criteria by use of light and electron microscopy. None of the 6 premature lambs treated with EGF and allowed to breath showed evidence of hyaline membrane disease, while 3 untreated control lambs developed typical hyaline membranes when delivered by cesarean section after maternal hypotension. All untreated control animals showed more severe clinical symptoms of respiratory distress than did the EGF-treated animals. (Am J Pathol 1980, 100:707-726)

HYALINE MEMBRANE DISEASE (HMD) is an important specific pathophysiologic process of premature infants. The susceptibility of infants to this disease process is inversely related to gestational age, but, more specifically, is directly related to the structural and functional levels of lung development.

A number of factors have been identified that have been shown to modify the pattern of morphologic and functional development of lung during fetal life. These include naturally occurring hormones such as ACTH,[1,2] glucocorticoids,[3-6] thyroid hormone,[7] prolactin,[8] and insulin,[9] as well as pharmacologic agents including heroin,[10] β-mimetic agents such as isoxuprine,[11] and phosphodiesterase inhibitors such as aminophylline.[12] We have shown that mouse epidermal growth factor (mEGF), when injected into 24-day rabbit fetuses, induced accelerated maturation of the lung, as demonstrated by both morphologic changes and changes in pressure volume curves toward mature patterns.[13]

This study was designed to assess the ability of mEGF infused into one of twin pairs of lamb fetuses to protect against the clinical and pathologic changes associated with HMD and to measure the effects of mEGF on

From the Departments of Pediatrics and Pathology, Vanderbilt University School of Medicine, Nashville, Tennessee.

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Address reprint requests to Mildred T. Stahlman, MD, Division of Neonatology, Department of Pediatrics, School of Medicine, Vanderbilt University, Nashville, TN 37232.

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morphologic indices of lung and airway development. The mEGF will be referred to as EGF in the experiments to be described.

Materials and Methods

General

There are several important morphologic differences between lamb and human lung at comparable percentages of total gestation. The most striking of these is early alveolarization of the lamb lung. However, the effects of EGF on lung and airway maturation can be evaluated when twin pairs of lambs are used. In the experiments to be reported we used 10 pairs of twin fetal lambs at various gestational ages, 2 pairs of newborn lambs, and 2 singleton newborn lambs. One member of each twin pair and the 2 singletons were infused intravenously with EGF over a 5-day period unless otherwise indicated. In 5 twin pairs, the control lamb was similarly infused with normal saline for 5 days. Techniques for dating the pregnancies, surgical procedures, chronic *in utero* infusion techniques, sequential blood sampling, and analysis for plasma cortisol have been described.[14] The lambs were divided into 3 groups according to management (Table 1).

Group I consisted of 6 EGF-infused lambs and their twin control animals, ranging in age at time of delivery from 128 to 131 days of gestation. All 6 ewes were made hypotensive the day before delivery, a procedure that enhances the probability of development of hyaline membrane disease in premature lambs.[15] All lambs in this group were delivered by cesarean section and resuscitated as necessary.

Group II consisted of 4 EGF-infused lambs and their twin controls, which were delivered by cesarean section at gestational ages ranging from 124 to 131 days. The heads of the animals were submerged in saline-filled bags to prevent air breathing, and they were killed immediately with intracardiac potassium chloride.

Group III consisted of 4 EGF-infused normal newborn lambs, two of which had twin control animals.

After they were killed, all lambs were weighed and measured, and the lungs, adrenals, pituitary, thyroid, gonads, and thymus were excised and weighed. Blocks of lung were processed for light- and electron-microscopic examination. Tissues including the salivary gland, trachea, esophagus, heart, stomach, small intestine, large intestine, kidney, liver, pancreas, spleen, bladder, ureter, wooly and nonwooly skin, large vessels, and lymph nodes were processed for light-microscopic examination. The nonrespiratory tissue will be the subject of a subsequent communication.

Morphologic Studies

Tissue blocks, fixed in Gendre's fluid, were dehydrated, embedded in Paraplast (Sherwood Medical Industries, St. Louis, Mo), and sectioned at 3 μ for light microscopy. Sections were stained with hemotoxylin and eosin and the periodic acid–Schiff technique with and without pretreatment with diastase.

Small blocks of lung tissue (1 cu mm) were prefixed in 2.5% phosphate-buffered glutaraldehyde (pH 7.2), transferred to 7.5% phosphate-buffered sucrose, then postfixed in Millonig's osmium tetroxide for electron-microscopic examination.[16] Dyhydration and embedding were done by Luft's procedure.[17] Sections 1 μ thick were mounted on glass slides and stained with 1% toluidine blue. Thin sections were mounted on naked grids and stained with alcoholic uranyl acetate and lead citrate.[18] The grids were examined with a Philips 300 electron microscope.

Results

General Effects of EGF Infusion

Table 1 compares body size, weight, and organ weight/body weight in EGF-treated lambs and their control animals. The breed of sheep appeared to have no effect on these measurements.

Although both body weight and crown–rump length tended to be smaller in the EGF-treated lambs, the differences were not significant by paired t test. The largest discrepancies appeared in lambs given amounts of EGF over 0.9 mg/5 days. Dry lung weight per kilogram in treated animals was not significantly different from that of controls by paired t test. EGF treatment had no consistent effect on thymic weight per kilogram. Thyroid weight per kilogram was higher in all treated lambs ($P < .005$). Adrenal weight per kilogram was also significantly greater in treated animals ($P < 0.025$). Other endocrine weights did not show differences between treated and untreated pairs. Plasma cortisol levels were measured on 38 occasions in 13 pairs of lambs and showed no significant changes during EGF infusion from baseline values; nor were levels in treated lambs different from those in untreated control animals by paired t test. CT_4, FT_4, and TSH were measured in 4 lamb pairs during treatment and showed no differences between pairs; nor were they related to the day of treatment with EGF.

All 14 of the EGF-treated animals showed evidence of epithelial hyperplasia of the conducting airways, typical of the effect of EGF, whereas control animals failed to show similar changes.

Pathologic Changes in Resuscitated Lambs (Group I)

Pathologic changes were studied in the resuscitated animals of Group I by light- and electron-microscopic examination. Lungs were judged for degree of alveolarization; atelectasis or loss of alveolar volume; integrity of cuboidal cells of respiratory bronchioles and alveolar ducts (Clara cells); presence and location of hemorrhage; presence and location of lymphatic dilatation and constriction or dilatation of small arterioles, veins, and capillaries; and the presence of hyaline membranes.

Table 2 shows the time of either spontaneous death from HMD or sacrifice of each of the resuscitated lambs. The general status during life and the lung pathology are also shown. There were clear-cut differences in the ease of resuscitation and the requirements for continued high inspired O_2 levels and ventilatory assistance between treated animals and their control twins. All five of the control animals required 60–100% O_2 and ventilatory support during life. Persistent metabolic acidosis and a gradually

Table 1—Comparison of Body Size and Organ Weight in Fetal and Newborn Lambs in Relation to EGF Treatment

Lamb pair number	Gestational age at delivery	EGF dose (mg) and duration	Crown–rump (cm)	Body weight (kg)	Lung dry weight (g/kg)	Adrenals wet weight (mg/kg)	Pituitary wet weight (mg/kg)	Thymus wet weight (g/kg)	Thyroid wet weight (mg/kg)	Ovaries wet weight (mg/kg)	Testicles wet weight (mg/kg)
Group I*											
1T	128	1.5 mg/5 days	40	1787	5.2	211	36	2.7	644	33	—
1C	128	NS/5 days	41	1871	5.2	155	60	4.3	230	14	—
2T	129	1.9 mg/5 days	41	1701	7.4	294	46	4.3	497	—	304
2C	129	—	48	3515	5.4	103	30	3.3	284	12	—
3T	130	1.5 mg/5 days	42	2565	4.9	205	41	2.9	267	—	335
3C	130	NS/5 days	50	3147	4.4	138	38	7.0	156	—	309
4T	131	0.4 mg/5 days	—	2098	5.6	181	26	2.3	359	16	—
4C§	131	NS/5 days									
5T	131	0.4 mg/5 days	39	2126	3.6	148	46	2.9	203	—	331
5C	131	—	40	1673	6.8	153	38	4.3	252	13	—
6T	131	0.4 mg/5 days	43	2254	4.7	155	35	4.2	393	14	—
6C	131	NS/5 days	45	2778	4.0	137	31	1.7	229	17	—
Group II†											
7T	124	.8 mg/5 days	41	1814	4.3	138	29	2.0	—	—	250
7C	124	—	39	1956	8.6	74	23	3.2	237	15	—
8T	126	.9 mg/3 days	38	2240	2.5	167	17	5.2	264	20	—
8C	126	NS/3 days	40	2126	3.1	164	35	3.8	214	—	366
9T	130	1.6 mg/5 days	46	3090	3.1	130	23	1.6	328	—	218
9C	130	—	47	3247	4.7	110	22	4.2	212	—	265
10T	131	0.8 mg/5 days	47	3062	4.1	151	39	6.2	310	7	—
10C	131	—	46	3175	5.4	147	38	6.8	239	11	—
Group III‡											
11T	Term	1.8 mg/5 days	49	4444	8.3	169	—	3.0	291	—	236
11C	Term	—	50	4359	4.6	145	—	4.2	106	—	251
12T	Term	3.2 mg/5 days	53	3770	6.3	231	35	2.6	403	24	—
12C	Term	—	53	7031	3.0	108	12	4.0	112	8	—
13T	Term	.8 mg/4 days	52	4536	5.2	213	24	3.2	265	29	—
14T	Term	.8 mg/4 days	52	4082	3.5	152	16	1.0	160	—	379

* Resuscitated and ventilated fetal lambs after cesarean section.
† Fetuses killed at birth after cesarean section.
‡ Normal newborn lambs.
§ Died *in utero* on Day 1 of treatment.

Table 2—Outcome of Lambs in Group I

Lamb*	Gestation age (days)	Outcome†	Time at death (hours)	Status while alive	Lung pathology (light microscopy)	Bronchial epithelial hyperplasia (EGF effect)
1T	128	S	4 1/2	Decreasing amounts of O_2 pressure over first 3 hours, extubated on room air	Alveolar duct and respiratory bronchiolar epithelium intact, with no membrane formation Alveolar volume normal	++
1C	128	D	4 1/2	Acidotic and unsaturated despite 100% O_2 high pressures throughout	Massive slough of alveolar duct and respiratory bronchiolar epithelium, with membrane formation and loss of alveolar volume	0
2T	129	S	5 1/2	Improving ventilatory status, extubated on 60% O_2	Alveolar duct and respiratory bronchiolar epithelium intact, with no membrane formation	+
2C	129	D	5 1/2	Unsaturated and acidotic on 100% O_2 and ventilated throughout	Massive slough of alveolar ducts and respiratory bronchioles, with membrane formation and loss of alveolar volume	0
3T	130	S	6	O_2 decreased from 60% to room air over 5 hours, ventilated 1 1/2 hours	Alveolar duct and respiratory bronchiolar epithelium intact, with no membrane formation Alveolar volume normal	+-+
3C	130	D	1/2	Accidental death at 1/2 hour on ventilator and O_2	Normal lung without membrane	0
4T	131	S	5	Required no O_2 or ventilatory assistance. Stable over 5 hours' study	Alveolar duct and respiratory bronchiolar epithelium intact with no membrane formation Alveolar volume normal	+

Table 2 continued

Lamb*	Gestation age (days)	Outcome†	Time at death (hours)	Status while alive	Lung pathology (light microscopy)	Bronchial epithelial hyperplasia (EGF effect)
4C‡	131					
5T	131	S	5	100% oxygen, ventilator and buffers used over 5-hour period	Alevolar duct and respiratory bronchiolar epithelium intact, without membrane formation Alveolar volume normal Alveolar edema present	+
5C	131	S	5	Required resuscitation with O_2 and ventilator with improvement at 1 hour. Gradually deteriorated for 4 hours with progressive acidosis and unsaturation in 100% O_2	Alvelolar duct and respiratory bronchiolar epithelium sloughing, with membrane formation and loss of alveolar volume	0
6T	131	S	7	Improving ventilatory status over 1 1/2 hours with decreasing use of O_2 and ventilator Over next 6 hours required 40% O_2 and 4–6 cm CPAP	Alveolar duct and respiratory bronchiolar epithelium intact, with no membrane formation Alveolar volume normal	+
6C	131	S	7	Required 60–100% O_2 and ventilator throughout life and repeated infusion of bicarbonate for metabolic acidosis	Pulmonary edema and loss of alveolar volume without epithelial slough or membrane formation	0

* T = treated; C = control.
† S = sacrificed; D = died spontaneously.
‡ Died *in utero*.

worsening clinical course occurred in these animals prior to death. One of the control animals (3c) accidently died 1 half hour after birth, at which time it was requiring 100% O_2 and ventilatory support. The lungs were essentially normal morphologically. The lungs of three of the control animals (1c, 2c, and 5c) showed typical findings of HMD with massive alveolar duct and respiratory bronchiolar cell lining slough with membrane formation and loss of alveolar volume. Membranes lined denuded alveolar ducts and respiratory bronchioles, with minimal involvement of airways lined by Type I epithelial cells (Figure 1). The fifth control lamb (6c) had edema in terminal conducting airways and alveoli and loss of alveolar volume without true membrane formation.

Five of the EGF-treated animals (1T, 2T, 3T, 4T, and 6T) required either no supplemental O_2 or had decreasing oxygen requirements from 60% down to room air within 3–5 hours after birth. The sixth EGF-treated animal had evidence of sepsis at autopsy and required O_2 and ventilatory assistance throughout life (5T). None of the 6 EGF-treated lambs in Group I had slough of Clara cells or membrane formation, and none had apparent loss of alveolar volume. Although the EGF dosage in this group of lambs varied between 0.4 and 1.9 mg/5 days, protection was apparently adequate, even at the lowest dose (Figure 2).

Histologic Effects of EGF on the Respiratory Tract of Lambs Killed at Birth and of Newborn Lambs (Groups II and III)

Table III shows the histologic changes associated with EGF treatment in the respiratory tract of lambs in Group II. In the trachea, the morphologic features and number of cell layers in the epithelium, the frequency of mitoses seen, and the morphologic features of the submucosal glandular epithelium were evaluated. In the smaller conducting airways within the lung, the presence of hypertrophy or hyperplasia of their epithelial lining, both ciliated and nonciliated cells, the presence and amount of glycogen in these cells, and the number of mitoses seen were judged. Alveolarization was judged as incomplete and immature or mature, and Type II alveolar cells were judged for prevalence, the amount of glycogen present, and the number of lamellar bodies per cell.

Tissue from the trachea was available on 3 of the 4 twin pairs of animals in Group II. All 4 pairs had lung tissue examined by light and electron microscopy. The tracheal epithelium of untreated control lambs was normal pseudostratified ciliated columnar in type with a well-defined single basal layer of nuclei, one intermediate cell layer, and a normal-appearing ciliated border. Mitoses were not seen. The tracheas of EGF-treated animals showed apparent piling up of epithelial cells with nuclei 5–6 layers in

Table 3—Histologic Characteristics of the Respiratory Tract in Group II (Light and Electron Microscopy)

Lamb*	Gestational age in days	Trachea	Conducting airways	Respiratory bronchioles and alveolar ducts	Alveolarization	Alveolar Type II cells
7T	124	—	Hyperplastic nonciliated epithelium, glycogen ++	Normal	Incomplete and intermediate	Scarce and immature
7C	124	—	Normal, immature, without hyperplasia, glycogen ++++	Normal	Incomplete and intermediate	Scarce and immature
8T	126	Hyperplasia of nonciliated epithelium	Hyperplastic nonciliated epithelium, glycogen ++	Normal	Normal, mature, well alveolarized Alveolar volume well established	Plentiful and mature, containing many lamellar bodies/cell
8C	126	Normal epithelium	Normal, immature, without hyperplasia, glycogen ++++	Normal	Incomplete and immature	Scarce and immature
9T	130	Hyperplasia of nonciliated epithelium	Hyperplastic nonciliated epithelium, glyocgen ++++	Normal	Normal, mature, well alveolarized Alveolar volume well established	Plentiful and mature, containing many lamellar bodies/cell
9C	130	Normal epithelium	Normal, immature, without hyperplasia, glycogen ++++	Normal	Incomplete and immature	Scarce and immature
10T	131	Slightly thickened epithelium	Immature, hyperplastic nonciliated epithelium with infolding, glycogen ++++	Normal	Normal, mature, well alveolarized Alveolar volume well established.	Plentiful and mature, containing many lamellar bodies/cell
10C	131	Normal epithelium	Normal, immature, without hyperplasia, glycogen ++++	Normal	Incomplete, with more interstitial volume to alveolar walls	Not as numerous, with few lamellar bodies/cell

* T = treated; C = control.

depth. The basal layer was especially prominant, appeared 2–3 layers of nuclei thick, and contained mitotic figures. The intermediate layers of cells appeared pale and vacuolated, and the ciliated border was flattened and discontinuous (Figures 3 and 4). Cells of the tracheal glands in both treated and untreated twins were filled with secretory granules, but the EGF animals showed crowding of lining cells with formation of folds.

The morphologic characteristics of the intrapulmonary bronchi in control animals were normal. Epithelium in similarly sized airways in EGF-treated twins was thickened and folded (Figures 5 and 6). By electron microscopy, the bronchiolar epithelium in both EGF-treated and control lambs consisted primarily of undifferentiated, glycogen-filled cells with few organelles, interspersed with ciliated cells. In treated animals, cell crowding resulted in the formation of deep folds. The nonciliated cells were the predominant cell type, almost covering the tops of the rare ciliated cells (Figure 7). Alveolar duct epithelium appeared relatively unaffected by EGF treatment.

With the exception of the most immature pair (7T), alveolarization had a more mature pattern, as judged by thinning and loss of cellularity of alveolar septa and by development of normal alveolar volume in all EGF-treated lambs when compared with their untreated twins (Figures 8 and 9). Type II alveolar cells were scarce in untreated lambs, and immature in appearance, with few lamellar bodies and abundant glycogen. By contrast, Type II cells of treated twins were easily identified by electron-microscopic examination, plentiful in number, and mature in appearance, with abundant lamellar bodies per cell (Figures 10 and 11).

The epithelium of trachea and conducting airways of lambs in Group III showed essentially the same changes associated with EGF treatment as those in Group II. Table IV shows these histologic changes in Group III lambs whose tracheal epithelium was even more markedly thickened, with 15–20 apparent cell layers appearing in the animal with the highest EGF dose, 3.25 mg/5 days (12T). There were possible evidences of toxicity in this animal, because it fed poorly and ceased to gain weight during treatment.

Discussion

EGF is a biologically active polypeptide first described in 1962 by Cohen,[19] who observed that the daily injection of an extract of male mouse submaxillary gland into immature mice resulted in precocious tooth eruption and eye opening. Subsequent analysis of the salivary gland extract showed that the biologically active component was a heat-stable, nondialyzable, single polypeptide chain of 53 amino acid residues with a mo-

Table 4—Histologic Characteristics of Airways in Group III (Light and Electron Microscopy)

Lamb*	Age at autopsy (days)	Trachea	Conducting airways
11T	6	Thickened and infolded epithelium with numerous mitoses in basal cells	Bronchial and bronchiolar epithelium hypertrophied with crowding of cells with glycogen + + present Mitoses readily seen Alveolar duct and repiratory bronchiolar epithelium unaffected
11C	6	Normal respiratory epithelium	Bronchial, bronchiolar, and alveolar duct epithelium normal, glycogen + present
12T	6	Markedly thickened and infolded epithelium with numerous mitoses in basal cells.	Bronchial and bronchiolar epithelium hypertrophied with crowding of cells Mitoses readily seen Alveolar duct and respiratory bronchiolar epithelium unaffected
12C	6	Normal respiratory epithelium	Bronchial, bronchiolar, and alveolar duct epithelium normal
13T	5	Thickened with mitoses in basal cells	Bronchial and bronchiolar duct epithelium moderately hypertrophied, with crowding of cells Mitoses readily seen Alveolar duct and respiratory bronchiolar epithelium unaffected
14T	6	Thickened and infolded	Bronchial and bronchiolar duct epithelium moderately hypertrophied, with crowding of cells Mitoses readily seen Alveolar duct and respiratory bronchiolar epithelium unaffected

* T = treated, C = control.

lecular weight of 6045 daltons.[20] The biologic effects of EGF have been shown to be primarily those of general epithelial growth and keratinization. These effects of mouse-derived EGF (mEGF) have been demonstrated in other species, including dogs, rats, and rabbits.[21] Mitogenic effects have been shown in mammary epithelial cells; chondrocytes; glial cells; vascular smooth muscle; uterine, vaginal, ureteral, and ductus deferens epithelial cells; prostatic cells; and various fibroblasts.[22,23,24] Recently it was demonstrated that EGF was also present in man [25] and that human EGF, when tested in organ culture systems, appeared to act in a manner identical to that of mEGF.[26]

Because of its demonstrable powerful effect on epithelial growth, EGF was used in this study in fetuses and newborn animals to evaluate its effects on lung maturation and possible protection against HMD. Twin fetal lambs whose mothers had been made hypotensive 24 hours previously were delivered by cesarean section at a gestational age at which the de-

velopment of HMD is assured in unprotected lambs. Four of five untreated animals in Group I developed clinical and pathologic evidence of HMD, the fifth animal dying accidentally at 1-half hour. Treated animals ran benign clinical courses with improvement in respiratory status during life, and none showed the pathologic changes of HMD.

The mechanism by which protection of alveolar duct and respiratory bronchiolar epithelium from massive slough with protein exudation and membrane formation after birth is achieved following EGF treatment is unknown. Cells lining this level of airway do not appear to be morphologically different in treated and untreated lambs of Group II. One can speculate that there are changes in pulmonary blood flow similar to those associated with ACTH treatment that protect the fetal lung alveolar duct and respiratory bronchiolar cells, in particular, from ischemia during fetal stress.[27]

Terminal airways in treated animals appear to undergo a more accelerated maturation than their control twins, with differentiation of both Type I and Type II alveolar cells. It is this alveolarization, with the development of a blood gas interface, and effective maturation of the surfactant system, that apparently reduces the susceptibility of treated lambs to HMD development. It has been shown that certain other hormones, notably cortisol, are capable of accelerating the changes in fetal lungs at susceptible gestational ages toward morphologic, biochemical, and physiologic maturity. These changes, when inducible, have been shown to protect both human infants [28,29] and experimental animals of appropriate gestational ages against the development of hyaline membrane disease.[2] There was no evidence, however, of EGF affecting levels of cortisol production or of increasing levels of thyroid hormone in these animals. The increase in treated animals of the adrenal and thyroid gland weight per kilogram body weight over that of their twin controls apparently reflects increases in cell number and/or cell size rather than changes in cell function by differentiation.

After the successful demonstration that susceptible fetal lambs could be protected against the development of HMD when infused with EGF, a series of fetal animals of two different gestational ages and a series of normal newborn animals were similarly treated with EGF for study of the morphologic changes in the developing lung and conducting airways. Several different dosage schedules were used, because the optimal dose was unknown.

The principal morphologic effects of EGF on the respiratory tract appeared to be on the growth of those cells of the tracheal and bronchial epithelium that were least differentiated. Cell proliferation, as in other

epithelia,[21] was most active in the undifferentiated basal cells, those cells that appeared more differentiated being crowded toward the luminal surface. This greatly increased number of relatively undifferentiated cells became thrown up into folds and formed deep crypts in the intrapulmonary conducting airways, while fully differentiated cells, such as those with cilia, appeared unaffected by this growth factor. These changes in cell growth are compatible with the findings of Rheinwald and Green,[30] who have reported that in cell cultures of human keratinocytes, EGF delayed the onset of terminal differentiation and increased propagation *in vitro* by a factor of 10^{10}. In the respiratory tract, as elsewhere, EGF appears to have little, if any, effect on fully differentiated cell populations. Those portions of the fetal and newborn lung still capable of growth and differentiation appear maximally affected. The trachea and conducting airways showed cell growth as a primary effect, whereas in the distal airways, which are already undergoing rapid differentiation, the EGF effect appeared to be that of continued terminal differentiation.

EGF has been shown to be a human growth hormone. The undifferentiated tissues of the fetus and those cell populations in the older individual that continue to undergo rapid cell turnover would be the expected targets for EGF effect. Further study may elucidate its role in human development.

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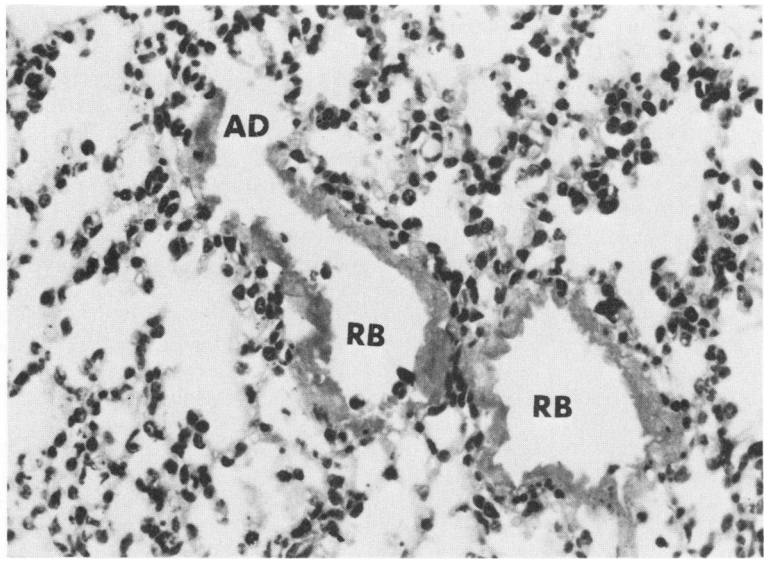

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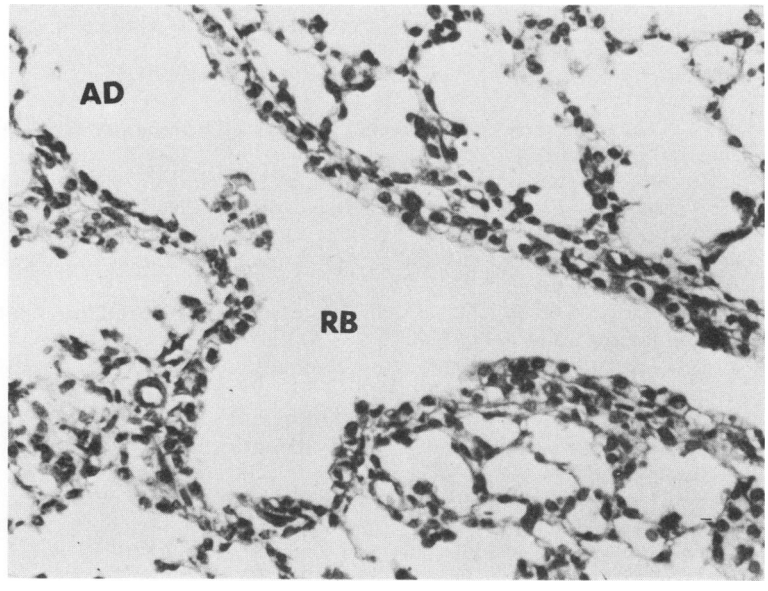

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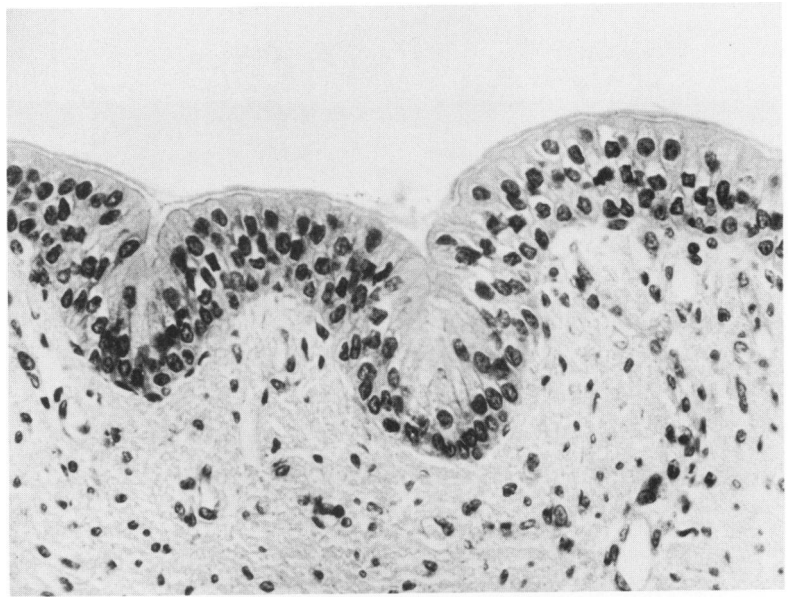
Figure 1—Fetal lamb 1C. Section of lung from a saline-infused control fetal lamb of 128 days' gestation that died 4.5 hours postnatally. There is diminished alveolar volume. Respiratory bronchioles (*RB*) and alveolar ducts (*AD*) have lost their epithelium and are lined with hyaline membranes made up of sloughed epithelial cells and a proteinaceous fluid. (H&E, ×400) **Figure 2**—Fetal lamb 1T. Section of lung from an EGF-infused fetal lamb of 128 days' gestation that was killed 4.5 hours postnatally. In comparison with Figure 1, there is more advanced alveolarization, with thinning of interalveolar septa and diminution of cellularity. Respiratory bronchiole (*RB*) and alveolar ducts (*AD*) are lined by intact epithelium, and there is no evidence of hyaline membrane formation. (H&E, ×400)



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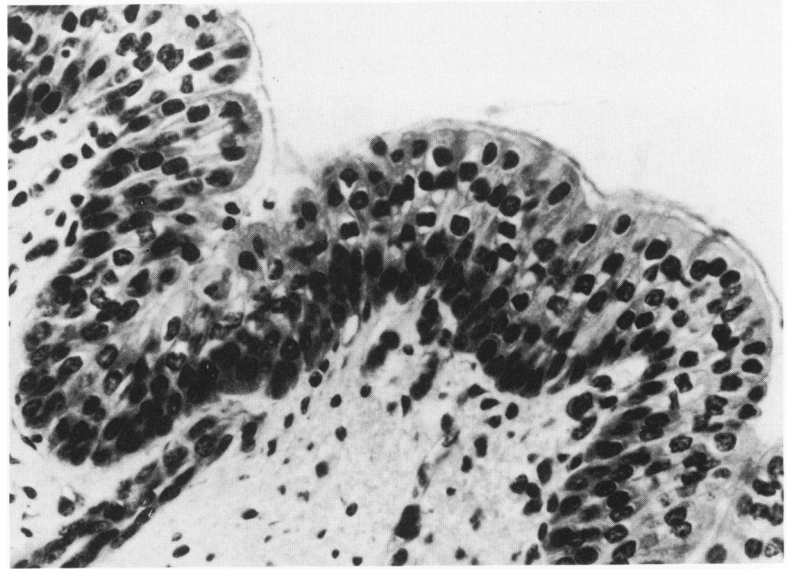

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Figure 3—Fetal lamb 8C. Section of trachea from a saline-infused control fetal lamb of 126 days' gestation, delivered by cesarean section and killed at birth without breathing. The pseudostratified columnar ciliated epithelium of the mucosa is normal in appearance, with two to three rows of nuclei. (H&E, ×400) **Figure 4**—Fetal lamb 8T. Section of trachea from an EGF-infused fetal lamb of 126 days' gestation, delivered by cesarean section and killed at birth without breathing. The epithelium of the mucosa is thickened markedly. There are five to six rows of nuclei. There are at least two rows of nuclei belonging to basal cells, the cytoplasm of which is more basophilic than that of similar cells in the trachea of the control lamb. Whether this epithelium has retained its pseudostratified characteristics or is undergoing squamous metaplasia cannot be determined. However, the luminal surface is still ciliated. The underlying connective tissue of the lamina propria appears to be more cellular than that in the trachea of the control animal of Figure 3. (H&E, ×400)

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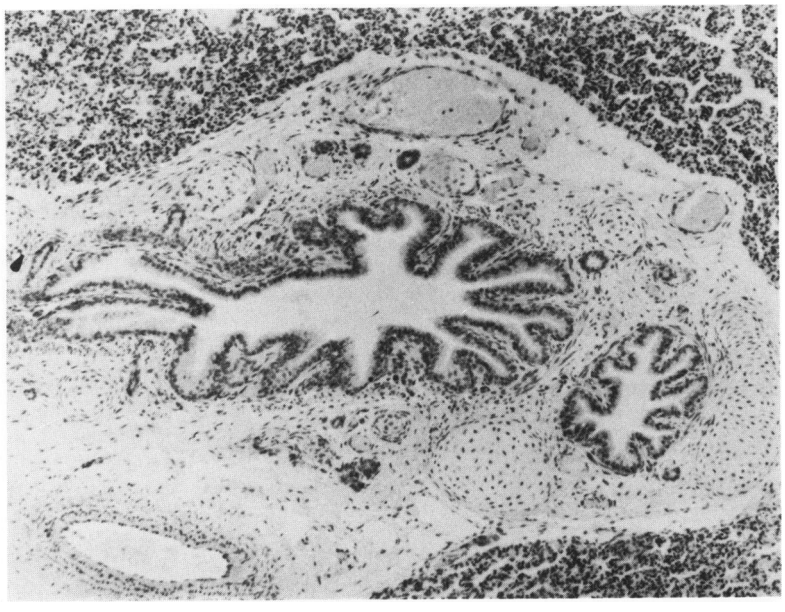

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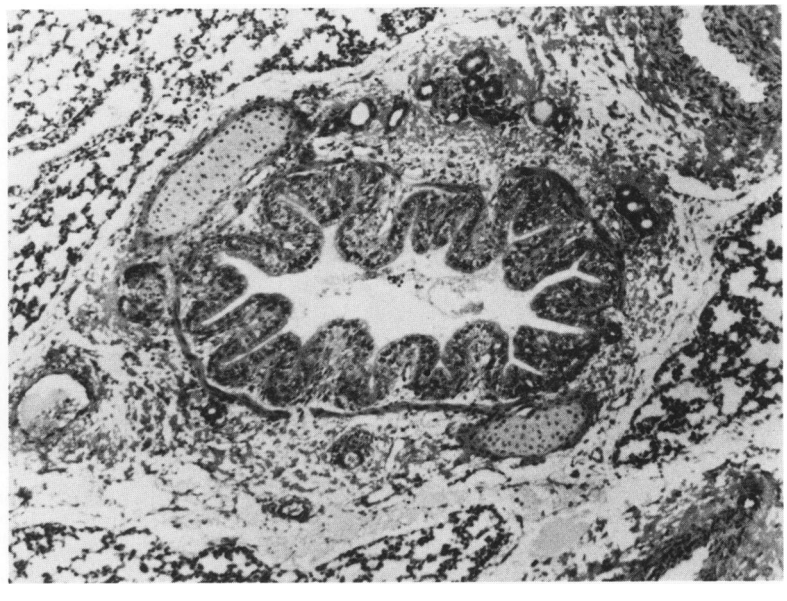

Figure 5—Fetal lamb 8C. Section of an intrapulmonary bronchus from a saline-infused control fetal lamb of 126 days' gestation, delivered by cesarean section and killed at birth without breathing. The pseudostratified columnar ciliated epithelium of the mucosa is thin, with only one or two rows of nuclei visible. (H&E, ×107) **Figure 6**—Fetal lamb 8T. Section of an intrapulmonary bronchus from an EGF-infused fetal lamb of 126 days' gestation, delivered by cesarean section and killed at birth without breathing. The epithelium of the mucosa is thickened, with two to three rows of nuclei apparent. (H&E, ×107)

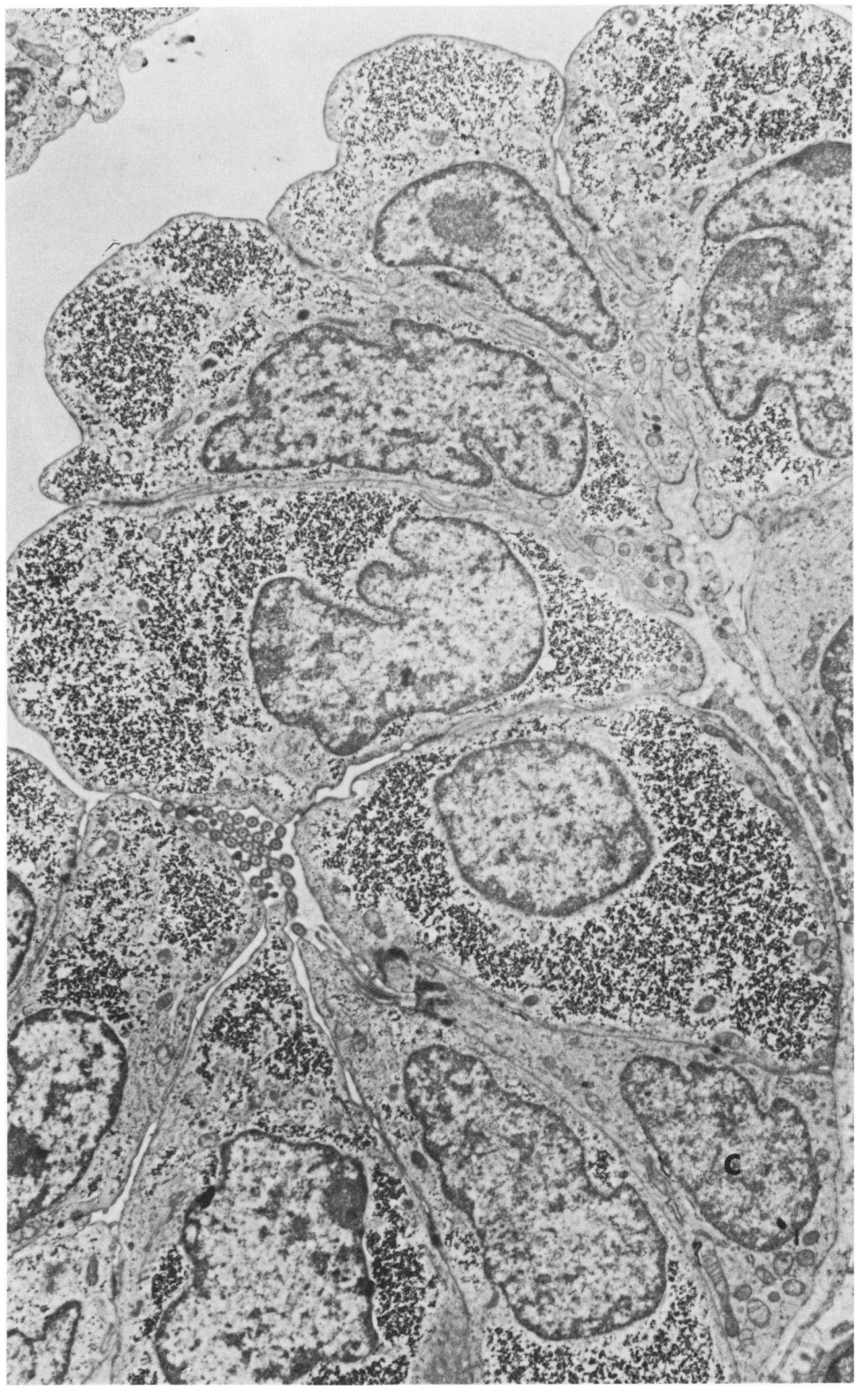

Figure 7—Fetal lamb 10T. Electron micrograph of a small bronchiole of lung from an EGF-infused fetal lamb of 131 days' gestation, delivered by cesarean section and killed at birth without breathing. The mucosa is thrown into deep folds. The epithelial lining is composed primarily of glycogen-filled, cuboidal cells. The few organelles they contain are located peripherally. These cells are so numerous and so crowded that the few ciliated cells (*C*) are almost covered. The ciliated cells contain no glycogen, but numerous organelles. (Uranyl acetate and lead citrate, ×7350)

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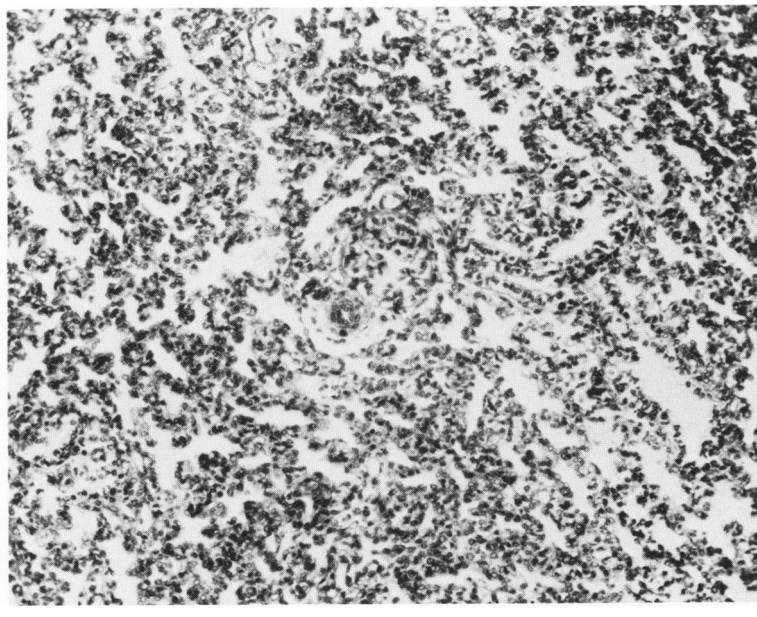

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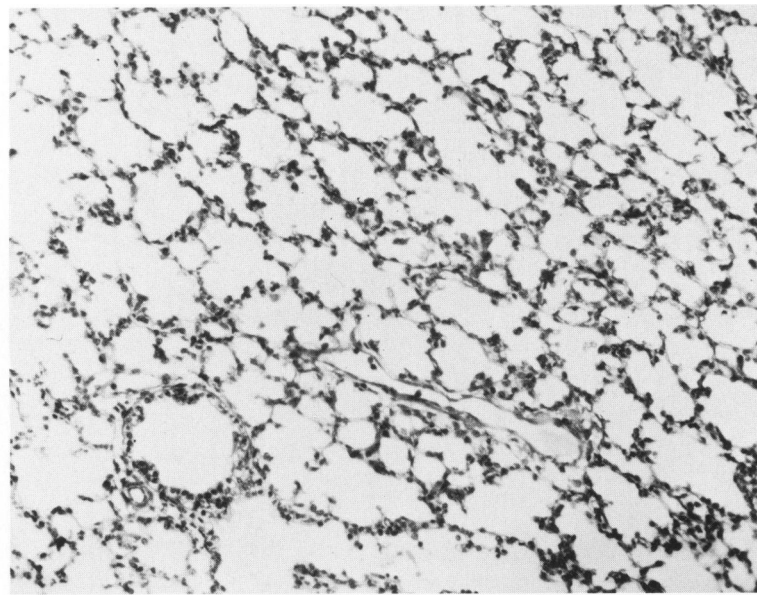
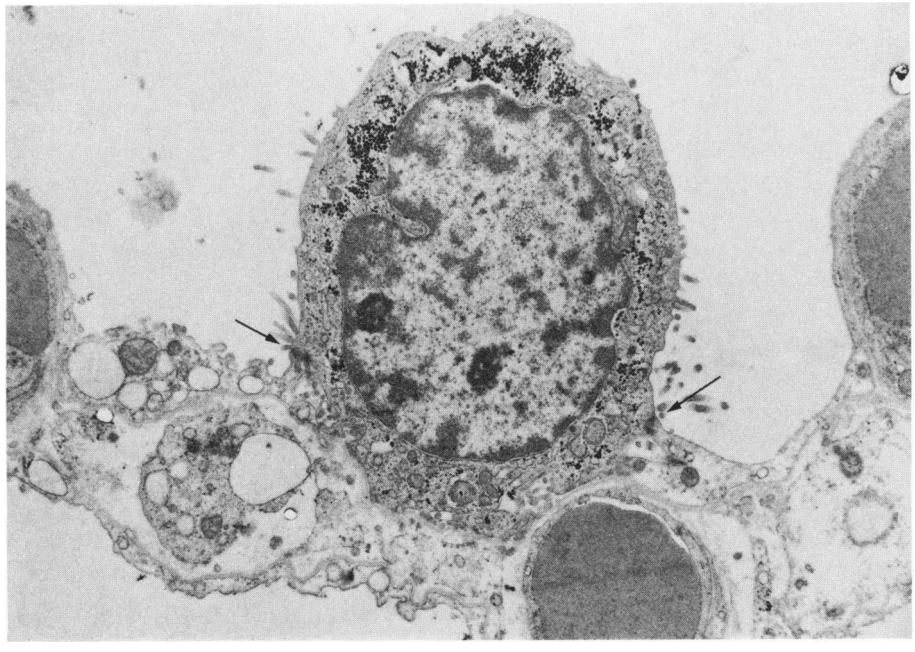


Figure 8—Fetal lamb 8C. Section of lung from a saline-infused control lamb of 126 days gestation, delivered by cesarean section and killed at birth without breathing. This is an immature fetal lung, with many airways still lined by undifferentiated cuboidal epithelium. (H&E, ×160) **Figure 9**—Fetal lamb 8T. Section of lung from an EGF-infused fetal lamb of 126 days' gestation, delivered by cesarean section and killed at birth without breathing. There is advanced alveolarization, with thinning of interalveolar septa providing potential for adequate blood–air interface. (H&E, ×160)



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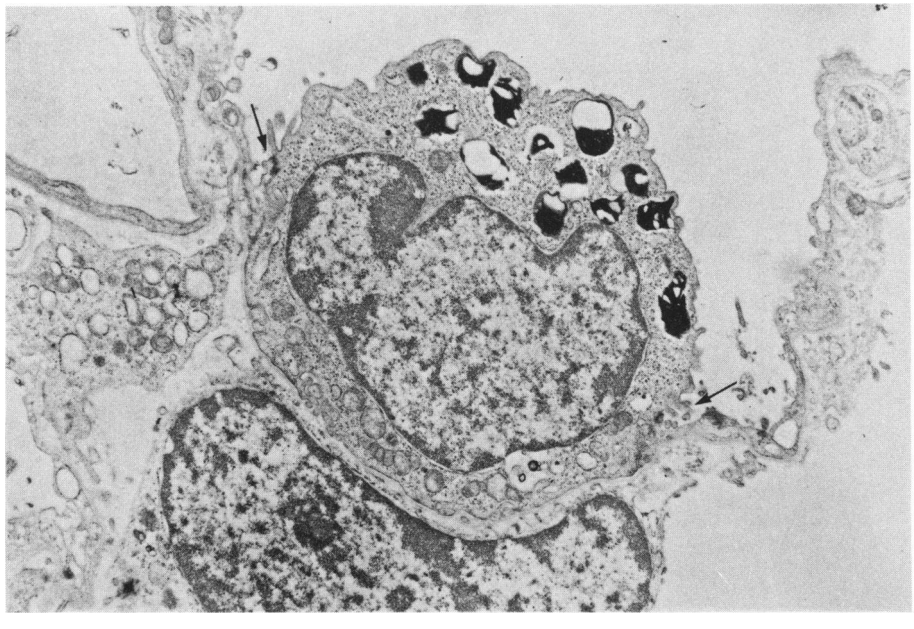

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Figure 10—Fetal lamb 10C. Electron micrograph of an alveolar Type II cell from lung of a saline-infused control fetal lamb of 131 days' gestation, delivered by cesarean section and killed at birth without breathing. This is an immature glycogen-rich Type II cell, devoid of lamellar bodies. Slender microvilli are scattered over the surface but appear to be more numerous near the junctions with adjacent Type I cells (*arrows*). (Uranyl acetate and lead citrate, ×8950) **Figure 11**—Fetal lamb 10T. Electron micrograph of an alveolar Type II cell from lung of an EGF-infused fetal lamb of 131 days' gestation, delivered by cesarean section and killed at birth without breathing. This Type II cell is more mature than the one in Figure 10 but is still immature, in that it contains abundant glycogen. The lamellar bodies are at the luminal surface. Mitochondria are at the base. The microvilli are prominent only near junctions with adjacent cells (*arrows*). (Uranyl acetate and lead citrate, ×8950)

[*End of Article*]