Glucocorticoid Enhances Surfactant Proteolipid Phe and pVal Synthesis and RNA in Fetal Lung*

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Two newly described surfactant proteolipids (SPL), Phe and pVal, are produced by proteolytic processing of distinct precursors of Mr = 40,000 and 22,000, respectively. These proteins are structurally related and intimately associated with surfactant phospholipids. We now demonstrate the expression of both SPL(Phe) and SPL(pVal) in explants of human fetal lung from 16–24 weeks of gestation. Content, synthesis, and mRNA for the proteolipids were low prior to organ culture of fetal lung. Induction of synthesis of the proteolipids occurred rapidly in explant culture in the absence of exogenous hormones and was enhanced by addition of dexamethasone. Increased synthesis of the proteolipids was detected by enzyme-linked immunosorbent assay and by [35S]methionine incorporation into the glycosylated Mr = 40,000–43,000 SPL(Phe) precursor. The response to dexamethasone occurred rapidly and contrasted with effects of dexamethasone on the expression of surfactant-associated protein-35 (SAP-35), a distinct surfactant glycoprotein. 8-Br-cAMP did not significantly increase proteolipid content but markedly increased synthesis of SAP-35 in identical cultures. Increased proteolipid content was associated with increased mRNA for each protein as determined by the Northern blot analysis. Proteolipid RNA was also increased by 8-Br-cAMP, however, not to the extent observed with the glucocorticoid. Immunohistochemical analysis of fetal lung with anti-proteolipid antiserum confirmed that the dexamethasone-enhanced synthesis of the proteins by Type II epithelial cells. The time and hormone dependence of the regulation of expression of both SPL(Phe) and SPL(pVal) precursors were distinct from that of SAP-35. Expression of the surfactant proteolipids increased during explant culture of human fetal lung and was further enhanced by glucocorticoid. Developmental and hormonal regulation of the surfactant proteolipids may be important factors in surfactant function at birth.

Surfactant-associated hydrophobic proteins of Mr = 6,000–14,000 have been identified in mammalian surfactant and are associated with the dramatic surface active properties of phospholipids in surfactant extracts used clinically to treat hyaline membrane disease (1–4). These human proteins consist of small molecular weight hydrophobic peptides, one of which has been identified as SPL(Phe)* on the basis of the NH₂-terminal phenylalanine and which arises from a 40,000-dalton precursor protein (5); a second proteolipid SPL(pVal) has been identified on the basis of its unique polyvaline domain and is encoded by distinct cDNA which encodes a 22,000-precursor protein. Synthesis of proteolipid precursors has been identified in human lung, and antiserum generated against the surfactant proteolipid recognizes both the Mr = 40,000 and 22,000 precursors (6). Recent studies from this laboratory demonstrated the synthesis and processing of pulmonary surfactant protein SAP-35 (8, 9) and proteolipid SPL(Phe) in fetal lung tissue. Increased expression of SAP-35 and SPL(Phe) was demonstrated during explant culture of human fetal lung (5, 8, 9). Developmental expression of surfactant and of surfactant-associated proteins has been recently subjected to intense study. Synthesis of surfactant phospholipids is enhanced by corticosteroid, thyroid hormones, cAMP phosphodiesterase inhibitors, and epidermal growth factor (10, for review). In recent studies, expression of SAP-35 was enhanced by cAMP (8, 11) and epidermal growth factor (12); however, contrasting effects of dexamethasone have been recently reported (8, 11–13). The present study was designed to determine whether glucocorticoids alter the expression of SPL(Phe), SPL(pVal), or SAP-35 precursors during explant culture of human fetal lung.

EXPERIMENTAL PROCEDURES

Purification of Hydrophobic Surfactant Proteins—Surfactant was purified by differential centrifugation and the hydrophobic proteins extracted by chloroform/methanol or ether/ethanol extraction as previously reported (1, 2). Protocols for use of human tissue were approved by the Human Research Committee, University of Cincinnati College of Medicine. Human preparations contain two surfactant peptides, SPL(Phe) and SPL(pVal), identified by their distinct NH₂-terminal amino acid sequences (5). The N-terminal sequence of SPL(Phe) peptide Phe-Pro-Ile-Pro, etc., represented approximately ½ of the sequence obtained in the human preparation as previously reported (5). The remainder consisted of SPL(pVal): Ile-Pro-Cys-Cys, etc. Antibody was generated in rabbits by repeated injection of the bovine surfactant proteolipid prepared by chloroform/methanol extraction of surfactant (1, 2). This antiserum (bovine surfactant proteolipid antiserum) immunoprecipitates both the 40,000–43,000-

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1 The abbreviations used are: SPL, surfactant proteolipid; ELISA, enzyme-linked immunosorbent assay; SAP, surfactant-associated protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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SPL(Phe) and SPL(pVa1) Synthesis

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dalton SPL(Phe) and the 22,000-dalton SPL(pVa1) precursors after
[35S]methionine labeling (5). The antisera reacts with protein of
M, 6,000-14,000 in immunoabsolts of organic extracts of bovine,
human, and canine surfactants (1, 2).

**Explant Culture**—Tissue was obtained from consenting donors
from pathologic specimens from the National Diabetes Research
Institute, (Philadelphia, PA), with a protocol approved by the
Human Research Committee, University of Cincinnati
College of Medicine. Tissue was immediately placed in ice-cold
essential medium, (GIBCO) at 4 °C and transported on ice to this
laboratory within 24 h. Tissues were incubated in 1 × 10^6 pieces
using a McElvain tissue chopper and placed on scraper surfaces of
60-mm plastic dishes to which was added Weymouth’s media with
2% carbon-stripped fetal calf serum (14) containing 100 units/ml
penicillin and 0.1 pg/ml gentamicin. Explant cultures were placed on
a rocker platform at 3 cycles per minute, to immerse and expose the
explants during the culture period, described by Gross, et al. (15).

Dexmethylone, 8-Br-cAMP, or triiodothyronine (T3) (Sigma) were
added to stated concentrations and were present throughout the
culture period. Media was changed on the second or third day of 4–5
days of culture. Metabolic labeling of tissues and cells was performed
in methionine-deficient medium (1 mg/liter of unlabeled methionine)
in the absence of added cysteine. Culture media was replaced with 1.5%
bovine serum albumin. Following a 30-min equilibration period, [35S]
methionine (New England Nuclear), specific activity = 1000 Ci/
mmol, was added to a final concentration of 150 μCi/ml and the tissues
were incubated for 5 h. [35S]Methionine-labeled tissues were
prepared for autoradiography in 100% N-lauroyl sarcosine, 20 mM
sodium phosphate-buffered saline, pH 7.2, containing 10 mM EDTA and 1
mM phenylmethylsulfonyl fluoride, and 68 mM Tris-HCl, pH 7.4. Duplicate aliquots of the sonicate were precipitated
with trichloroacetic acid for determination of [35S]methionine incorporation.
[35S]Methionine incorporation was normalized among the
samples prior to immunoprecipitation by the method of Anderson
and Blobie (16). Labeling with [3H]Phenylalanine was performed as above
except that the media was deficient in phenylalanine; [3H]
phenylalanine (450 mCi/mmol) was present at 150 μCi/nl.

**Protein Methods**—Protein samples were analyzed under reducing
conditions by SDS-polyacrylamide gel electrophoresis (13%) or by
two-dimensional isoelectric focusing (pH 3.5–5.8) polyacrylamide gel
electrophoresis (13%), as described by Garrison and Wagner (17).
Proteins were electrophoretically transferred to nitrocellulose which
was subsequently dipped in 20% 2,5-diphenyloxazole in toluene and
then performed using the specific antiserum at a dilution of 1:500 in
bovine serum albumin. Following a 30-min equilibration period,
the media was deficient in phenylalanine; [3C] methionine-labeled tissues were
prepared for autoradiography in 100% N-lauroyl sarcosine, 20 mM
sodium phosphate-buffered saline, pH 7.2, containing 10 mM EDTA and 1
mM phenylmethylsulfonyl fluoride, and 68 mM Tris-HCl, pH 7.4. Duplicate aliquots of the sonicate were precipitated
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except that the media was deficient in phenylalanine; [3H]
phenylalanine (450 mCi/mmol) was present at 150 μCi/nl.

**Northern Blot and Slot Blot Analysis**—Northern blot analysis
was performed using a 1.5-kilobase SPL(Phe) cDNA clone (214.1), a 0.3-kilobase SPL(pVa1) cDNA clone (354.2), or a 0.5-
kilobase SAP-35 cDNA clone (7–1) isolated from a Agt111 expression
library generated from adult human lung poly(A)+ RNA (5). The
cDNA probes were labeled with [α-32P]dCTP using a random primer-labeling kit (Pharmacia). In some experiments, filters were washed free of radioactivity and rehybridized in an identical fashion with the
SAP-35 cDNA which contains nearly the entire coding region of
SAP-35, as previously reported (8, 12).

For Northern blot analysis, 15 μg of total RNA was prepared from
100 mg of tissue and was separated on a 1.2% agarose-formaldehyde
gel (22) and transferred to nitrocellulose. The filter was baked at
80 °C for 2 h. Nitrocellulose "slot" blots were made by applying 500
or 250 ng of formaldehyde-denatured RNA to nitrocellulose using a
slot blot manifold (Schleicher & Schuell), Inc.). Optimal conditions
for hybridization were determined in preliminary experiments varying
the method of RNA preparation, hybridization, and washing conditions.
The filters were prehybridized in 46% deionized formamide, 5 × SSC
(1 × SSC is 0.15 M NaCl, 0.15 M sodium citrate) 50 mM NaH2PO4,
pH 7.0, 5 × Denhardt's solution (1 × Denhardt's is 0.02% bovine
serum albumin, 0.02% BSA, 0.02% polyvinyl pyrrolidone), 0.2% SDS,
and 100 μg/ml denatured salmon sperm DNA at 41 °C overnight.
Filters were hybridized in the same solution containing 10% dextran
sulfate and cDNA probes at approximately 5 × 10^6 cpm/ml at 41 °C
overnight. Following hybridization, filters were washed four times at
room temperature with 2 × SSC, 0.2% SDS, once at 41 °C with the
same solution and once with 0.2 × SSC, 0.2% SDS at 50 °C. Filters
were placed between two sheets of Saran wrap and exposed to Kodak
XAR-2 film.

ELISA Assay—ELISA of the surfactant proteolipid was performed
using the anti-proteolipid antibody which recognizes both SPL(Phe)
and SPL(pVa1). Competition curves were generated with bovine SPL.
The standard completely inhibited immunoreactivity of the antibody
under assay conditions. Standard SPL protein was estimated after silver
staining and in relationship to previous amino acid analysis
after HCl hydrolysis (5). This antisera is reactive with both proteo-
lipid precursors in immunoprecipitation assays. ELISA was per-
formed by competition assay using purified, delipidated SPL which
was adsorbed to each of the plastic wells. Purified SPL inhibited the
reactivity of the antisera in a dose-dependent manner (10–10,000
ng) and duplicates generally varied less than 10%. Assays of the tissue
homogenates were performed at two or three different dilutions of the
sample in 10% propylene glycol to assure parallel dilution in the
competition curve. Explant samples were homogenized immediately
or frozen at −70 °C for 1–2 weeks prior to homogenization in
phosphate-buffered saline, pH 7.2, containing 10 nm EDTA and 1
mM phenylmethylsulfonyl fluoride. Sample and anti-bovine proteo-
lipid antiserum (1:30,000) were preincubated overnight at 37 °C prior
to addition to the well. The reaction was then developed with
imunoperoxidase conjugated goat anti-rabbit IgG (1:1000) and
D-phenylephedrine.

**RESULTS**

Competition ELISA assay of the surfactant proteolipid in
fetal lung of 16–21 weeks gestation demonstrated low or
undetectable levels of activity prior to organ culture. Proteo-
lipid content increased during organ culture in the absence of
exogenous hormones, Table I. Continuous exposure to dex-
methasone enhanced content of the proteolipids (Table I) and
was readily detected as early as 24 h in culture (not shown).
Stimulatory responses to the glucocorticoids contrasted sharply with inhibitory effects of dexamethasone on SAP-35
expression in identical cultures (8). At 4 days in culture, 8-
Br-cAMP (100 μM) had no detectable effect on the content of

|       | Preculture (16–21 weeks) | Control (day 6) | Dexmethasone (10 nm) | Dexmethasone (100 nm) | Dexmethasone (1 μM) | 8-Br-cAMP (100 μM) |
|-------|--------------------------|----------------|---------------------|----------------------|-------------------|------------------|
| ND    | 4.6 ± 1.0                | 36 ± 7.5       | 85 ± 21             | 102 ± 21             | 85 ± 21           | 102 ± 21          |
| (n)   | (n)                      | (n)            | (n)                 | (n)                  | (n)               | (n)              |
| β     | 0.017                    | 0.014          | 0.018               | 0.018                | 0.017             | 0.038            |

*ND = none detected.*
FIG. 1. Synthesis of SPL precursors in fetal lung explant culture. Explants were labeled with \[^{35}S\]methionine for 5 h after 4 h (lane 1), 1 day (lane 2), 2 days (lane 3), and 4 days (lane 4) in culture. After normalization of \[^{35}S\]methionine-labeled protein, lysates were immunoprecipitated. Synthesis of \(M_r = 42,000\) and 25,000 D proteins increased rapidly and reached maximal levels after 24–48 h of culture. Immunoprecipitation with nonimmune rabbit serum did not detect these proteins (lane 5).

Surfactant proteolipids by the ELISA assay, Table I.

Culture-dependent increases in SPL(Phe) were confirmed by \[^{35}S\]methionine-labeling and immunoprecipitation of 40,000–43,000 glycosylated peptides, Fig. 1. After 2–4 days in culture, synthesis of the proteolipid precursors was high under all conditions and not further enhanced by addition of corticosteroid. Similar to the findings in the ELISA studies, there were no detectable effects of 8-Br-cAMP on synthesis of the SPL precursor protein at 3–5 days (not shown); however, synthesis of SAP-35 was markedly enhanced by 8-Br-cAMP in these explants as previously reported (8).

Smaller molecular weight proteins, \(M_r = 6,000\)–14,000, which co-migrated in SDS-PAGE with the proteolipids from surfactant, were immunoprecipitated from the explants but were present in relatively low abundance, Fig. 2. Migration of the \(M_r = 6,000\)–14,000 proteolipids in SDS-PAGE in the absence of \(\beta\)-mercaptoethanol resulted in migration of the labeled protein as a single band \(M_r = 18,000\), Fig. 2. Incorporation of \[^{3}H\]phenylalanine, an amino acid present in SPL(Phe) but not the SPL(pVal) peptide, was also observed in both the \(M_r = 6,000\) and 12,000 proteins (not shown).

Identification of SPL(Phe) and SPL(pVal) precursors was confirmed by hybrid arrested translation assay which identified the preproteins as \(M_r = 40,000\) and 22,000, respectively, Fig. 3, a and b. The presence of N-linked carbohydrate on SPL(Phe) was demonstrated by treatment of the immunoprecipitates with endoglycosidase F which decreased the size and size heterogeneity of the 42,000–43,000 proteolipid precursors, Fig. 4. Endoglycosidase-F also trimmed immunoprecipitable protein of \(M_r = 25,000\) which may represent a proteolytic fragment of SPL(Phe). These findings confirm previous demonstration of a consensus sequence for N-linked carbohydrate on the SPL(Phe) precursor (5).

Immunohistochemical identification of the proteins was performed using both surfactant proteolipid antisera and SAP-35 antisera. Staining for the proteolipid was detected in

\(^4\) J. Whitsett, unpublished observation.

FIG. 2. Synthesis of \[^{35}S\]methionine-labeled SPL. Fetal lung explants of 20 weeks gestation were labeled with \[^{35}S\]methionine after 4 days in explant culture. Total labeled protein was normalized prior to immunoprecipitation, SDS-PAGE, and autoradiography. a, electrophoresis under reducing conditions identified precursors with nominal \(M_r = 42,000\) and 25,000 and processed proteolipids with nominal \(M_r = 6,000\) and 14,000. b, electrophoresis under nonreducing conditions resolves processed proteolipids as a single band with \(M_r = 18,000\).

FIG. 3. Hybrid arrested translation of the proteolipid precursors. Human lung poly A(+) RNA was hybridized with SPL(Phe) cDNA (a) or SPL(pVal) cDNA (b). Half of each sample was melted (lane 1) while the other half was maintained in the hybrid form (lane 2). All samples were translated in wheat germ extracts, immunoprecipitated, and analyzed by 10–20% SDS-PAGE and autoradiography. the samples prior to culture, but intensity increased dramatically after 1–2 days. Treatment of the explants with dexamethasone resulted in enhanced immunoreactive material at early time points in culture (1–2 days), Fig. 5. However, after 4–5 days in culture there was marked induction of proteolipid

\[\text{Fig. 1. Synthesis of SPL precursors in fetal lung explant culture}.
\text{Explants were labeled with }[^{35}\text{S}]\text{methionine for 5 h after 4 h (lane 1), 1 day (lane 2), 2 days (lane 3), and 4 days (lane 4) in culture. After normalization of }[^{35}\text{S}]\text{methionine-labeled protein, lysates were immunoprecipitated. Synthesis of }M_r = 42,000\text{ and 25,000 D proteins increased rapidly and reached maximal levels after 24–48 h of culture. Immunoprecipitation with nonimmune rabbit serum did not detect these proteins (lane 5).}
\]
FIG. 4. Glycosylation of SPL precursors. Fetal lung explants were labeled with [35S]methionine on day 4 of culture and immunoprecipitated with bovine surfactant proteolipid antiserum. Protein A-Sepharose containing the radiolabeled precursors was divided into two samples, one of which was digested with endoglycosidase F (lane 1) and the other which served as the control (lane 2).

FIG. 5. Immunohistochemistry of the surfactant proteolipids. Immunoperoxidase staining was performed on lung explants at 22 weeks gestation using rabbit anti-proteolipid antiserum. Increased reactivity was noted in the apical (solid arrows) and basilar (open arrows), regions of the epithelium. Bar = 10 μm. Examples are representative of at least three separate experiments with tissue of 16–22 weeks gestation.

RNA Studies—Northern blot analysis demonstrated that the abundance of SPL(Phe) and SPL(pVal) RNA was low in fetal compared to adult lung tissue, Fig. 6. There was a progressive increase in expression of both SPL(Phe) and SPL(pVal) from 16–20 weeks gestation, Fig. 6. Lungs from later gestation (22–24 weeks) showed significant variability, some lungs expressing the proteolipid RNAs at relatively high levels, (not shown). Northern blot analysis of the RNA from the explant cultures demonstrated the mRNAs for SPL(Phe) at 2.0 kilobases, SPL(pVal) at 1.0 kilobases and SAP-35 at 2.15 kilobases, Figs. 6 and 7. Increased mRNA for both proteolipids was observed in the presence of 10 nM dexamethasone, Fig. 7. 8-Br-cAMP also resulted in a smaller but consistent enhancement of SPL(pVal) and SPL(Phe) RNA in the Northern and slot blot analysis especially after 2–5 days in culture. Relative effects of the hormones were somewhat variable since rapid and variable induction occurs in the absence of added hormones, especially in the tissue of older gestational age. Induction of the proteolipid RNA in the
FIG. 7. Northern blot analysis of SPL(pVal), SPL(Phe), and SAP-35. RNA as extracted from explants of a single lung at 16 weeks gestation after culture in presence of 10 nM dexamethasone (DEX) or 100 μM 8-Br-cAMP (cAMP). Replicate blots of 15 μg RNA were probed for SPL(pVal) in lanes 1–3; SPL(Phe) (4–6) after 48 h in culture and SAP-35 (7–9) after 96 h. Lanes 1, 4, and 7 are control; lanes 2, 5, and 8 are 10 nM dexamethasone, and lanes 3, 6, and 9 are 100 μM 8-Br-cAMP. Size (kilobases) is listed on the right of the figure. Figures are representative of similar experiments with at least three separate lungs. Weak hybridization of SPL(pVal) cDNA to larger RNA was detected in fetal but not adult lung and its origin is unclear.

FIG. 8. Slot blot analysis of SPL(Phe) and SPL(pVal) RNA during 1–5 days in explant culture. Slot blots were prepared from total RNA, applying 500 or 250 ng of RNA/well. RNA was prepared from fetal lung explants at time 0 and 1–5 days in culture and were probed with 32P-labeled SPL(Phe) (A) or SPL(pVal) cDNA (B). Explants were cultured in presence or absence of 10 nM dexamethasone (DEX), 100 μM 8-Br-cAMP (cAMP), or 5 nM T3. CON, control.

FIG. 9. Comparison of SPL(Phe) and SAP-35 RNA in fetal lung explant cultures. RNA from fetal lung (22 weeks gestation) was prepared prior to culture and after culture for 12, 24, and 48 h in presence of 10 nM dexamethasone (DEX) or 100 μM 8-Br-cAMP (cAMP). RNA (500 or 250 ng) was applied to the nitrocellulose and hybridized with 32P-labeled SPL(Phe) cDNA (A) or 32P-labeled SAP-35 cDNA (B) and subjected to autoradiography. CON, control.

DISCUSSION

These studies demonstrate glucocorticoid induction of newly described proteolipids SPL(Phe) and SPL(pVal) in explant cultures of human fetal lung. Increased proteolipid content during culture was associated with increased [35S] methionine incorporation into glycosylated precursor protein of SPL(Phe) of M, = 40,000–43,000. Increases in proteolipid content and RNA were observed during explant culture in the absence of exogenous hormones and were further enhanced by dexamethasone. The time course of induction and glucocorticoid control of proteolipid expression were distinct from that of SAP-35 expression in these cultures.

Surfactant proteolipids are intimately associated with surfactant phospholipids, and their developmental regulation is prepared from fetal lung explants at time 0 and 1–5 days in culture and were probed with 32P-labeled SPL(Phe) (A) or SPL(pVal) cDNA (B). Explants were cultured in presence or absence of 10 nM dexamethasone (DEX), 100 μM 8-Br-cAMP (cAMP), or 5 nM T3. CON, control.
likely to be vital to surfactant function during respiratory adaptation to postnatal life. There was detectable immuno-
reactivity prior to culture of lung explants (between 15–24 weeks of gestation) although abundance of RNA and immu-
nocytotoxic abundance was low compared to cultured explants or that in adult lung. Prior to culture, SPL(pVal) and SPL(Phe) RNA was variable but tended to be higher in lung tissue from older fetal lungs, generally increasing from 16 to 24 weeks of gestation. In the present study, surfactant synthesis of proteolipids increased very rapidly during explant culture. In contrast to the proteolipids, induction of SAP-35 mRNA occurred more slowly (8, 12, 13). SPL(Phe) and SPL(pVal) RNA was rapid (1–2 days) and coordinate, but was distinct from that of SAP-35 expression which reached maximal levels only after 3–5 days of explant culture (8, 12, 13). Enhancement of SPL(Phe) and SPL(pVal) expression by dexamethasone was detected at concentrations which were similar to those previously described for the stimulatory effects of corticosteroids on the phospholipid synthesis by fetal lung and consistent with the affinity of dexamethasone for pulmonary corticosteroid receptors (6, 22). In contrast to the marked effects of 8-Br-cAMP on SAP-35 synthesis (8, 11), 8-
Br-cAMP had no detectable effect on SPL expression or content although some enhancement of proteolipid RNAs by 8-Br-cAMP was detected. Influences of 8-Br-cAMP on surfactant proteolipid RNA occurred in a time frame similar to that observed with the effects of 8-Br-cAMP on SAP-35 synthesis. However, effects of 8-Br-cAMP on proteolipid RNA were less than those observed for dexamethasone and were not associated with increased proteolipid content.

SPL(Phe) and SPL(pVal) arise from larger precursors. The SPL(Phe) precursor was recently shown to be a glycoprotein containing asparagine-linked carbohydrate sensitive to endo-
glycosidase F (5). Both preproteins must undergo further proteolytic processing to generate the smaller molecular weight proteins (proteolipids) detected in surfactant. The site and nature of such proteolytic processing remains to be clarified. Small amounts of [35S]methionine-labeled protein of $M_r = 6,000–14,000$ (which co-migrated with the proteolipids in surfactant extracts) were immunoprecipitated from the explants after culture; however, whether SPL (pVal), SPL(Phe) or both migrate at $M_r = 6,000$ or $14,000$ remains to be clarified. Since labeling of both of the small peptides of $M_r = 6,000–$14,000 by $[3H]$phenylalanine (an amino acid unique to SPL(Phe)) was observed, the precise relationships among the $6,000–14,000$ peptides and the SPL(Phe) or SPL(pVal) pre-
cursors remains unknown. The smaller proteins ($M_r = 6,000–$14,000) migrated at $M_r = 18,000$ in the absence of β-
mercaptoethanol, supporting their sulfhydryl-dependent ag-
gregation (1, 2). The presence of a surfactant protein of $M_r = 18,000$ was recently reported by Hawgood et al. (7) who identified a canine surfactant protein with high homology to human SPL(Phe) presently described (5).

Immunohistochemistry was used to demonstrate the localization of the proteolipids in Type II epithelial cells. The intracellular distribution of the proteolipids was similar but distinguishable from that of SAP-35. SAP-35 was previously shown to be concentrated primarily in lamellar bodies in the apical region of the cells; immunoreactivity of the proteolipids was noted both in the basilar and apical regions of the Type II cells. Immuno staining of the proteolipid increased rapidly in culture in a time frame distinct from that of SAP-35 (8, 12). The proteolipid antiserum utilized in the present work recognizes both SPL(Phe) and SPL(pVal). The hydrophobic N-terminal regions of the small molecular weight peptides are structurally similar (5). It is therefore unclear whether the material detected by immunohistochemistry and the ELISA assay represents both SPL(Phe) and SPL(pVal). Likewise, it is unclear whether proteolipid precursors and the proteolyti-
cally processed peptides are equally reactive. Small peptides ($M_r = 6,000–14,000$) were detected after [3H]phenylalanine or [14C]phenylalanine-labeling but were present at low levels in the explant cultures. Thus, it is likely that the immunoreac-
tivity detected in the acinus of distal respiratory structures comprises SPL(Phe) or SPL(pVal) precursors which are se-
creted intact into the lumen, suggesting that proteolytic pro-
cessing occurs after their secretion. It is also possible, how-
ever, that some proteolytic processing is not expressed under the explant conditions utilized in this study.

Corticosteroids have dramatic effects on maturation of developing lung cells and are used clinically for the prevention of hyaline membrane disease in premature infants. The present study demonstrates marked effects of dexamethasone on the expression of SPL(Phe) and SPL(pVal) in vitro and supports the hypothesis that enhancement of surfactant pro-
teolipid synthesis may be important for adaptation to air breathing in postnatal life.

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