Influence of growth hormone on the lung growth response to tracheal obstruction in fetal sheep

L. NARDO, I. R. YOUNG, AND S. B. HOOPER
Department of Physiology, Monash University, Clayton, Victoria 3168, Australia

Nardo, L., I. R. Young, and S. B. Hooper. Influence of growth hormone on the lung growth response to tracheal obstruction in fetal sheep. Am. J. Physiol. Lung Cell. Mol. Physiol. 278: L453–L459, 2000.—Obstructing the fetal trachea is a potent stimulus for fetal lung growth, but little is known about the factors that regulate this process. Our aim was to determine the role of growth hormone (GH) in regulating the increase in lung growth induced by obstruction of the trachea in fetal sheep. Twenty chronically catheterized fetal sheep, nine of which were hypophysectomized, were divided into four experimental groups: 1) control group (n = 4), 2) a group in which the fetal trachea was obstructed for 3 days (3-day obstructed; n = 6), 3) a 3-day obstructed group in which the pituitary was removed [hypophysectomized (HX)], and the fetus was given maintenance infusions of ACTH, thyroxine, and human GH (hGH; HX hGH 3-day obstructed; n = 5), and 4) a HX 3-day obstructed group in which the fetus was given maintenance infusions of ACTH and thyroxine (n = 5). Tracheal obstruction significantly increased fetal lung liquid volumes from 37.2 ± 3.2 ml/kg in control fetuses to 75.6 ± 9.0 ml/kg in 3-day obstructed fetuses, and the presence or absence of GH did not affect this increase. Similarly, the presence or absence of GH did not affect the increase in lung weight or protein content induced by 3 days of tracheal obstruction. However, in the absence of GH, 3 days of tracheal obstruction failed to increase total lung DNA content above unobstructed control values (107.9 ± 5.3 and 94.1 ± 7.0 mg/kg for control and HX 3-day obstructed groups, respectively). In contrast, 3 days of tracheal obstruction increased total lung DNA content to a similar extent in fetuses with an intact pituitary and HX fetuses that received GH replacement (126.0 ± 4.4 and 126.7 ± 4.0 mg/kg for 3-day obstructed and HX hGH 3-day obstructed groups, respectively). These data indicate that the absence of GH either abolishes or delays the acceleration in cell division caused by an increase in fetal lung expansion.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
lung expansion induced by obstruction of the fetal trachea.

METHODS

Surgery was performed on 20 pregnant ewes and their fetuses (Border-Leicester X Merino) at 117–121 days of pregnancy. Anesthesia was induced with thiopental sodium (1 g iv) and was maintained, after tracheal intubation, with 1.5% halothane in O₂: N₂O (50:50 v/v). In each fetus, two silicone rubber cannulas were inserted into the fetal trachea and secured with silk ties to ensure that there was no leakage of lung liquid (14). Polyvinyl catheters were also implanted into a fetal carotid artery and jugular vein for blood sampling. In addition, 16 fetuses were hypophysectomized (HX) as previously described (20). The fetal head was exposed, and a hole was drilled in the sphenoid bone to reveal the pituitary gland, which was removed by microdissection and aspiration. Antibiotics (900 mg of Depomycin, Intervet, Castle Hill, Australia) were administered intramuscularly to the fetus before the uterine incision was closed. All fetal catheters were exteriorized through an incision made in the ewe’s right flank. Ewes and fetuses were allowed to recover for 5 days after surgery. Fetal well-being was monitored on a daily basis by measuring fetal arterial PO₂, arterial PCO₂, pH, arterial O₂ saturation, total hemoglobin, and hematocrit (ABL30, Radiometer).

Experimental protocol. The animals were divided into four treatment groups, and tracheal obstruction was achieved by obstructing the exteriorized tracheal loop. In group 1, the pituitary was left intact and lung liquid flow through the trachea was unimpeded for the duration of the experiment (control; days 117–131; n = 4). In group 2, the pituitary was left intact and the fetal trachea was obstructed for 3 days (3-day obstructed; days 128–131; n = 6). In group 3, the pituitary was removed, the fetus was given intravenous infusions of ACTH (1.0 μg·24 h⁻¹·kg⁻¹), thyroxine (T₄; 40 μg·24 h⁻¹·kg⁻¹, 0.6 ml/h, pH 11) and human GH (hGH; 1.5 mg·24 h⁻¹·kg⁻¹), and the trachea was obstructed for 3 days (HX hGH 3-day obstructed; days 128–131; n = 5). In group 4, the pituitary was removed, the fetus was given intravenous infusions of ACTH (1.0 μg·24 h⁻¹·kg⁻¹) and T₄ (40 μg·24 h⁻¹·kg⁻¹, pH 11), and the trachea was obstructed for 3 days (HX 3-day obstructed; days 128–131; n = 5).

Fetal body weights were estimated on the basis of gestational age (18). Saline was infused (0.9% NaCl, 0.6 ml/h iv, pH 11) in control and 3-day obstructed fetuses. All infusions commenced immediately after surgery and continued for the duration of the experimental period (days 117–131). As much liquid as possible was drained from the lungs immediately before the ewe and fetus were humanely killed with an overdose of pentobarbital sodium (135 mg/kg iv). The fetus was removed and weighed, and the fetal lungs, heart, liver, right and left adrenals, and pituitary (where present) were removed and weighed (wet weight). Portions of the left lung were rapidly frozen in liquid nitrogen for biochemical analysis, and the right fetal lung was prepared for histological analysis. All experimental procedures on the animals were approved by the Monash University (Clayton, Australia) Standing Committee for Ethics in Animal Experimentation by complying with the requirements of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Analytic methods. Twelve milliliters of fetal arterial blood were collected on days 122, 124, 127, and 131 of gestation, and the plasma was stored at −20°C. Fetal plasma samples were analyzed for ACTH (ACTH 125I-RIA kit, IncStar), cortisol (5), T₄ (ImmuChem, ICN Biomedicals, Costa Mesa, CA), prolactin (PRL) (19), ovine GH (oGH) (32), and hGH (ImmuChem double-antibody hGH 125I-RIA kit, ICN Biomedicals). Lung liquid secretion and volume measurements were performed with an established method (14) of impermeant indicator dilution (blue Dextran 2000, Pharmacia Chemical). These measurements were made at 128 days of gestation just before tracheal obstruction and at the end of the obstruction period (131 days). The concentration of indicator in each lung liquid sample was measured with a multichannel absorbance meter (Titertek Multiscan, Flow Laboratories) set at a wavelength of 620 nm.

Soluble protein and total DNA contents of the fetal lung were determined in all treatment groups with established techniques (14, 24). Tissue and luminal space volumes, alveolar diameter, number, wall thickness, and surface area were determined for all fetal lungs with techniques previously described (11, 36). The volume of the right lung was estimated with the Cavalieri method (22), and tissue shrinkage was calculated to be 7%. The reported values of total volume; tissue volume; luminal volume; and alveolar surface area, density, and number and for the right lung only, and the values for alveolar surface area, density, and number have been adjusted for tissue shrinkage.

Statistical analysis. Data in the text are means ± SE. Differences in blood gases and pH, hormone concentrations, lung liquid volumes, and secretion rates were analyzed by three-way analysis of variance for repeated measures with treatment group, gestational age, and animals as factors. When an interaction was found between treatment and gestational age, the data were analyzed by two-way analysis of variance with either gestational age and animals or treatment and animals as factors. Differences between body weight, crown-to-rump lengths, lung organ weights, and pulmonary DNA and protein contents were analyzed with two-way analysis of variance with animals and treatment as factors. Morphometric measurements were compared with a nested analysis of variance with treatment and animal within treatment as factors. When a significant difference was found, the differences between individual means were analyzed with a least significance difference test, with α = 0.05.

RESULTS

Fetal outcome. All fetuses were considered healthy according to their arterial blood gas and acid-base values measured before and during the experiments (pH 7.36 ± 0.01, arterial Pcco₂ 46.2 ± 0.5 mmHg, arterial Pco₂ 21.2 ± 0.39 mmHg, arterial O₂ saturation 64.2 ± 0.9%, total hemoglobin 9.1 ± 0.2 g/dl, and hematocrit 27.5 ± 0.5%). Body weights and crown-to-rump lengths were not different between the control and experimental groups at 131 days of gestation; the mean values for the control fetuses were 3.52 ± 0.48 kg and 58.7 ± 1.0 cm, respectively.

Plasma hormone concentrations. Circulating concentrations of ACTH (in pg/ml), cortisol (in ng/ml), T₄ (in ng/ml), PRL (in ng/ml), oGH (in ng/ml), and hGH (in ng/ml) were not affected by gestational age and, therefore, have been presented as mean values spanning the duration of the experiment. Circulating concentrations of ACTH, cortisol, and T₄ were not significantly different among all experimental groups; mean plasma concentrations in control fetuses were 2.7 ± 2 pg/ml, 4 ± 1 ng/ml, and 69 ± 9 ng/ml, respectively. Arterial PRL concentrations were undetectable in all HX fetuses,
indicating that the hypophysectomy was complete, whereas values were detectable and similar in control (28 ± 5 ng/ml) and 3-day obstructed (23 ± 5 ng/ml) fetuses. Circulating oGH concentrations were similar in the control (62 ± 4 ng/ml) and 3-day obstructed (57 ± 4 ng/ml) fetuses but were undetectable in both groups of HX fetuses. Only HX hGH 3-day obstructed fetuses contained measurable concentrations of hGH (169 ± 38 ng/ml).

Lung liquid volume and secretion rates. Before tracheal obstruction (at 128 days gestational age), lung liquid volumes adjusted for fetal body weight were not different between treatment groups (Fig. 1A), and in control fetuses, the volumes were similar at 128 (36.9 ± 3.2 ml/kg) and 131 (37.2 ± 3.2 ml/kg) days of gestation (Fig. 1A). After 3 days of tracheal obstruction, fetal lung liquid volumes were increased to 75.6 ± 9.0 ml/kg in the 3-day obstructed fetuses, 89.0 ± 10.7 ml/kg in HX hGH 3-day obstructed fetuses, and 81.3 ± 12.5 ml/kg in HX 3-day obstructed fetuses. The increase in lung liquid volume was similar in these three groups of fetuses (Fig. 1A).

Before tracheal obstruction (128 days), lung liquid secretion rates were similar in all treatment groups (control, 2.58 ± 0.78 ml·h⁻¹·kg⁻¹; 3-day obstructed, 3.02 ± 0.78 ml·h⁻¹·kg⁻¹; HX hGH 3-day obstructed, 3.46 ± 0.64 ml·h⁻¹·kg⁻¹; HX 3-day obstructed, 2.92 ± 0.48 ml·h⁻¹·kg⁻¹) as well as in control fetuses at 131 days of gestation. However, in all trachea-obstructed fetuses, lung liquid secretion rates at the end of the obstruction period were undetectable (Fig. 1B).

Organ weights. The wet pituitary-to-body weight ratios were similar in control and 3-day obstructed fetuses, whereas pituitary tissue was absent in HX hGH 3-day obstructed and HX 3-day obstructed fetuses, which confirms the completeness of the hypophysectomy. Neither hypophysectomy nor tracheal obstruction significantly affected the wet organ-to-body weight ratios of the fetal heart, liver, or right and left adrenal glands. On the other hand, wet lung-to-body weight ratios after 3 days of tracheal obstruction (3-day obstructed, 51.55 ± 3.48 g/kg; HX hGH 3-day obstructed, 46.98 ± 2.31 g/kg; HX 3-day obstructed, 43.96 ± 2.65 g/kg) were greater than the control value (34.09 ± 1.45 g/kg; Fig. 2). The presence or absence of GH in HX fetuses had no effect on wet lung-to-body weight ratios after tracheal obstruction, although in both groups, the ratios tended to be lower than the values obtained in the intact 3-day obstructed fetuses (Fig. 2).

Fetal lung protein and DNA content. The total protein content of the lung adjusted for fetal body weight was increased to a similar extent in all fetuses exposed to 3 days of tracheal obstruction (3-day obstructed, 1,982.7 ± 162.7 mg/kg; HX hGH 3-day obstructed, 1,776.6 ± 16.4 mg/kg; HX 3-day obstructed, 1,682.9 ± 92.6 mg/kg). These values were significantly greater than the value from control fetuses (1,199.2 ± 114.6 mg/kg; Fig. 3A) and were not affected by the presence or absence of GH in HX fetuses (Fig. 3A).

Total DNA content of the lungs adjusted for body weight (Fig. 3B) was significantly greater than the control value (107.9 ± 5.3 mg/kg) after 3 days of tracheal obstruction if the pituitary remained intact (3-day obstructed, 126.0 ± 4.4 mg/kg) or if GH was administered after hypophysectomy (HX hGH 3-day obstructed, 126.7 ± 4.0 mg/kg). On the other hand,
Lung morphometry

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>3-Day Obstructed</th>
<th>HX hGH 3-Day Obstructed</th>
<th>HX 3-Day Obstructed</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Tissue, %</td>
<td>22.9±4.0*</td>
<td>23.3±4.9*</td>
<td>22.6±4.0*</td>
<td>25.4±2.8*</td>
</tr>
<tr>
<td>Luminal space, %</td>
<td>77.1±4.0*</td>
<td>74.6±2.8*</td>
<td>77.4±4.0*</td>
<td>76.8±4.9*</td>
</tr>
<tr>
<td>Right lung volume, cm³/kg</td>
<td>98.0±15.4*</td>
<td>161.0±11.7†</td>
<td>188.9±13.7†</td>
<td>146.5±17.2†</td>
</tr>
<tr>
<td>Tissue volume, cm³/kg</td>
<td>581.8±106.8*</td>
<td>965.9±117.5†</td>
<td>1,030.5±193.9†</td>
<td>1,041.6±242.1†</td>
</tr>
<tr>
<td>Luminal volume, cm³/kg</td>
<td>1,983.0±174.1*</td>
<td>2,844.3±205.2†</td>
<td>3,665.9±300.6†</td>
<td>3,644.9±377.1†</td>
</tr>
<tr>
<td>Tissue-to-luminal space ratio</td>
<td>0.3±0.1*</td>
<td>0.4±0.1*</td>
<td>0.3±0.1*</td>
<td>0.3±0.1*</td>
</tr>
<tr>
<td>Interalveolar wall thickness, µm</td>
<td>4.7±1.0*</td>
<td>5.7±0.8*</td>
<td>5.5±1.2*</td>
<td>5.5±1.3*</td>
</tr>
<tr>
<td>Alveolar number, ×10⁶</td>
<td>7.4±1.0*</td>
<td>8.4±1.0*</td>
<td>9.3±1.1*</td>
<td>7.8±1.0*</td>
</tr>
<tr>
<td>Alveolar surface area, m²/kg</td>
<td>1.6±0.2*</td>
<td>2.0±0.2*</td>
<td>2.3±0.2*</td>
<td>1.8±0.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. 3-Day obstructed, fetuses exposed to 3 days of tracheal obstruction; HX, hypophysectomized fetuses; hGH, fetuses given human growth hormone replacement. Data were collected from fetuses at 131 days gestational age. Values that do not share a common symbol are significantly different from each other; P < 0.05.

DISCUSSION

In pre- and postnatal animals, increased lung expansion initiates a series of events that ultimately leads to an increase in lung tissue growth as well as extensive tissue remodeling. However, the mechanisms by which physical stimuli such as alterations in lung expansion are transduced into signals that induce growth and tissue remodeling are poorly understood. This experiment provides evidence to suggest that GH may be an important factor regulating the type of lung growth that is induced after an increase in fetal lung expansion. In particular, we have shown that although the degree of lung expansion was similar in GH-deficient fetuses as demonstrated by the similar increase in lung liquid volumes, the absence of circulating GH prevented pulmonary hyperplasia. Alveolar structure, as compared with control fetuses, 3 days of tracheal obstruction did not significantly increase total lung DNA content in HX fetuses in the absence of GH (control, 107.9±5.3 mg/kg; HX 3-day obstructed, 94.1±7.0 mg/kg; Fig. 3B). DNA content in HX 3-day obstructed fetuses was, therefore, significantly lower than the values obtained in 3-day obstructed and HX hGH 3-day obstructed fetuses (Fig. 3B).

Lung morphometry. The percentage of tissue or luminal space was not different among the four groups of fetuses (Table 1). Thus the increase in right lung volume after 3 days of tracheal obstruction was due to a similar increase in both luminal and tissue volumes as indicated by normal tissue-to-volume ratios. Similarly, the interalveolar wall thickness was not different among the four groups of fetuses (Table 1).

Alveolar diameter, as determined using the mean linear intercept, was increased in HX hGH 3-day obstructed (55.6±2.2 µm), 3-day obstructed (55.6±2.3 µm), and HX 3-day obstructed (46.8±2.2 µm) fetuses compared with that in control fetuses (42.4±2.4 µm), although the values from HX 3-day obstructed fetuses were not significant (Fig. 4A). The increase in alveolar diameter was reflected by a significant reduction in alveolar density (number/unit volume) in all fetuses after 3 days of tracheal obstruction (control, 7.66±1.1×10⁶/cm³; 3-day obstructed, 5.01±0.70×10⁶/cm³; HX hGH 3-day obstructed, 5.19±0.56×10⁶/cm³; HX 3-day obstructed, 5.00±0.83×10⁶/cm³; Fig. 4B). The total number of alveoli in the right lung was not different among the four groups of fetuses, although the number tended to be greater in 3-day obstructed and HX hGH 3-day obstructed fetuses compared with that in control fetuses (Table 1). When corrected for body weight, alveolar surface area was also not different among the different groups, although the values tended to be greater in 3-day obstructed and HX hGH 3-day obstructed fetuses (Table 1).
indicated by alveolar number and surface area, also appeared to be affected by the presence or absence of GH in response to a short period of tracheal obstruction.

GH and lung growth. The stimulation of lung growth induced by tracheal obstruction in fetuses and by PPNX in postnatal animals is thought to be a consequence of increased tissue stretch resulting from increased lung expansion. In both instances, lung growth occurs rapidly and is due to hyperplastic growth (9, 14, 28), indicating that the underlying molecular and cellular mechanisms may be similar. Indeed, the present study demonstrates that the hyperplasia induced by tracheal obstruction in fetal sheep is dependent on the presence of circulating GH, which is believed to play a similar role after PPNX (8). Because the degree of lung expansion induced by tracheal obstruction was unaffected by the presence or absence of circulating GH, the failure of fetal lung DNA content to increase in the absence of GH could not be attributed to differences in the degree of fetal lung expansion. In contrast to the increase in DNA content, the increase in protein content and wet lung weight after tracheal obstruction appears to be independent of GH. Thus it appears that the presence of GH is not required to detect the stretch stimulus and initiate a cellular response, as indicated by increased protein synthesis, but is required for the cells to progress through the cell cycle. Indeed, it is possible that GH acts as a competence factor that allows the cells to initiate cell division in response to a stretch stimulus. Our finding that total lung DNA content tended to be reduced in GH-deficient fetuses even after 3 days of tracheal obstruction compared with that in control fetuses suggests that lung growth may have ceased immediately after the hypophysectomy in GH-deficient fetuses. However, it is possible that the absence of GH has simply delayed rather than prevented the lung growth response to tracheal obstruction.

GH receptors have been located on fetal rat lung epithelial cells and fibroblasts in culture, although functional activity was only apparent in the epithelial cells (4). It is unlikely that the absence of hyperplastic lung growth after tracheal obstruction in GH-deficient fetuses was simply due to a reduction in the division of epithelial cells. Indeed, Nardo et al. (25) have previously found that increased lung expansion stimulates most major cell types within the lung to divide, indicating that numerous cell types contribute to the cellular hyperplasia. However, if epithelial cells are the only cell type to have functional GH receptors in vivo, it is possible that epithelial cells are the primary sensors of the stretch stimulus and transmit the response to other cell types via the release of intermediary factors (e.g., growth factors). Indeed, epithelial cells are ideally situated to detect changes in lung expansion. Thus in the absence of GH, if epithelial cells were unable to respond to an increase in lung expansion, the growth response may be prevented.

Some actions of GH are mediated via intermediate substances such as insulin-like growth factor (IGF-I) (33). Thus the effect of GH on lung cell proliferation after tracheal obstruction may be due to alterations in the levels of “intermediate” hormones or paracrine factors such as IGF-I. After PPNX in rats, the initial increase in lung weight is preceded by an increase in IGF-I concentration and continued lung growth is accompanied by increased levels of GH and IGF-I (16). IGF-I is a progression factor that is known to have receptors in the fetal lung and appears to primarily control growth of the pulmonary mesenchyme (31). Furthermore, circulating IGF-I concentrations are reduced (by ~50%) by hypophysectomy in fetal sheep (21), and defects of the GH receptor are associated with low plasma IGF-I concentrations in humans (29). Thus it is possible that reduced IGF-I concentrations and/or altered levels of IGF binding proteins, which regulate the bioactivity of IGF-I, may be responsible for the failure to stimulate lung cell proliferation after tracheal obstruction in the absence of GH.

GH and lung structure. Elevated GH levels in acromegalic adults are associated with an increase in lung volume and a proportional increase in the diffusing capacity of the lung (34, 35). These findings have led to
the suggestion that increased GH results in the formation of new alveoli or the expansion of existing alveoli. The finding in this study that tracheal obstruction in the presence of GH tended to increase alveolar number and surface area compared with those in control and trachea-obstructed fetuses in the absence of GH is consistent with this view. Although these values were not quite significant, previous findings by Nardo et al. (25) indicate that the increase in alveolar number and surface area are time dependent, indicating that given a longer period of tracheal obstruction, an effect of GH on alveolar structure and number may have become apparent. If correct, this would not only indicate that the increased cellular proliferation and the process of alveolarization are GH dependent but may also indicate that the process of alveolarization is dependent on cell division, not just growth of existing cells and extracellular matrix reorganization.

Hypophysectomy and changes in fetal plasma hormone concentrations. The lack of pituitary tissue at postmortem and the absence of PRL and oGH from the fetal circulation confirms that the fetal hypophysectomies were complete. Furthermore, because circulating concentrations of ACTH, cortisol, and T4 were similar to the control values in all groups, changes in the levels of these hormones cannot account for the differences in lung liquid volume and secretion rates, lung growth, and alveolar structure observed in this study.

Previous studies have reported that GH concentrations in fetal sheep are ~120 ng/ml during the last weeks of gestation (20) and that continuous infusions of GH at 0.5–1.5 mg·kg⁻¹·day⁻¹ are necessary to maintain normal GH levels in HX fetal sheep (26, 30). We chose the higher dose of 1.5 mg·kg⁻¹·day⁻¹ to avoid the risk of GH deficiencies caused by inadequate GH replacement. However, although hGH infusions restored GH concentrations to values that have been previously reported (20), circulating hGH concentrations were higher than the oGH concentrations we measured in fetuses with an intact pituitary. Although hGH has a high sequence homology with oGH and is biologically active in sheep (17, 37), the higher levels of circulating hGH in experimental animals did not affect body growth or lung development. This may have been because the total treatment period was relatively brief (~2 wk) and/or that circulating GH concentrations in GH-treated fetuses were physiological.

GH and fetal lung liquid secretion rates. A previous study (27) has indicated that GH reduces lung liquid production in fetal guinea pig lungs in vitro. However, our finding that lung liquid secretion rates were unaffected by the presence or absence of circulating GH indicates that GH does not regulate lung liquid production in fetal sheep in vivo. The reduction in lung liquid secretion rates during the period of tracheal obstruction most probably results from the increase in luminal pressure associated with tracheal obstruction as previously discussed (24). Similarly, although PRL administration into lung liquid has been shown to increase fetal lung liquid secretion rates in anesthetized and exteriorized fetal goats (10), intravenous infusions of PRL (80 μg/h) had no effect in fetal sheep (12). Our findings that the presence or absence of PRL had no effect on the volume or secretion rate of fetal lung liquid support the findings of the latter study.

In conclusion, we have shown that the increase in DNA content after obstruction of the fetal trachea for 3 days is GH dependent. However, it is not known whether the absence of GH has abolished or simply delayed the lung growth response to tracheal obstruction. The actions of GH appear to directly influence the cellular events that initiate cell division and are not the result of alterations in the degree of lung expansion. Furthermore, it is possible that increases in alveolar number and surface area that occur with longer periods of tracheal obstruction may be prevented by the absence of GH because the inability of the lung to form new cells may ultimately compromise the formation of new respiratory units. Our results also provide some evidence to suggest that GH may play an important role in normal fetal lung development.

We are indebted to Alex Satragno for assistance with the surgical preparation of animals; Jan Loose for performing the ACTH, cortisol, and prolactin assays; and Alison Thiel for expert technical assistance. We thank Prof. M. Waters (University of Queensland, Brisbane, Australia) for providing some of the growth hormone and Dr. Iain Clarke (The Prince Henry’s Institute for Medical Research, Victoria, Australia) for conducting the ovine growth hormone assays.

This work was funded by the National Health and Medical Research Council of Australia.

Received 23 July 1999; accepted in final form 28 October 1999.

REFERENCES


11. Crone, R. K., P. Davies, G. C. Liggins, and L. Reid. The effects of hypophysectomy, thyroidectomy, and postoperative...