PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 167, 62–69 (1981)

Humoral Immune Response of Kidney Transplant Recipients to Pneumococcal Vaccine (41126)

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Abstract. We immunized 63 renal transplant patients and 8 control subjects with a pneumococcal vaccine containing 14 capsular polysaccharides. The antibody levels to 12 of these polysaccharides were determined by a radioimmunoassay technique. The control subjects had antibody levels determined prior to and after immunization. The patients were divided into three groups on the basis of their underlying renal disease—Group 1, those with glomerulonephritis; Group 2, those with pyelonephritis; and Group 3, those with other renal diseases. Twenty-four Group 1, 9 Group 2, and 18 Group 3 patients had antibody levels determined before, 1 month and 1 year after immunization. The overall immune response of the patients 1 month after vaccination was not significantly different from that of the control subjects. The geometric mean antibody level at 1 year had declined by 13.8, 37.1, and 32.7% from the 1-month level for Groups 1, 2, and 3, respectively. An increasing dose of azathioprine resulted in a decreasing antibody response only in patients whose underlying disease was glomerulonephritis. An increasing dose of prednisone had a similar depressant effect in patients who had no apparent immunological disorder as the cause of failure of their own kidneys. Immunization did not adversely affect renal function. To date, no cases of pneumococcal pneumonia or bacteremia have occurred in the vaccinated patients; but, because of the small number of patients, a much larger period of observation is needed to determine the vaccine's efficacy.

The risk of pneumococcal pneumonia in the general population appears to be one to two cases per 1000 per year (1, 2). In elderly and chronically ill patients, it is much higher—14.2 cases per 1000 per year (3). The rate of pneumococcal pneumonia in renal transplant patients is close to this latter figure (4). Most of the pneumococcal infections in Linnemann's study occurred in patients who had undergone splenectomy in addition to transplantation, and three of the four patients with pneumococcemia of sudden onset died (4).

A polyvalent pneumococcal vaccine is now available for general use and has been recommended for individuals with splenic dysfunction, or with a variety of chronic diseases, and for those more than 50 years of age (5). The vaccine has been shown to be safe, antigenic, and effective in a nonimmunosuppressed population (6).

The sudden death from community-acquired pneumonia of two renal transplant patients in our center led to a decision to immunize all the renal transplant patients with pneumococcal vaccine. We undertook this study to determine if this group of patients receiving immunosuppressive therapy would respond to the vaccine and if there was an adverse effect of vaccination on graft function. A further long-term aim (in view of the small number of patients) was to determine the effectiveness of the pneumococcal vaccine.

Materials and Methods. *Study population.* The study population consisted of 63 renal transplant patients and of 8 controls selected from hospital laboratory staff. The characteristics of the patient population are shown in Table 1. The patients were divided into three groups based on their underlying disease: Group 1—32 patients with glomerulonephritis; Group 2—10 patients with pyelonephritis; and Group 3—21 patients with a variety of other underlying diseases including 5 patients with polycys-

0037-9727/81/050062-08$01.00/0

TABLE I. CHARACTERISTICS OF THE STUDY POPULATION

	Group			
	1	2	3	4
		Underlying disease		
	Glomerulonephritis	Pyelonephritis	Other[a]	Control subjects
Number	32	10	21	8
Males/females	17/15	3/7	13/7	5/3
Mean age (years)	36.6	33	41.2	31.6
Source of graft—living related donor/cadaver	10/22	5/7	3/18	—
Mean time from transplant until vaccination (months)	50	35.6	31.7	—
Mean dose of azathioprine (mg/kg/day) at time of vaccination	1.57	1.58	1.82	—
Mean dose of prednisone (mg/kg/day) at time of vaccination	0.15	0.19	0.16	—
No. of patients with a rejection episode within the three months following vaccination	2	1	1	

[a] As defined in the text.

tic disease, 5 with congenital or hereditary renal disease, 4 with nephrosclerosis, 2 each with analgesic nephropathy, Fabry's disease, or diabetic glomerulosclerosis, and 1 male patient in whom the etiology of renal failure was unknown.

Fifty-six patients were receiving both azathioprine and prednisone, five azathioprine alone and two prednisone alone at the time of vaccination. Five Group 1 patients were receiving alternate-day prednisone as were two Group 2 patients and three Group 3 patients. Thirteen received antilymphocyte globulin—six Group 1 patients, two Group 2 patients, and five in Group 3. These patients had received the antilymphocyte globulin more than 1 year prior to immunization; hence this factor was not evaluated as a variable that might influence the immune response. Splenectomy was not performed in any of our patients.

Patients were immunized at various times after transplantation with means of 50, 35.6, and 31.7 months for Groups 1, 2, and 3, respectively (range 3 to 99 months).

Antibody levels of 51 of the 63 patients were measured 1 month and 1 year after immunization. Two of those missing at 1 year had died.

Two patients had not completed 1 year after immunization at the time the study ended, and three were unavailable for testing. An additional five were excluded because they did not have a determination at 1 month.

The patients and controls received tetradecavalent pneumococcal vaccine containing 50 μg of each capsular polysaccharide (Danish Types 1, 2, 3, 4, 6A, 7F, 8, 9N, 12F, 14, 18C, 19F, 23F, 25—Merck Sharp & Dohme) in 0.5 ml subcutaneously in the deltoid area. Serum was obtained before and 4 weeks and 1 year after immunization, and stored at $-20°$ until assayed. (The 1-year samples from the control subjects were not assayed.)

Antibody determinations. Serum antibodies to each of 12 pneumococcal polysaccharides were determined by a radioimmunoassay as previously described (7).

Data analysis. Data analysis was performed after the appropriate data file had been compiled in the computer. A principal component analysis was run of the logarithms of the antibody levels to all 12 antigens assayed after immunization. The first principal component accounted for 58.6% of the variability and had weights for the various antigens ranging from 0.6 to 0.91. This finding suggested that the geometric mean was a reasonable summary measure. The Statistical Package for the Social Sciences (SPSS) was used in all subsequent statistical analyses (8). To determine if any difference existed among the three groups of transplant patients and the controls, a one-way analysis of variance was used. This method requires an additive model, and the dependent variable used was the average of the logarithms (L TOTAL), of the antibody levels, rather than the geometric mean, which is the antilog of L TOTAL. A multiple regression analysis of L TOTAL with the following possible predictor values—azathioprine, prednisone, preimmunization serum creatinine level, and time from transplantation until immunization, was carried out by using the SPSS subroutine regression.

Percentage responders were calculated by determining those individuals having antibody responses 1.4-fold and greater.

Comparison of the antibody levels at 1 month to those at 1 year was performed by using the *t* test only for those patients whose antibodies had been assayed on both occasions.

Results *Antibody response to the vaccine.* The percentage of renal transplant patients and of control subjects who responded to immunization with a 1.4-fold and greater increase in antibody levels to each of the 12 pneumococcal polysaccharide antigens assayed is shown in Table II. Note that patients had a lower response rate to immunization with pneumococcal polysaccharide (PPS) Types 1 and 12F, and control subjects to PPS Types 12F and 18C, compared with that to the other antigens in the vaccine.

The geometric mean antibody concentrations (GMT) of the renal transplant patients and of the control subjects to 12 type-specific pneumococcal polysaccharides before immunization, at 1 month and at 1 year after immunization, are shown in Table III. Antibody levels at 1 year after immunization were not determined for the control group. While the mean antibody levels were generally somewhat lower, both before and at 1 month after immunization in the patient groups, overall, the 1 month postimmunization geometric mean antibody responses to the 12 antigens as shown in Table IV were not significantly different from those of controls.

One month after immunization the antibody levels were significantly higher than the preimmunization level ($P < 0.0005$) for all antigens except Types 4 and 12F (Table III).

All three patient groups showed a poor response to Type 12F while only Group 1 showed a poor response to Type 4. For the

TABLE II. PERCENTAGE RESPONDERS (DEFINED AS A 1.4-FOLD INCREASE IN ANTIBODY) AT 1 MONTH AFTER IMMUNIZATION OF RENAL TRANSPLANT PATIENTS AND CONTROL SUBJECTS WITH PNEUMOCOCCAL CAPSULAR POLYSACCHARIDES

	Pneumococcal polysaccharide type											
	1	3	4	6A	7F	8	9N	12F	14	18C	19F	23F
Percentage responders 58 renal transplant patients	57	90	72	74	74	90	86	45	81	74	83	84
Percentage responders 8 control subjects	87	100	87	87	87	100	100	75	87	50	87	100

TABLE III. GEOMETRIC MEAN ANTIBODY CONCENTRATIONS (IN ng OF PROTEIN N/ml) TO 12 TYPE-SPECIFIC PNEUMOCOCCAL POLYSACCHARIDES IN THREE GROUPS OF RENAL TRANSPLANT PATIENTS AND IN CONTROLS—PREIMMUNIZATION, AT 1-MONTH AND 1-YEAR AFTER IMMUNIZATION

	Pre	1 mo	1 yr	Pre	1 mo	1 yr	Pre	1 mo	1 yr
Group		Type 1			Type 3			Type 4	
1	554	1064	926	81	361	285[a]	1306	1887[b]	2971
2	415	1081	1096	51	344	60[a]	473	2673	2178
3	690	1096	984	87	677	203[a]	1713	3881	3351[a]
4	727	2365	ND[c]	92	779	ND	963	2904	ND
Group		Type 6A			Type 7F			Type 8	
1	198	516	501	158	378	364	133	544	481[a]
2	123	362	396	108	426	352	158	961	266[a]
3	192	457	322[a]	192	543	428[a]	203	918	591[a]
4	290	981	ND	265	699	ND	192	935	ND
Group		Type 9N			Type 12F			Type 14	
1	338	1061	870	249	341[b]	642	363	794	1145
2	194	944	523	287	388[b]	651	257	887	769
3	358	1358	1078[a]	416	494[b]	353	549	1377	1207[a]
4	465	1807	ND	308	598	ND	449	1832	ND
Group		Type 18C			Type 19F			Type 23F	
1	484	950	1071	81	209	246	939	2349	3589
2	374	1348	1193	69	251	197[a]	1052	2693	3133
3	659	1475	1380[a]	102	285	199[a]	1216	4335	3258[a]
4	961	1761	ND[c]	85	194	ND	950	5176	ND

Group 1: Twenty-four patients with glomerulonephritis as their underlying disease and reason for transplantation.
Group 2: Nine patients with pyelonephritis as their underlying disease.
Group 3: Eighteen patients with various other underlying diseases as given in the text.
Group 4: Eight control subjects.

[a] *Decrease* statistically significant compared with 1-month antibody level. All other values at 1 year did not show a significant decrease compared with the 1-month antibody level.

[b] *Increase not* statistically significant compared with the preimmunization antibody level. All other values at 1 month are significantly higher than the preimmunization level $P < 0.0001$.

[c] Not done.

control group, the 1-month-fold increase in antibody to Type 12F was lower than that to any of the other type-specific polysaccharides. The control subjects had a lower 1-month antibody level to Type 19F than did the patients. This antigen was the only one for which the fold increase and absolute antibody concentration of the control subjects were less than those of the patients.

Group 1 patients showed increased antibody levels at 1 year compared with 1 month for antigens 4, 12F, 14, 18C, 19F, and 23F. Group 3 patients showed a significant decline in antibody concentration at 1 year compared with the 1-month value to 10 of the 12 antigens. All groups showed such a decline of antibody to Types 3 and 8. In spite of such a decline, the 1-year antibody levels were above preimmunization levels for all antigens except Type 3 in Group 2 patients. The overall geometric mean antibody concentration 1 year after immunization is shown in Table IV.

One patient in Group 1 died from cryptococcal meningitis 4 months after immunization. She had very low antibody concentrations prior to immunization and had failed to respond at all to 6 of the 12 antigens by 1 month after immunization. Another Group 1 patient died 6 months after immunization from a histiocytic lymphoma. Her antibody response at 1 month after

TABLE IV. GEOMETRIC MEAN ANTIBODY CONCENTRATION (IN ng OF PROTEIN N/ml) TO ALL 12 TYPE-SPECIFIC PNEUMOCOCCAL POLYSACCHARIDES BEFORE, 1 MONTH, AND 1 YEAR AFTER IMMUNIZATION

Group	N	Geometric mean aggregate antibody concentration			
		Before immunization	1 Month after immunization	Fold increase	1 yr after immunization
1	24	322 (231–447)"	816 (502–1326)"	2.2	703 (44–1113)
2	9	184 (69–493)	770 (477–1242)	4.2	484 (187–1251)
3	18	379 (260–553)	1005 (739–1368)	2.6	698 (452–1080)
1 to 3	51	305 (231–403)	872 (684–1112)	2.8	659 (499–871)
4	8	360 (247–521)	1230 (897–1686)	3.4	Not done

Note. Groups 1 to 4 are as previously defined.
" 95% confidence interval is indicated in parentheses.

immunization was similar to that of the other Group 1 patients.

Effect of different variables on the antibody response. A multiple-regression analysis was performed to determine the effect of the following variables on the antibody response: daily dose of azathioprine, daily dose of prednisone, serum creatinine, and the duration from transplantation at the time of immunization. As the daily dose of azathioprine increased, the antibody response decreased. In addition, the antibody response decreased, but not significantly ($P = 0.06$), the longer the interval between transplantation and immunization. Further analysis revealed that the antibody response of Group 1 patients only was influenced by the daily dose of azathioprine (Fig. 1). An increasing daily dose of prednisone was associated with a decrease in the antibody response only of Group 3 patients (Fig. 2).

The antibody response of Group 2 patients was not influenced by any of the variable noted above.

Effect of the concentration of serum creatinine at the time of immunization on the antibody response. The effect of the concentration of creatinine in serum on the antibody response was also analyzed by dividing patients into three groups according to their serum creatinine concentration at the time of immunization. As shown in Table V, the GMT was lower for the group with serum creatinine levels of 1–3 mg/dl, as compared with those who had creatinine levels of <1 mg/dl, but not significantly so. Only two patients had a serum creatinine of ≥3 mg/dl at the time of immunization.

Effect of immunization on renal function. The differences in serum creatinine levels 1 month and 1 year after vaccination from the preimmunization values are shown in Fig. 3. At 1 month, the serum creatinine increased in 24 patients, decreased in 26, and remained unchanged in 12, when levels were compared with the preimmunization level. As can be seen from the figure, in most patients the change was minimal. Four patients experienced a rejection episode within 3 months of immunization. One year after immunization the creatinine had increased in 21, decreased in 27, and remained unchanged in 13, when compared with the preimmunization level.

Only one patient who had stable renal function prior to immunization experienced a rejection episode within 3 months of immunization. This patient, a 26-year-old female, had received her kidney 96 months prior to immunization. One other patient whose kidney had been transplanted 65 months prior to vaccination had three rejection episodes, but the first one followed manipulation of a dental abscess without antibiotic therapy.

Discussion. In this study, we have demonstrated that renal transplant patients mount an adequate humoral immune response to pneumococcal vaccine. Any population of renal transplant patients contains individuals whose native kidneys have failed as the result of a variety of diseases. Since some of these diseases are

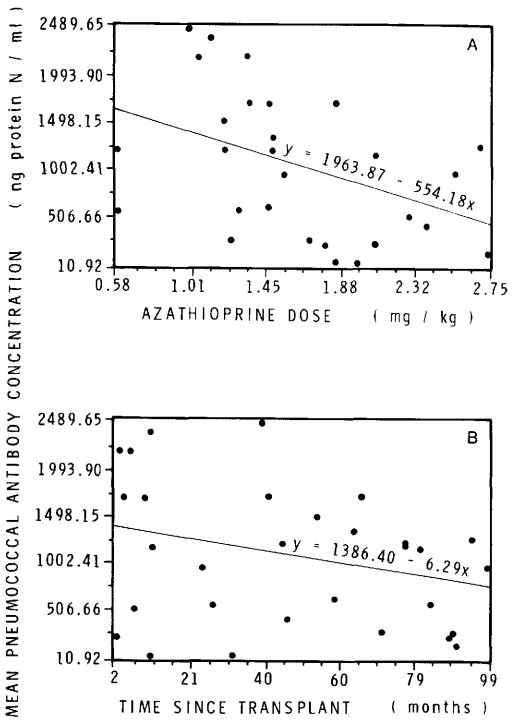

FIG. 1. Mean pneumococcal antibody concentrations of the glomerulonephritis group of renal transplant patients 1 month after immunization with pneumococcal vaccine containing 14 pneumococcal polysaccharide antigens. Antibodies to 12 of the 14 antigens were determined by radioimmunoassay. Panel A shows the effect of an increasing daily dose of azathioprine on the antibody response at 1 month after immunization. These patients were receiving this drug at the time of immunization. The equation of the regression line is given in Panel A: $r = -0.42$, $P = 0.011$. Antibody levels ranged from 25.25 to 2480.43 ng Protein N/ml. Panel B shows the effect of the time since transplantation on the antibody response of 30 patients. The equation of the regression line is given in Panel B: $r = -0.28$, $P = 0.06$. The range of antibody levels is the same as that in Panel A.

immunological in nature and may affect the response to a vaccine, we divided our patients into three groups. Group 1, the glomerulonephritic patients; had an immunological disorder as the basis for their renal failure and subsequent transplantation. Group 3 patients had a variety of disorders, but none had an immunological cause for his or her initial renal failure. Group 2 patients all had pyelonephritis and, for this reason, were analyzed separately.

Overall, the antibody levels of the patients 1 month after immunization were not significantly different from those of the control group—a finding similar to that of Dailey *et al.*, for 61 renal allograft recipients, 57 of whom had undergone splenectomy (9). In a recent study, Silberman *et al.*, using an indirect hemagglutination technique, found that renal transplant patients had a response to pneumococcal vaccine that was not different from that of control subjects (20). We found a gradation of antibody levels, both before immunization and 1 month after immunization among the three groups. Patients comprising Group 3 had higher levels at each of these two sampling periods, and their overall geometric mean antibody concentration 1 month after vaccination was very close to that of the control group.

An analysis of variables that could influence the immune response revealed differences among the three groups. In Group 1 patients, higher doses of azathioprine, and the longer the duration since transplantation at the time of immunization both had a moderately negative effect on the antibody response, although the latter was of a doubtful significance. Dailey and coworkers found no differences in antibody levels when patients receiving >0.2 mg/kg/day of prednisone and >1.5 mg/kg/day

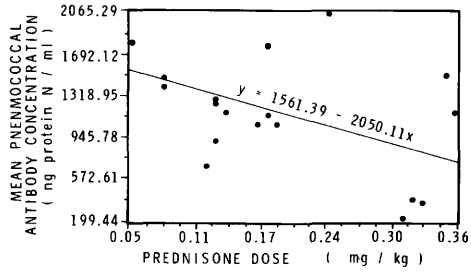

FIG. 2. Effect of the daily dose of prednisone at the time of immunization on the 1 month postimmunization aggregate pneumococcal antibody concentration of renal transplant patients with diseases other than glomerulonephritis and pyelonephritis. The patients were immunized with tetradecavalent pneumococcal vaccine and antibodies to 12 of these antigens were determined by a radioimmunoassay. There is a negative effect of increasing daily prednisone dose on the antibody response as shown by the regression line: $r = -0.40$, $P = 0.04$. Antibody levels ranged from 225.50 to 2065.29 ng Protein n/ml.

TABLE V. EFFECT OF THE LEVEL OF SERUM CREATININE AT THE TIME OF IMMUNIZATION ON THE ANTIBODY RESPONSE AT 1 MONTH AFTER IMMUNIZATION

Creatinine	*N*	Geometric mean antibody concentration to all 12 pneumococcal polysaccharides	95% confidence interval
≤1 mg/dl	16	1026	758–1388
1–3 mg/dl	40	756	545–1050
≥3 mg/dl	2	1456	Not reliable With $\eta = 2$

of azathioprine were compared to those receiving lower doses (9). The role of drugs such as azathioprine and prednisone in the suppression of humoral immunity is not well defined. Patients in one study on doses of 3 mg/kg/day of azathioprine for 4 months had IgG synthesis decrease by 33% and IgM synthesis by 41% (10). Most of the patients in that study had elevated rates of immunoglobulin synthesis before treatment. Corticosteroids in large doses have been shown to inhibit cellular and humoral immune responses (11, 12) and immunoglobulin synthesis (13). Increasing doses of prednisone were associated with a decreased antibody response only in Group 3 patients.

The response of renal transplant patients to a variety of influenza vaccines has been studied with conflicting results (14–17). Some (14, 17) found a response not different from that of controls; others found a decreased response (15, 16). Cytotoxic agents were observed not to influence the antibody response (14–17); in addition, conflicting results were noted regarding the effect of allograft function on the response; however, vaccination did not adversely affect allograft function (14–17).

We did not measure creatinine clearance so we were unable to determine accurately the effect of allograft function on the immune response. Those with preimmunization serum creatinine levels ≥1 mg/dl did not have a significantly different response from those with creatinine levels <1 mg/dl. In Dailey's study, patients with a creatinine clearance of >50 ml/min had significantly higher geometric mean titers of antibodies to 9 of the 12 serotypes than those with creatine clearances of <50 ml/min (9). As in our study, there was no adverse effect of vaccination on allograft function.

We found that the antibody levels of all three groups of patients were well maintained for 1 year. Group 3 patients showed a statistically significant decline in levels of antibody to 10 of the 12 serotypes 1 year after immunization compared with levels 1 month after immunization. This finding

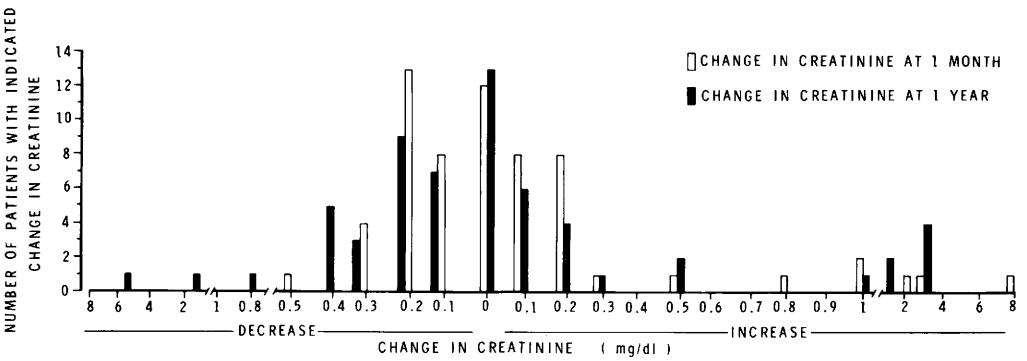

FIG. 3. Change in creatinine at 1 month and 1 year postimmunization from the value immediately before immunization. Values are for all transplant patients who were vaccinated with pneumococcal vaccine containing 14 capsular pneumococcal polysaccharides.

compares with such a decline of antibody against three serotypes in the other two groups. The Group 3 response may have been related to their higher levels of antibody 1 month after immunization. In nonimmunosuppressed individuals 3 years after immunization, levels of antibody range between one-half and one-third the peak level (6). In some instances (Table III) antibody levels 1 year after immunization were higher than the levels 1 month after immunization. We can only speculate that more than 1 month is necessary for the peak immune response to some antigens in these patients who are receiving immunosuppressive drugs.

The postimmunization aggregate level of antibody in our control subjects, 1230 ng of antibody N, for 12 types is comparable to postimmunization levels for control subjects of 1200–1700 ng of antibody N reported in other studies (18, 19). The decision of whether or not an immune response is adequate to protect a patient against pneumococcal infection must ultimately be based on what is determined to be a protected level of antibody in the presence of optimum leukocytic function.

The protective efficacy of the vaccine in this group of patients remains to be determined. None of the vaccinated patients has had pneumococcal pneumonia or bacteremia over the follow-up periods which range from 8 months to 2 years. Over this same 2-year period, 4 of 62 hemodialysis patients developed pneumococcal bacteremia. This observation suggests that hemodialysis patients should be vaccinated; however, they respond poorly to the vaccine (18).

Supported in part by a Grant-in-Aid from the Nova Scotia Kidney Foundation, and in part by Contract 1 AI02647 from the National Institute of Allergy and Infectious Diseases. We wish to thank Ms. Carol Swantee for technical assistance and Ms. Wilma Mays for typing the manuscript. Ms. Jennifer MacDonald helped with the statistical analysis. To Doctors A. S. MacDonald, P. Belitsky, and S. Lannon, transplant surgeons, Victoria General Hospital, who provided the impetus for this study, we are grateful. Dr. Bortolussi is supported by a grant from the Medical Research Council of Canada.

1. Austrian, R., J. Infect. Dis. **131**, 474 (1975).
2. Foy, H. M., Wentworth, B., Kenny G. E., Kloeck, J. M., and Grayston, J. T., Amer. Rev. Respir. Dis. **111**, 595 (1975).
3. Valenti, W. M., Jenzer, M., and Bentley, D. W., Amer. Rev. Respir. Dis. **117**, 233 (1978).
4. Linnemann, C. C., Jr., JAMA **241**, 2619 (1979).
5. Recommendations of the Public Health Service Advisory Committee on Immunization Practices: Pneumococcal Polysaccharide Vaccine. Morbidity & Mortality Weekly Report, **27**, 25 (1978).
6. Austrian, R., J. Infect. Dis. **136**, S38 (1977).
7. Schiffman, G., Douglas, R. M., Bonner, M. J., Robbins, M., and Austrian, R., J. Immunol. Methods **33**, 133 (1980).
8. "Statistical Package for the Social Sciences" (N. H. Nie, C. H. Hull, and J. G. Jenkins, eds.) McGraw–Hill, New York (1976).
9. Dailey, M. P., Schiffman, G., Piering, W. F., Hoffman, R. G., and Rytel, M. W., Current Chemotherapy and Infectious Disease. Proceedings of the 11th ICC and the 19th ICAAC. Amer. Soc. Microbiol. **II**, 854 (1980).
10. Levy, J., Barnett, E. V., and MacDonald, N. S., J. Clin. Invest. **51**, 2233 (1972).
11. Faüci, A. S., Dale, D. C., and Dalow, J. E., Ann. Int. Med. **84**, 304 (1976).
12. Schwartz, R. S., Prog. Allergy **9**, 246 (1965).
13. Butler, W. T., and Rossen, R. D., J. Clin. Invest. **52**, 2629 (1973).
14. Briggs, W. A., Rozek, R. J., Migdol, S. D., Shillis, J. L., Brackett, R. G., Brandon, F. B., Mahajon, S. V., and McDonald, F. D., Ann. Int. Med. **92**, 477 (1980).
15. Pabico, R. C., Douglas, G., Betts, R. F., McKenna, B. A., and Freeman, R. B., Ann. Int. Med. **85**, 431 (1976).
16. Kumar, S. P., Ventura, A., and Vander Werf, B., JAMA **239**, 840 (1978).
17. Carroll, R. N. P., Marsh, S. D., O'Donoghue, E. P. N., Breeze, D. C., and Shackman, R., Brit. Med. J. **2**, 701 (1974).
18. Simberkoff, M. S., Schiffman, G., Katz, L., Spicehindler, J., Moldover, N. H., and Rahal, J. J., Current Chemotherapy and Infectious Disease. Proceedings of the 11th ICC and the 19th ICAAC. Amer. Soc. Microbiol. **II**, 851 (1980).
19. Siber, G. R., Weitzman, S., Aisenberg, A. C., Weinstein, H. J., and Schiffman, G., N. Engl. J. Med. **298**, 442 (1978).
20. Silberman, H., Overturf, G. D., Field, R. J., Butler, J., Berne, T. V., and Witt, R., Ann. Surg. **192**, 199 (1980).

Received September 8, 1980. P.S.E.B.M. 1981, Vol. 167.