

Bronchopulmonary fluids in pulmonary alveolar proteinosis

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The alveoli of patients with pulmonary alveolar proteinosis (P.A.P.) become filled with a proteinaceous material, the origin of which is uncertain. The purpose of this study was to examine bronchopulmonary washings from four adults and one child with this disease who had total lung lavage for treatment of the hypoxemia caused by the disease process. In each instance a liter or more of lavage fluid was concentrated approximately four hundred fold by ultrafiltration for protein analysis. Quantitative determinations of the proteins in this fluid and in serum were performed in quadruplicate by the Lowry method, radial diffusion, or radioimmunoassay. There was a selective increase of IgG in washings from all five patients with P.A.P., and there was a lesser increase of IgA, suggesting either a local production or a mechanism for concentrating these immunoglobulins. Albumin usually comprised a similar proportion of the total protein in serum and washings, but alpha-2 macroglobulin comprised less than half that in serum in each instance, suggesting that equal transudation of large and small proteins did not occur. There was considerable variation in the relative proportion of IgM, IgD, and IgE in washings compared to serum, suggesting that each of these immunoglobulins is metabolized (synthesized, transported, or catabolized) by an independent mechanism in the lung. Secretory piece was demonstrated in the bronchial washings of each patient, including controls, as were two soluble nonserum proteins. Fibrinogen was identified in the washings of the three patients with P.A.P. Large amounts of insoluble surfactant-like material were also present in the washings of each of the patients with P.A.P.

In 1959 Rosen, Castleman, and Liebow¹ reported a 5 year experience with a chronic disease of the lung that they termed pulmonary alveolar proteinosis (P.A.P.). They reported a characteristic histologic pattern that consisted of the deposition of P.A.S.-positive proteinaceous material, rich in lipids, within the alveoli of these patients, with little cellular reaction. The disease is usually manifested by dyspnea, cough, weight loss, and easy fatigability and bears some histologic and clinical resemblance to *Pneumocystis carinii* infection of the lung. More than 190 cases have been reported in adults and some 34 cases in children. In childhood the disease is usually fatal within 12 to 24 months, but the course is much more variable in adults. Peak age incidences are under one year and between 20 and 50 years. Although nothing is known as yet

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regarding the etiology of the condition, Ramirez-R²⁻⁵ and others have done much to elucidate the nature of the material filling the alveoli.

Therapeutic approaches have been developed by Ramirez-R, Schultz, and Dutton⁶ and Wasserman, Blank, and Fletcher,⁸ employing multiple lavages of an entire degassed lung in the adult, and by Seard and associates,⁷ who described simultaneous lavage of both degassed lungs in young children. Lavage of the whole degassed lung permits recovery from the bronchial tree of large amounts of particulate matter with the histologic and histochemical characteristics of the alveolar material found in the tissue sections of patients with P.A.P. Stansifer and Bourgeois,⁹ Larson and Gordinier,¹⁰ and Taxay, Montgomery, and Wildish¹¹ concluded, on the basis of immunochemical analyses, that the proteins were derived from plasma and therefore suggested that the presence of these proteins in the alveolar washings may represent mere transudation of serum proteins. These studies have, however, been largely qualitative. The purpose of our study was to examine bronchopulmonary washings from four adults and one child with P.A.P. in an effort to determine if the material is indeed the result of simple transudation of serum proteins.

MATERIALS AND METHODS

Subjects

Case 1 was a boy 7 years of age reported previously.⁷ He was one of two siblings with P.A.P., both of whom underwent simultaneous bilateral lung lavage with partial cardiopulmonary bypass. Four additional previously unreported adults with P.A.P., a 36-year-old man (Case 2), a 23-year-old woman (Case 3), a 37-year-old man (Case 4), and a 25-year-old man (Case 5) also underwent total lung lavage. All patients were in active stages of their disease, and the lavage was performed for therapeutic reasons using a modification of a technique developed previously,⁸ the modification being that the lavage fluid consisted of isotonic saline alone, without heparin or acetyl cysteine. The diagnosis of P.A.P. was established by open lung biopsy in the five patients who underwent bilateral lavage. For comparison, bronchopulmonary fluid was obtained by segmental lung lavage from a 60-year-old woman with bronchiectasis (Case 6) and a 64-year-old man with carcinoma (Case 7).

Protein determinations

One of the first few liters of lavage fluid was obtained, and the soluble proteins were concentrated approximately four hundred fold using in succession Amicon model 2000 and model 8MC ultrafiltrators. Quantitative determinations of total protein were performed simultaneously on sera and concentrated washings by the method of Lowry and associates.¹² A commercial protein standard (Armour crystalline bovine albumin) was used for the Lowry determinations.

Albumin, α_1 , α_2 , β , and total gamma globulins were determined by cellulose acetate electrophoresis of serum and washings and read on a Beckman Microzone model R-110 Densitometer. Concentrations of IgG, IgA, IgM, IgD, ceruloplasmin, α_2 macroglobulin, transferrin, and B₁C (C₂) were determined in all fluids by radial immunodiffusion¹³ employing immunodiffusion plates and standards obtained from Hyland Division of Travenol Laboratories, Costa Mesa, California. Concentrations of individual proteins were expressed not only in absolute values but also in percentages of the total protein concentration of the respective fluid and as a ratio of the percentage concentration in bronchial washings to serum.

The concentration of IgE was determined by radioimmunoassay utilizing the double antibody technique described by Gleich, Averbek, and Swedlung,¹⁵ purified E myeloma protein (PS),* rabbit anti-IgE and goat antirabbit gamma globulin as the second antibody. The P.A.P.

*Kindly supplied by Dr. O. R. MacIntyre, Hanover, N. H., and isolated by Dr. S. Kochwa, New York, N. Y.

samples were also tested by radioimmunoassay employing sheep anti-IgND obtained from Pharmacia Laboratories, Inc., with rabbit anti-sheep gamma globulin as the second antibody. Results were similar using either antiserum system, indicating that with the standards and materials used it made little difference whether the antiserum was raised from one or the other of the two E myeloma proteins (ND or PS). E myeloma protein was radiolabeled with I^{125} according to Hunter and Greenwood¹⁴ to a specific activity of $44 \mu\text{c}/\mu\text{g}$. The labeled IgE was refiltered through Sephadex G-50 prior to each use. Antiserum to IgE was prepared by injecting rabbits with purified E myeloma protein (PS) and absorbing the pooled antisera with serum from a pool of ten healthy persons (IgE level of pool: 70 I.U. per milliliter). Standards consisted of three sera of known IgE content (kindly tested for us by Dr. S. G. O. Johansson, of Uppsala, Sweden, and by Drs. Bazaral and Hamburger, of La Jolla, California) and the current WHO reference standard (obtained from Dr. W. D. Terry, Bethesda, Maryland). Calculations for IgE assume that one I.U. (of IgE) is equivalent to one ng., an assumption that may well be erroneous.¹⁸ Nevertheless, the relative amounts of IgE in specimens from the several patients are unaffected by the absolute weight equivalent of the international unit (I.U.).

Search for nonserum proteins

Three New Zealand white rabbits were each immunized with 5 mg. of protein contained in concentrated bronchial washings from a different patient with P.A.P. A fourth rabbit was immunized with 1 ml. of centrifuged washed insoluble surfactant-like sediment subsequently solubilized by the addition of 0.2 ml. of 0.1 N NaOH. A fifth rabbit was immunized with 5 mg. of whole serum protein from the 23-year-old P.A.P. patient (Case 3). In each instance the material used for immunization was made up to 1 ml. in 0.1 N phosphate buffer, pH 7.4, was emulsified in 1.5 ml. of complete Freund's adjuvant, and one third of the emulsion was injected into footpads, one third into the peritoneum, and one third subcutaneously. Rabbits were given booster injections of the same emulsion at one month and were bled weekly thereafter.

Antisera from a given rabbit were pooled and examined for antibodies to the immunizing antigen by immunoelectrophoresis and Ouchterlony analyses. Aliquots of each pool were absorbed with one part pooled normal human serum to five parts antiserum. The absorbed antisera were shown to be devoid of antibodies to serum proteins and were tested for antibodies to nonserum proteins by immunoelectrophoresis and Ouchterlony analyses.¹⁷ Even though all detectable antibodies to serum proteins had been removed by the initial absorption, each antiserum was absorbed a second time with one fifth volume of pooled human serum and was then analyzed for antibodies to nonserum constituents of the bronchial washings.

Antiserum to "secretory piece" was prepared in the following manner: A rabbit was immunized with saliva from an IgA-deficient patient (in a method identical to the above), and the resulting antiserum was absorbed twice with one-tenth volume of pooled normal human serum. The absorbed antiserum was shown to be free of antibodies to serum proteins and contained antibodies to a single constituent of saliva. This antiserum, when reacted with saliva or breast milk in Ouchterlony plates, produced a reaction of identity with antiserum specific for secretory piece obtained from Drs. M. South and R. A. Good.

Cultures

Aliquots of the bronchial washings used for protein determinations were sent at the time of lavage for bacterial, fungal, and viral cultures.

RESULTS

Protein electrophoresis

Cellulose acetate protein electrophoresis of the sera and bronchial washings is shown in Fig. 1. There was a relative increase in the gamma globulin concentration in bronchial washings as compared to the serum of the patients with P.A.P. that was not apparent in washings from the patient with bronchiectasis or the patient with carcinoma.

TABLE I. Individual protein concentrations in sera and bronchial washings (BW)

	Case 1		Case 2		Case 3	
	Serum	BW	Serum	BW	Serum	BW
Total protein (mg.%)	9,400	5,000	6,800	3,300	8,300	8,300
Albumin (mg.%)	4,350	2,140	3,400	1,600	4,100	3,100
IgG (mg.%)	900	1,120	810	750	1,175	1,800
IgA (mg.%)	350	210	155	125	430	600
IgM (mg.%)	53	16	115	15	85	80
IgD (mg.%)	8	2	4	3	2	2
IgE (I.U./ml.)	50	37	2,000	240	120	140
Ceruloplasmin (mg.%)						
Transferrin (mg.%)	200	21	120	27	160	130
C ₃ (B ₁ C, B ₁ A) (mg.%)	225	34	180	65	210	210
Alpha ₂ macroglobulin (mg.%)	150	26	145	42	120	82
	490	32	168	31	120	8

N.D. = Not done.

TABLE II. Individual proteins in sera, and bronchial washings (BW) expressed as a per cent of

	Per cent of					
	Case 1		Case 2		Case 3	
	Serum	BW	Serum	BW	Serum	BW
Albumin	47	43	50	48	49.5	37
IgG	9.6	22.5	12	23	14	22
IgA	3.7	4.2	2.3	3.8	5.2	7.2
IgM	0.6	0.3	1.7	0.5	1.0	1.0
IgD	0.09	0.04	0.06	0.09	0.02	0.02
IgE	0.53	0.74	29	7.3	1.4	1.7
Ceruloplasmin	2.1	0.4	1.8	0.8	1.9	1.6
Transferrin	2.8	0.7	2.6	2.0	2.5	2.5
C ₃ (B ₁ C, B ₁ A)	1.6	0.5	2.1	1.3	1.4	1.0
Alpha ₂ macroglobulin	5.2	0.6	2.5	0.9	1.4	0.1
Total protein	9,400	5,000	6,800	3,300	8,300	8,300

N.D. = Not done.

*The value for IgE represents % $\times 10^4$ and assumes one I.U. is equivalent to one ng.†According to Schultze and Heremans,³² the proteins measured should constitute roughly 80% the lesser constituents, as well as the presence of nonserum proteins, in bronchial washings**Total protein**

Final total protein concentrations of bronchial washings were somewhat arbitrary, depending on the amount of solution used and the degree to which each was concentrated by pressure dialysis. In three patients with P.A.P. the total protein content of the concentrated bronchial washings was equivalent to that of the serum (Cases 3 to 5). In the other two patients concentrated bronchial washings contained approximately half as much total protein as serum (Cases 1 and 2). The concentrations of the individual proteins studied can be seen in Table I. They are also expressed as a percentage of the total protein in Table II and as the ratio of the per cent composition in bronchial washings to that in serum in Table III.

Albumin

The ratio of per cent albumin in the bronchial washings to per cent in serum was similar (0.7 to 1.0) in all patients. However, the patients with P.A.P. had a

Case 4		Case 5		Case 6		Case 7	
Serum	BW	Serum	BW	Serum	BW	Serum	BW
6,500	7,000	8,500	8,500	7,700	5,500	6,500	1,200
3,380	3,820	4,800	4,820	2,100	990	2,300	420
770	1,550	1,800	2,400	2,450	1,475	1,250	250
170	435	140	220	850	390	150	60
100	150	240	74	650	40	75	5
< 2	19	34	34	2	2	2	< 2
1,800	820	1,300	1,100	60	N.D.	640	90
72	37	38	18	180	90	200	25
95	85	210	170	160	70	80	13
85	37	145	130	120	50	140	8
270	40	370	30	180	78	220	8

total protein concentration*

total protein†

Case 4		Case 5		Case 6		Case 7	
Serum	BW	Serum	BW	Serum	BW	Serum	BW
52	54	56	57	27	18	35	35
12	22	21	28	32	27	19	21
2.6	6.2	1.6	2.6	11	7.1	2.3	5
1.5	2.1	2.8	0.9	8.4	0.7	1.2	0.4
< 0.03	0.27	0.4	0.4	0.03	0.04	0.03	< 1.7
28	12	15	13	0.78	N.D.	10	7.5
1.1	0.5	0.4	0.2	2.3	1.6	3.1	2.1
1.5	1.2	2.5	2.0	2.1	1.3	1.2	1.1
1.3	0.5	1.7	1.5	1.6	0.9	2.1	0.7
4.2	0.6	4.4	0.3	2.3	1.4	3.4	0.7
6,500	7,000	8,500	8,500	7,700	5,500	6,500	1,200

of the total serum proteins. Differences in techniques for measuring total protein, albumin, and (particularly Cases 6 and 7) may account for the additional variations observed.

higher per cent of albumin in both serum and bronchial washings than did the two other subjects. Since both cellulose acetate electrophoresis and immunoelectrophoresis of bronchial wash concentrates from P.A.P. patients closely resemble the serum patterns, it is clear that serum proteins constitute the bulk of the soluble proteins in the washings.

Other serum proteins

Ceruloplasmin, transferrin, and C₃ showed minor variations in the patients studied, none of which seemed unique to the patients with P.A.P. Alpha₂ macroglobulin was significantly decreased in the bronchial washings of all patients in relation to its concentration in serum.

Immunoglobulins

The proportion of IgG in the lavage fluid was increased in all five patients with P.A.P., but this was not the case in the other patients. Bronchial wash IgA

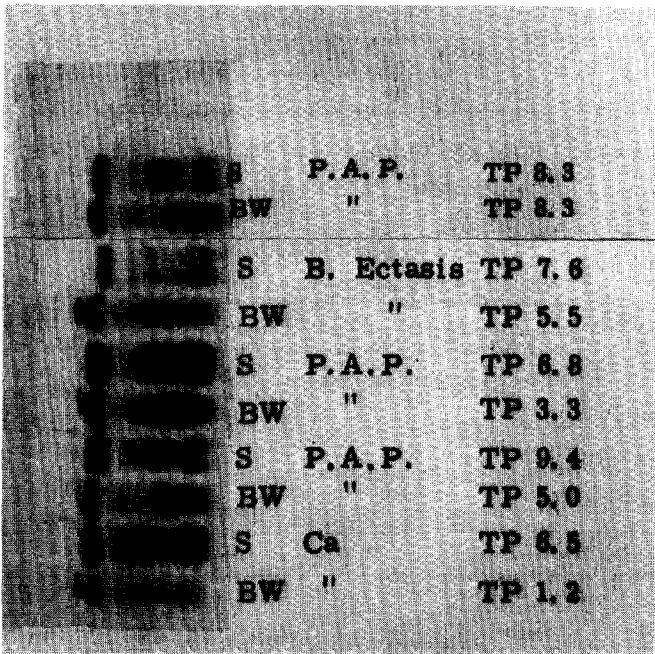


Fig. 1. Cellulose acetate protein electrophoresis on sera and bronchial washings (BW) of three of the patients with P.A.P., one with bronchiectasis, and one with carcinoma. TP = Total protein.

TABLE III. Ratio of per cent bronchial washing (BW) protein: per cent serum protein*

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Albumin	0.9	1.0	0.8	1.0	1.0	0.7	1.0
IgG	2.3	1.9	1.5	1.9	1.3	0.8	1.1
IgA	1.1	1.6	1.4	2.4	1.6	0.6	2.2
IgM	0.5	0.3	1.0	1.4	0.3	0.1	0.3
IgD	0.4	1.5	1.0	> 9.0	1.0	1.3	< 1.0
IgE	1.4	0.25	1.3	0.42	0.86	N.D.	0.76
Ceruloplasmin	0.2	0.4	0.8	0.45	0.5	0.7	0.7
Transferrin	0.3	0.8	1.0	0.8	0.8	0.6	0.9
C ₂ (B ₁ C, B ₁ A)	0.3	0.6	0.7	0.4	0.9	0.6	0.3
Alpha ₂ macroglobulin	0.1	0.4	0.1	0.1	0.1	0.6	0.2

N.D. = Not done.

*Ratios exceeding 1.0 indicate that protein constitutes a higher proportion in BW than in serum.

was somewhat increased in all five patients with P.A.P. but was also increased in the patient with carcinoma, though slightly decreased in the patient with bronchiectasis. Bronchial wash IgM was similar to alpha₂ macroglobulin, being decreased in most patients. Two patients, however, had a similar or increased per cent of IgM in the bronchial wash even though they had a much lower percentage of alpha₂ macroglobulin in their bronchial wash than in their serum. The bronchial wash to serum ratio of IgD was somewhat increased in two patients, markedly increased in Case 4, and reduced in two others. Less than 2 mg. per cent serum IgD was detectable in Case 4, but an elevated level of IgD (19 mg. per cent) was repeatedly demonstrated in the bronchial fluid. There was a

considerable decrease in the per cent of protein comprised of IgE in washings compared to serum in two patients with P.A.P. The IgE determinations were repeated several times, and each time duplicate determinations showed that the washings of these two subjects had less than half the serum value. Both had an elevated level of serum IgE, as did a third subject with P.A.P. Thus, three of the five P.A.P. subjects had elevated serum IgE values. The same three had the highest IgE concentrations in their bronchial fluids. The varying washings:serum ratios suggest differing synthesis or catabolism or both in each subject.

Secretory piece

The concentrated bronchial washings of all P.A.P. patients contained readily identifiable secretory piece that produced a reaction of identity on Ouchterlony analyses with secretory piece from normal saliva as well as with secretory piece from the bronchial washings of Patients 6 and 7.

Other nonserum antigens

One or two soluble nonserum proteins in addition to secretory piece were identified in each bronchial wash fluid. These were demonstrated as precipitate lines in Ouchterlony analyses using antiserum from any of the three rabbits immunized with bronchial wash concentrates after the antiserum had been exhaustively absorbed with pooled human serum. The observed precipitate lines were distinct from fibrinogen, not being affected by further absorption of the antiserum with either plasma or purified fibrinogen. In addition to these non-plasma proteins, the three P.A.P. bronchial washings tested with antiserum to fibrinogen were positive, each producing a reaction of identity with a line produced by the reaction of monospecific antifibrinogen with purified fibrinogen in Ouchterlony plates. Thus we have demonstrated secretory piece, fibrinogen, and two additional soluble nonserum proteins in P.A.P. bronchial fluids. We have not as yet been able to demonstrate that any antigen is found only in bronchial fluids of P.A.P. patients, since all antigens found in P.A.P. fluids also occurred in washings of Cases 6 and 7.

Cultures

Bacterial, viral, and fungal cultures of bronchial washings were negative in all patients with P.A.P.

DISCUSSION

The original report of P.A.P. by Rosen and associates¹ is perhaps the best clinical, epidemiological, and histochemical description of this disease. More recent reviews^{3, 5, 10, 19-24} have presented new hypotheses as to its pathogenesis. These are not well established and will not be discussed in detail.

Our results demonstrate a 1.3 to 2.3 fold increase in the percentage of total protein represented by IgG in the washings as compared to the serum of the five patients with P.A.P. This suggests that either a local production or a concentration of this immunoglobulin in bronchial fluids occurs in this disease. This was not the case in the patients with bronchiectasis and carcinoma. Albumin (M.W. 69,000) comprised a similar proportion of the total protein in both serum



Fig. 2. The first, second, and third bottles (reading from right to left) of bronchial lavage from the 23-year-old woman with P.A.P. **Top,** Bubbles created after vigorous shaking. **Bottom,** After sedimentation of the fluid for four hours.

and washings, but IgM (M.W. 900,000) and α_2 macroglobulin (M.W. 830,000) in the washings generally comprised less than half the proportion found in serum, while the concentrations of intermediate-sized molecules such as transferrin (M.W. 90,000), ceruloplasmin (M.W. 160,000), and C_3 (M.W. 187,000) were reduced to a lesser degree. Since two P.A.P. fluids contained an equal or greater proportion of IgM in bronchial fluids, it is likely that at least some subjects also produce IgM locally. Since transferrin is a smaller molecule than ceruloplasmin, one would anticipate that it might appear in equal or higher proportion in bronchial fluids than in serum. This was found to be true in the patients with P.A.P. (Table III).

In contrast to opinions expressed in previous reports,⁹⁻¹¹ the data suggest that equal transudation of large and small proteins does not occur. Rather, the situation is reminiscent of the mode of passage of albumin and globulins into lymph,²⁶ with the likelihood that there is an additional contribution of locally produced immunoglobulins to the bronchial secretions.

Rosen, Castleman, and Liebow¹ proposed that the alveolar filling material originated from altered and degraded alveolar lining cells. Larson and others,⁹⁻¹¹ however, proposed that the material was largely or wholly derived from passive transudation of plasma constituents.

There are chemical similarities between the insoluble portion of the alveolar filling material and a surface active lipoprotein (surfactant) secreted by the granular pneumatocyte. The surfactant-like material found in this disease, how-

ever, apparently does not have normal surface active properties.^{5, 24} It is highly insoluble and is present in greatest quantity in the first few liters of lavage fluid, decreasing in amount rapidly with succeeding lavages. Fig. 2 is a photograph of the first, second, and third bottles of fluid removed from the 23-year-old woman with P.A.P. (Case 3), and it demonstrates, in the first bottle, small stable (< 0.5 mm. in size) bubbles suggesting the presence of potent surface-active material. In the second and third bottles the bubbles become larger and less dense, indicating less surface-active material in spite of the fact that each lavage yielded about the same amount of sediment in each bottle.

One hypothesis is that the disease results from an overproduction of surfactant-like lipoprotein,¹⁰ perhaps of an altered composition. Ramirez-R and associates,^{5, 6} on the other hand, utilized the incorporation of palmitic-1-C¹⁴ acid into pulmonary lipid to demonstrate what they felt was a prolonged retention of the metabolic products of the alveolar lining cells. They postulated that the manifestations of alveolar proteinosis could result from an acquired dysfunction of the mechanism that clears metabolic products or surface-active material or both.

The significance of the elevated IgG and IgA levels in the bronchial washings of these patients is uncertain. If indicative of local production, they could represent pulmonary responses to a foreign macromolecule or antigen from the environment. Another possibility is that high immunoglobulin levels could represent autoantibodies to abnormal proteins produced by the alveolar lining cells—or they could represent the nonspecific production of increased amounts of immunoglobulins. If a local defect in immunoglobulin catabolism is at fault, it could be part of a more generalized defect in removing macromolecules from the lungs. We found an increased proportion of IgA in bronchial washings in all but one patient (Case 1) with P.A.P. There was not a close correlation between increased IgA and increased IgG concentrations, however, since in Case 1 the elevation of IgG was striking whereas in Case 4 the IgA elevation was more marked. There was an increased ratio of IgM in Case 4, of IgD in Cases 2 and 4, and of IgE in Cases 1 and 3. Of particular interest was the finding of IgD in high concentration (19 mg. per cent) in the bronchial washings of Case 4 while none was detectable in the serum (under 2 mg. per cent). This finding was confirmed by repeating the studies several times and suggests that at times large amounts of IgD may be synthesized in the lung and may be secreted into bronchial fluid. The variability in immunoglobulin ratios from patient to patient suggests that each immunoglobulin is regulated by an independent mechanism in individual patients and that different patients differ with respect to the rates of pulmonary synthesis or catabolism, or both, of the various immunoglobulins.

In support of the local production of immunoglobulin in the lung are the animal experiments of Hand and Contey,²⁵ who showed that when *Diplococcus pneumoniae* was aerosolized into the lower respiratory tract of normal rabbits, the lung synthesized immunoglobulin that was mostly IgG (approaching 50 per cent of the total protein!). Falk, Okinaka, and Siskind²⁷ examined bronchial fluids obtained at bronchoscopy in six patients with chronic obstructive lung disease, six normals and twenty-two others. In most it was possible to detect IgG and IgA but not IgM. The IgG/albumin and IgA/albumin ratios were sig-

nificantly higher in bronchial washings than in serum. The same is true of our specimens. In a study of normal human tracheobronchial washings by Keimowitz,²⁸ the IgG/albumin ratio was greater in bronchial washings than in serum in three of twelve samples, but similar in the other nine patients.

It is known that IgA is the predominant immunoglobulin in most external secretions^{29, 30}; yet according to the above studies, and our findings in patients with P.A.P., IgG may play the dominant role in secretions of the lower respiratory tract in contrast to the upper airways. In support of this are the observations of Hiramoto and Hamlin,³¹ who examined the cytokinetics of IgG and IgM responses to immunization in C3H mice. These authors found the lungs to be a prime site of localization of antibody-forming cells. They believed that these cells arrived via the blood primarily from lymphoid organs. They found only a minimal primary 19S antibody response in the lungs at the same time that a vigorous 19S response occurred in the spleen. The lung response paralleled the splenic response in both primary and secondary 7S antibody production.

Finally, our studies have demonstrated that there are at least two soluble proteins in bronchial fluid that are not derived from serum and that fibrinogen is also present. Attempts by others to identify soluble nonserum proteins in the bronchial washings of patients with P.A.P. have been unsuccessful.^{4, 9, 11, 24}

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