ever, the data seems to exclude the dorsal pallium, the hippocampal cortex and the pyriform cortex from having a direct role in the response.

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Immunoglobulin in Fetal Lamb Lung and Its Fluid* (33460)

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The fetal lamb lung was shown to produce a fluid (1) which completely fills the airway passages before the onset of respiration. Surface active material is normally present in fetal lung fluid before the first breath (2, 3). Lung extracts of patients dying with hyaline membrane disease have been shown to lack surface activity (4, 5). Thus, the physicochemical properties of the fetal lung fluid may be important during the onset of respiration. It seems possible then, that under conditions of fetal distress, changes in the composition of fetal lung fluid may occur, favoring the formation of hyaline membranes. Fetal lung fluid contains at least five protein fractions (6), including a globulin with gamma mobility, as detected by immunoelectrophoresis. The purpose of the present study was to determine the immunological properties and the source of the gamma globulin fraction previously described.

Materials and Methods. Eleven lambs at different gestational ages, ranging in weight from 1.4 to 5.2 kg were delivered by cesarean section. Special precautions were taken to maintain the placental circulation intact (2). The methods for handling and monitoring the mother and the fetus were identical to those previously described (6). Following removal of the lung fluid, the lambs were sacrificed and multiple lung biopsies were performed on six fetuses. In addition, sections were made of other fetal organs (i.e., thymus, liver, parotid, and kidney) as well as the lung in one.

Lung fluid. Immediately after collection, specimens were frozen without addition of preservatives and maintained at —20°. The total amount of fetal lung fluid collected was 323 ml. Prior to pooling and lyophilization, 2 ml of each specimen were removed and set aside for vacuum ultrafiltration concentration to approximately 3 g/100 ml (7). These 2 ml samples were later pooled. Prior to concentration, the total protein of the nonlyophilized samples was determined (8) to be 12.7 mg/100 ml.[1] Lyophilization was performed in the remaining 300 ml which rendered 4 ml of total protein residue.

Lung biopsy. Frozen biopsies were stored at —75°. Frozen sections, 4 μ in thickness, were cut on a Harris-International cryostat

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[1] The amount of total protein in tracheal fluid reported by one of us (FHA) previously was erroneously calculated and should have been about 0.1 the value, i.e., 30 mg/100 ml. (2).

(model CT) at −25° and dried at room temperature for about 36 hr. Lung sections were examined on a Zeiss fluorescence microscope; black and white 35 mm photographs were taken at a constant diaphragm setting with 25-sec exposure.

Immunoelectrophoresis (IEP). Immunoelectrophoresis (in agar gel) was performed on both pools of concentrated fetal lung fluid with Scheidegger's micromodification (9).

Immunogel diffusion (Ouchterlony). The Ouchterlony method of immunogel diffusion (10) was used on each specimen.

Antisera. (a) *Rabbit antisheep lung fluid (ALF)* was prepared as previously described (6).

(b) *Rabbit antisheep serum (ASS) and rabbit anti sheep gamma globulin (AGG)* were obtained from Hyland Laboratories.

(c) *Goat antirabbit serum globulins labeled with fluorescein (GAR-sg-Fl)* were obtained from Immunology Incorporated, Glen Ellyn, Illinois.

Cellulose acetate electrophoresis. Analysis of the electrophoretic components of the lung fluid proteins was performed on both concentrates using cellulose acetate as a supporting medium (11). Similar analysis was performed on maternal and fetal sheep sera.

Plasma fractions. Sheep gamma globulin (SGG) and rabbit gamma globulin (RGG), prepared by Cohn's fractionation, were obtained from Hyland Laboratories, Los Angeles, California and Pentex Laboratories, Kankakee, Illinois, respectively.

Fetal lung immunofluorescence. An indirect method of immunofluorescence was employed (12, 13), because a low concentration of gamma globulin in the fetal lung was suspected. After three 5-min rinses in phosphate buffered saline, the sections were treated with untagged antisera (AGG) in a moist chamber at room temperature for 45 min, followed by three additional 2-min buffer rinses; the tissue was then incubated with tagged antisera (GAR-sg-Fl) at room temperature in a moist chamber for 30 min. A 5-min rinse in 0.3*M* glycine was then followed by three final rinses in the buffer solution, after which the sections were mounted in buffered glycerine.

Three controls tested the specificity of the fluorescence: (i) Lamb tissue was incubated directly with GAR-sg-Fl as described above; (ii) tissue that had been incubated with AGG was then treated with blocked GAR-sg-Fl, incubated for 1.5 hr with enough RGG to completely eliminate the antirabbit gamma globulin from the tagged antisera (14); the blocked GAR-sg-Fl was centrifuged for 15 min at 17,000 rpm at 4° before application; and (iii) the AGG was analyzed by immunoelectrophoresis for the possible presence of other serum globulins.

Results. As previously reported (6), both pools of concentrated fetal lung fluid showed at least five protein fractions by immunoelectrophoresis when reacted against ALF or ASS (Fig. 1a and c). Fractions with prealbumin, albumin, alpha, beta, and gamma mobility were present in both pools analyzed by IEP and also by cellulose acetate electrophoresis. Precipitin lines in the prealbumin and gamma globulin area of the fetal lung fluid were detected only with ALF, showing no reaction against ASS (Fig. 1a). The ALF yielded a gamma globulin arc with both fetal lung fluid and SGG (Fig. 1c). A similar arc was observed against one of the components of the whole sheep gamma globulin fraction (Fig. 1b). No reaction was observed in the gamma area of the fetal lung fluid when ASS was used (Fig. 1a). Fetal lamb sera did not reveal the presence of gamma globulin (Fig. 1d). When repeatedly analyzed by IEP against AGG, neither concentrated pool of fetal lung fluid showed any visible lines in the gamma globulin region.

Ouchterlony analysis of both pools yielded a continuous precipitin line with fetal serum, SGG and ASS (Fig. 2a). The ALF showed a definite immunoprecipitin line with sheep gamma globulin by the same method (Fig. 2b).

Indirect fluorescence studies of the fetal lamb lung revealed a brightly stained uniform bronchiolar epithelium and bright bronchiolar glands (Fig. 3a). Fluorescence appeared at the periphery of the epithelial cells, particularly at the apical ends; fluorescence appeared to fill the glandular cells. Droplets of fluorescein stained material were also seen

Fig. 1

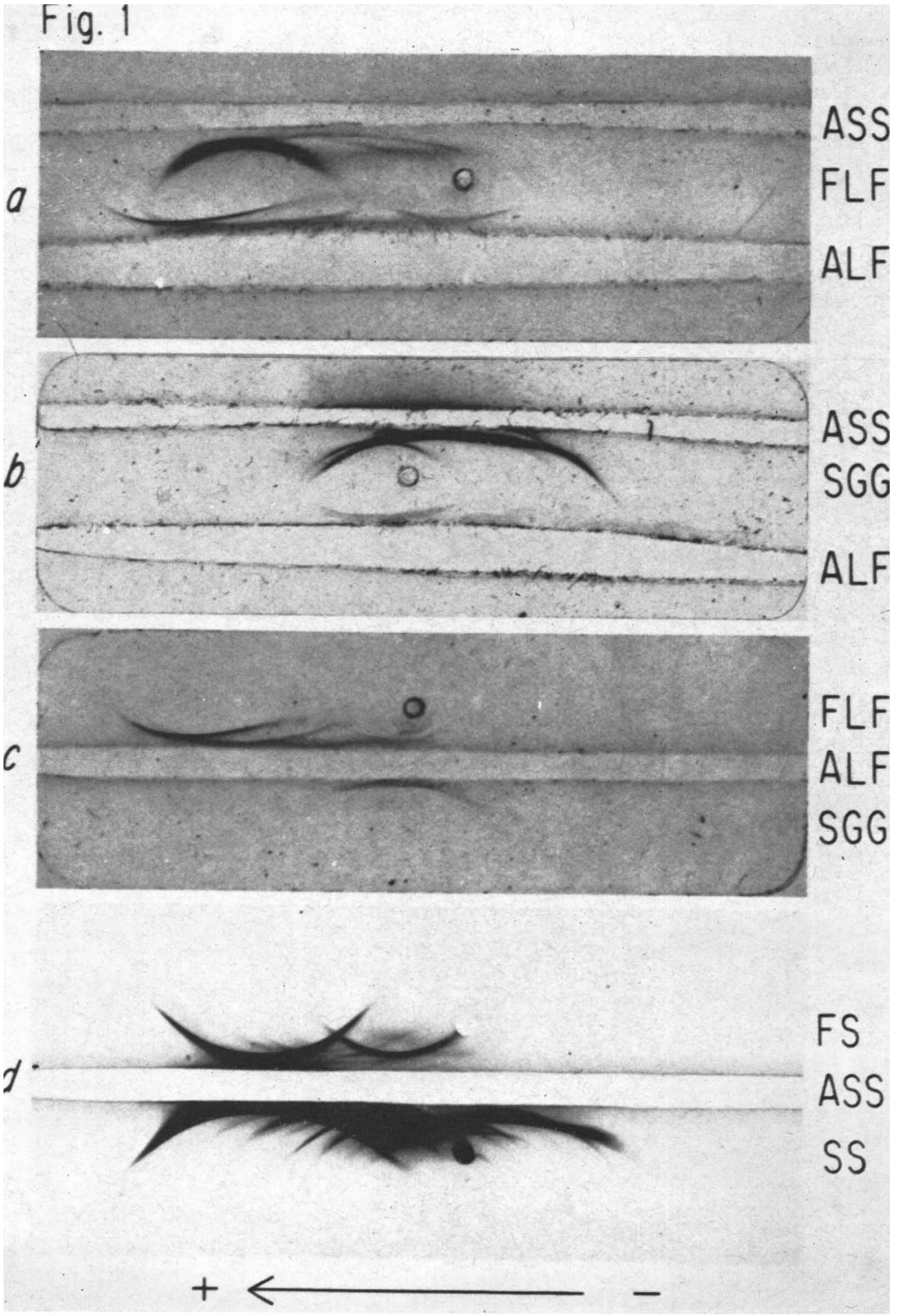

FIG. 1a. Immunoelectrophoresis of fetal lung fluid, middle well, developed with rabbit antisera against whole sheep serum (ASS) in upper trough and antiserum against fetal lung fluid (ALF) in lower trough. Notice the prealbumin and gamma globulin precipitin lines reacting only against ALF. (b) Immunoelectrophoresis of sheep gamma globulin (SGG) in middle well. Upper trough: antiserum against the whole sheep serum (ASS). Lower trough: specific antiserum (ALF). The specific antiserum (ALF) reacts only with the fastest component of the whole sheep gamma globulin. (c) Immuneolectrophoresis of fetal lung fluid, upper well, developed against specific antiserum (ALF) middle trough. Lower well: sheep gamma globulin. Identical immunoprecipitin arcs in the gamma globulin area can be observed in both sera. (d) Immunoelectrophoresis of fetal lamb serum (FS) upper well, developed with rabbit antisera against whole sheep serum (ASS). In lower well, whole sheep serum (SS). Notice the lack of gamma globulin in fetal serum. Abbrev. for all figures: ASS, antisera against whole sheep serum; ALF, antisera against fetal lung fluid; SGG, sheep gamma globulin; FS, fetal lamb serum; SS, whole sheep serum; FLF, fetal lung fluid; AGG, antisera to sheep gamma globulin and GAR-sg-Fl, antisera to rabbit serum globulins, fluorescein labeled.

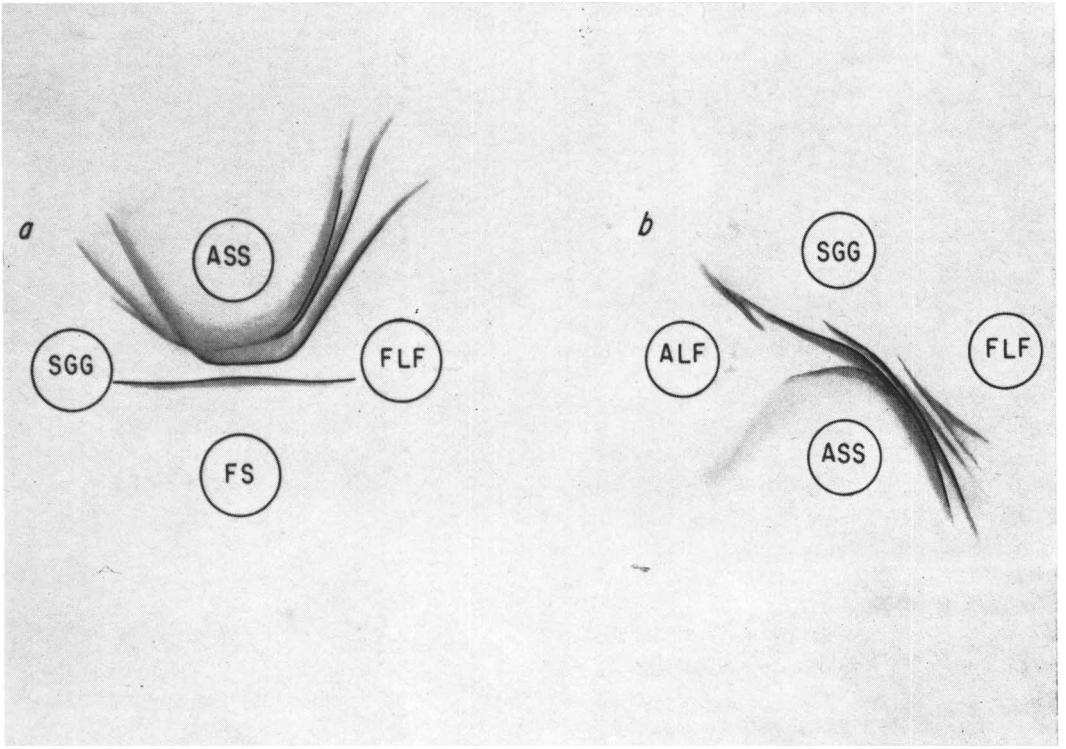

FIG. 2. Diagrams illustrating the precipitin patterns obtained in the Ouchterlony plates. (a) Reading clockwise from upper well: note the presence of several immunoprecipitin lines between the antisera against whole sheep serum (ASS) and fetal lung fluid. One band is shared by all fractions. The fetal serum (FS) shows several antigenically similar bands to fetal lung fluid (FLF), reacting against ASS. Note, however, the presence of one band between ASS and FS not shared by the other fractions. The sheep gamma globulin (SGG) shows three immunoprecipitin lines against ASS. (b) Reading clockwise from upper well: note the lack of precipitin lines between sheep gamma globulin (SGG) and fetal lung fluid (FLF). Several precipitin lines between FLF and ASS can be observed. Three immunoprecipitin lines are present between ASS and SGG, one of which is shared with FLF and partially with ALF. Note also the single precipitin line between ALF and SGG.

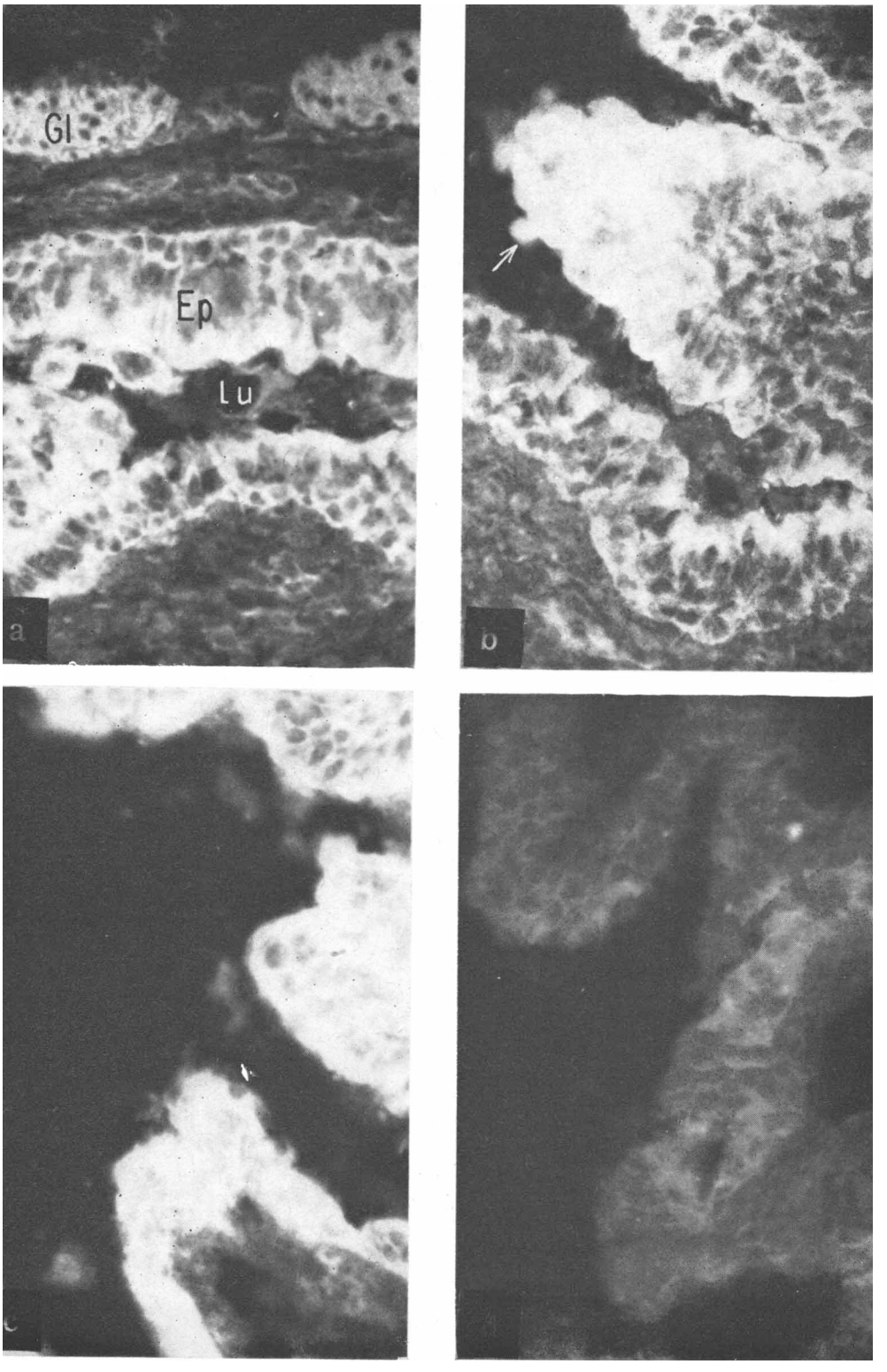
Gl
Ep
Lu
a
b

FIG. 3a. Gl-bronchiolar glands, Ep-bronchial epithelium, Lu-bronchiolar lumen. An even bronchiolar epithelium stained with AGG and GAR-sg-Fl. Note the fluorescence surrounding the epithelial cells, particularly at the apical end. The glandular cells exhibit specific fluorescence throughout with the exception of the nuclei. (b) At various positions along the epithelium, fluorescent droplets (arrow) were observed adjacent to the epithelial cells, extending into the bronchiolar lumen. (c) A section of bronchiolar epithelium treated with AGG and GAR-sg-Fl. (d) A consecutive section of bronchiolar epithelium treated with AGG and then GAR-sg-Fl from which the antirabbit gamma globulin had been absorbed. Note the specificity of the fluorescence in Fig. 3c as evidenced by the comparison.

protruding into the bronchiolar lumen (Fig. 3b).

Fetal lamb lung incubated with GAR-sg-Fl exhibited no specific fluorescence, eliminating the possibliity of a cross-reaction between the antirabbit sera and lamb tissue. Examination of tissues incubated with GAR-sg-Fl from which the antirabbit gamma globulin was absorbed revealed markedly diminished fluorescence (Fig. 3c and d). This blocking with homologous antigen indicated that the fluorescence is specific for gamma globulins. Only anti-gamma globulin was detected by immunoelectrophoresis of AGG against SS; thus, the possibility that GAR-sg-Fl conjugated with rabbit anti-alpha or anti-beta globulin contaminants was eliminated. Specific fluorescence with blocked goat anti-rabbit gamma globulin as a control was not observed in fetal lamb parotid, kidney, thymus, or liver. However, it was present in the same lamb's bronchiolar epithelium.

Discussion. These studies further describe the immunoelectrophoretic properties and immunological specificity of the gamma globulin fraction present in the fetal lung fluid. Immunofluorescent studies also revealed the presence of a gamma globulin fraction in fetal lamb lung tissue.

In a previous study (6), five protein fractions were detected in the fetal lung fluid, one of which showed a gamma mobility. It was postulated that the gamma globulin fraction detected in fetal lung fluid was a polymeric IgA, similar to that present in adult tracheal secretions (15–17). The present studies add further support to the hypothesis advocated by others (16, 18, 19) that secretory IgA is produced locally in the lungs. In accordance with our previous study (6) and those of others (20, 21), no gamma globulin could be detected in the fetal lamb sera. Although small amounts of gamma globulin have been described in the fetal lamb plasma (22, 23), the present findings suggest a higher concentration in the fetal lung fluid. Thus, a mechanism of local formation or selective pulmonary transport is likely.

The fetal lung fluid gamma fraction studied here shows a fast electrophoretic migration and appears to react only with ALF. The specificity of this fraction is further confirmed by the precipitin lines of fetal lung fluid and ALF shown in the immunodiffusion plates. Furthermore, the absence of a reaction of the fetal lung fluid with the AGG suggests the presence of a globulin other than IgG.

Immunofluorescent studies of the fetal lung indicate that specific gamma globulin fluorescence is present in the bronchiolar epithelium and bronchiolar glands, but not in the alveoli. Such findings suggest that these cells are either engaged in the production of gamma globulin or, alternatively, there is selective secretion of these proteins from the plasma through the glands and bronchiolar epithelium into the bronchiolar lumen (19).

The simultaneous presence of a gamma globulin in fetal lung fluid and lung tissue suggests a relationship between these fractions. Although an immunological identity was not established, the hypothesis of local production by the lung gains further support with these findings.

Based on the assumption that sheep immunoglobulins are similar to the human counterparts, and with the knowledge that a polymeric form of IgA is the main immunoglobulin in human mucoid secretions (16, 18, 19), it seems likely that the gamma globulin in fetal lung fluid and lung tissue is similar to IgA.

Summary. In this study, the protein frac-

tions present in lyophilized and nonlyophilized fetal lamb lung fluid were determined. Immunoelectrophoresis showed a gamma fraction with a fast electrophoretic migration which reacted only against antilung fluid. The specificity of this immunoglobulin was confirmed by immunodiffusion gel technique (Ouchterlony). Specific gamma fluorescence was present in the bronchiolar epithelium and glands, but was not present in the alveoli. The presence of a gamma globulin fraction in both lung tissue and lung fluid suggests a relationship involving local synthesis in the lung. The unidentified fetal lung fluid gamma globulin that was detected was not IgG and appeared to be peculiar to that biological fluid.

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