Clin. exp. Immunol. (1972) **10**, 257–265.

SERUM IgD CONCENTRATIONS IN SARCOIDOSIS AND TUBERCULOSIS

C. E. BUCKLEY AND HYLARY R. TRAYER*

Departments of Medicine and Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina

(*Received* 16 *June* 1971)

SUMMARY

Studies of the serum level of IgD in patients with tuberculosis and sarcoidosis reveal evidence of a difference in humoral immunity. Radial diffusion measurements were done of IgD in serums from fifty patients with active tuberculosis, fifty-three patients with sarcoidosis and 103 age, race and sex matched healthy controls. IgD was detected in serums from 20% more tuberculosis patients ($P<0{\cdot}0250$) and 20·7% fewer sarcoidosis patients than respective controls ($P<0{\cdot}0005$). Multivariate statistical analysis of $\log_e$ transformed IgD serum levels revealed significantly lower geometric mean IgD levels in sarcoidosis patients ($P=0{\cdot}0018$). The age dependence of serum IgD was highly significant ($P<0{\cdot}0001$). Age dependent disease effects were detected. High levels of IgD occurred predominantly in older tuberculosis patients while depression of IgD occurred in middle-aged sarcoidosis patients. It is suggested that the elevated levels of serum IgM in patients with sarcoidosis may represent a compensatory change associated with low levels of serum IgD.

INTRODUCTION

Pinner's (1946) provocative suggestion, that sarcoidosis may represent an anergic form of tuberculosis, remains unproven. The biomedical importance of contrasts of immunity in these two diseases lies in their relevance to our understanding of immunologic diseases. For example, a hypothesis (Buckley *et al.*, 1966) about the pathogenesis of sarcoidosis can be based on Mankiewicz & van Walbeek's (1962) unconfirmed (Bowman, 1968) observation of less mycobacteriophage antibody in serums from sarcoidosis patients than tuberculosis patients. Mankiewicz & van Walbeek reported that sarcoidosis patients excrete mycobacteriophage in their stool. Mycobacteria were demonstrated in tissues from patients with sarcoidosis by Mankiewicz in 1964 and this observation was confirmed in patients with sar-

* Present address: Department of Experimental Pathology, University of Birmingham, Birmingham 15.

Correspondence: Dr C. E. Buckley, Box 3804, Duke University Medical Center, Durham, North Carolina 27710, U.S.A.

coidosis-like-tuberculosis by other investigators (Kent & Schwartz, 1967; Vanek & Schwartz, 1970). These observations have heightened speculation about some intimate relationship between mycobacteria and sarcoidosis.

The possibility of some permissive role of comprised humoral immunity in sarcoidosis remains unexplored. Studies of the serum levels of IgG, IgA and IgM confirm the occurrence of hyperglobulinaemia in tuberculosis and sarcoidosis (Buckley & Dorsey, 1970a), but do not reveal differences between the three major immunoglobulins in the two diseases. Recent studies of the serum concentrations of a fourth serum immunoglobulin, IgD, provide evidence of a difference in humoral immunity between patients with sarcoidosis and tuberculosis.

MATERIALS AND METHODS

Serums from fifty patients with active tuberculosis and fifty-three patients with sarcoidosis were studied in comparison with serum from age, race and sex matched controls. Control serums were selected among samples collected from apparently healthy subjects. When more than one age, race and sex matched control serum was available, the selection of the control serum was random. Otherwise control serums at the most proximate age were selected. This method of selection allowed exact matches of the race and sex of patients and an approximate match of the patient's age. All serums were stored at $-20°C$ or retained at 4°C during the period of analysis. IgD antigen and goat antiserum containing globulin to IgD was prepared by methods described previously (Buckley & Dorsey, 1970b). A myeloma IgD paraprotein was used as antigen. Optimal conditions for single radial diffusion were established by measurement of the area of precipitation over a 72-hr period in antibody agar plates containing varying amounts of antibody. The laboratory secondary reference standard serum contained 49 mg/100 IgD in comparison with a reference serum (31 mg/100 ml) kindly provided by Dr William Terry through the National Cancer Institute Immunoglobulin Reference Center. Unknown serums or dilutions of serums reported did not yield a precipitin ring which exceeded the secondary reference serum. The area of precipitation observed with the undiluted secondary reference standard serum reached maximum size with an optimal amount of antibody in agar in 15 hr and was not different at 24 hr. Therefore, ring diameters were read at 24 hr. Diameters on each antibody-agar plate were measured directly on the moist agar and again on a photograph (Model 95B, Speedliner Land Camera, Polaroid Corporation, Cambridge 39, Massachusetts) of the indirectly illuminated agar plate after washing 30 min in 0·3 M saline and 10 min in 7% acetic acid. Estimates of minimum error were least with the direct measurements and are the basis of the observations in this report. Diffusion areas of the secondary reference serum dilutions were evaluated as previously described (Buckley & Dorsey, 1970b) by linear regression. An 0·3 ml aliquot of antibody globulin in 10 ml of 1% agar gel which precipitated an estimated 3·98 mg of IgD per 100 ml of antibody globulin (Becker, 1969) was used for these studies. The secondary reference standard serum was also quantitated with a rabbit anti-IgD serum provided by the National Cancer Institute Immunoglobulin Reference Center. Estimates of IgD concentration with the rabbit anti-IgD serum and the goat anti-IgD globulin preparation agreed within the limits of the precision of the method.

A chi-squared analysis was used to compare the frequency with which IgD could be measured in each patient group in relation to healthy controls (Siegel, 1956). Multivariate

analysis (Starmer & Grizzle, 1968) of $\log_e$ duplicate estimates of IgD concentrations was used to evaluate analytic variation and to compare age and disease effects on serum IgD levels of patients and healthy controls.

RESULTS

IgD could not be detected in a few serums from patients with tuberculosis and sarcoidosis. The lowest possible level of IgD that could be detected was calculated from the diameter of the antigen well and IgD in dilutions of the secondary reference serum. The calculated estimates of the limit of sensitivity of the method varied between 0·3 and 0·1 mg/100 ml. Precipitate rings close to the antigen well could not be accurately measured or distinguished from nonspecific changes about the rim of the well. The lower limit of reliable measurements within the useful range of dilutions of the secondary reference serum was 1·0 mg/100 ml. Detectable concentrations of IgD immediately below the limit of reliable measurements were suspected in only a few serums. Experiments with dilutions of serums of known IgD concentration suggested that serums in which IgD might be detected as a faint opacification about the antigen well had concentrations no less than 0·1 mg/100 ml or one-tenth the least measurable concentration of IgD. Few serums had detectable but not reliably measurable IgD levels. Therefore, all nonmeasurable IgD concentrations were arbitrarily assigned a concentration of 0·01 mg/100 ml for purposes of parametric statistical analysis. This assumption and classification was tested by comparison of nonparametric and parametric analyses of the data (Siegel, 1956). Statistical inferences derived from a chi-squared analysis of the frequency with which IgD was measurable in patients and in their respective age, race and sex matched controls are presented in Table 1. Nonparametric analysis revealed that IgD was measurable in serums from 20% more tuberculosis patients than in matched controls ($P<0·0250$). In contrast, 20·7% fewer serums from sarcoidosis patients had measurable

TABLE 1. Frequency of measurable serum IgD in tuberculosis, sarcoidosis and age, race and sex matched healthy controls

	Measurable IgD	
	Present	Absent
Tuberculosis*		
Patients	42	8
Controls	32	18
Sarcoidosis†		
Patients	40	13
Controls	51	2

* IgD was measurable in 20% more tuberculosis patients than in control subjects, $\chi_1^2=5·197$, $P<0·0250$.

† IgD was measurable in 20·7% fewer sarcoidosis patients than in control subjects, $\chi_1^2=9·396$, $P<0·0005$.

IgD than matched controls ($P<0{\cdot}0005$). These statistical inferences were compared with multivariate analyses of $\log_e$ IgD levels presented in Table 2. The geometric mean level of IgD in tuberculosis patients was greater than controls, but the difference was not significant ($P=0{\cdot}1010$). In contrast, the geometric mean level of IgD in sarcoidosis patients was significantly less than controls ($P=0{\cdot}0018$). The parametric comparisons revealed less significant differences than those based on an unambiguous nonparametric test. This suggests that the arbitrary classification of unmeasurable IgD levels for purposes of parametric multivariate analyses was conservative and that subsequent comparisons of age effects are likely valid. Sarcoidosis patients had a geometric mean IgD concentration of 8·1 mg/100 ml. This value was one-sixth the 49·1 mg/100 ml geometric mean IgD level observed in controls.

TABLE 2. Mean age and serum IgD levels

Group	Average age (years)	Serum IgD (mg/100 ml)	
		Mean ± S.E.	Geometric mean
Tuberculosis			
Patients	52·0 ± 2·1	2·7146 ± 0·6548	15·09
Controls	51·9 ± 2·1	1·1738 ± 0·6548	3·23
P	= 0·6295	= 0·1010	
Sarcoidosis			
Patients	34·8 ± 2·0	2·0942 ± 0·6360	8·11
Controls	34·6 ± 2·0	3·8944 ± 0·6360	49·12
P	= 0·2632	= 0·0018	

Variation in serum IgD concentrations unrelated to disease was anticipated (Buckley & Dorsey, 1970b). This expectation was confirmed. Race and sex matched controls were used. Similar precautions were taken with respect to age, but were not as easily accomplished. Therefore, the significance of age variation was tested. Table 2 presents contrasts of serum IgD levels and age variation in the two groups of patients and their respective controls. Tuberculosis patients were older than sarcoidosis patients, but age differences between each patient group and their respective controls were not significant. Thus, the information in this report is not at risk because of sampling differences in age, race or sex.

Duplicate observations on each patient and control provided a convenient means of estimating analytic error. A contrast of the difference between all duplicate observations revealed a log mean difference due to analysis of 0·09971. Differences between duplicate observations were not significant ($P=0{\cdot}9665$). The geometric mean IgD for the whole population of 206 patients and controls was 12·0 mg/100 ml. A coefficient of variation calculated from this mean and standard error of the mean (2·4845 ± 0·6451) was 26%. In contrast, a coefficient of variation calculated from the mean and standard error for analysis (2·4845 ± 0·0997) was 4%. This suggests that biologic variation in IgD was six-fold greater than analytical variation.

The importance of age matched controls was confirmed by testing the significance of differences in serum IgD levels between four sequential age spans. The distribution of

patients presented in Table 3 reflects the lack of direct comparability of the two diseases and the necessity for matched controls. Age differences in IgD between all age groups were highly significant ($P<0{\cdot}0001$). The effect of aging is presented in Fig. 1, which shows the range of the estimated mean for age grouped healthy controls. The effect of age on IgD

TABLE 3. Age, race and sex distribution of patients

Age range	Tuberculosis				Sarcoidosis			
	Whites		Blacks		Whites		Blacks	
(years)	Males	Females	Males	Females	Males	Females	Males	Females
14–25	1	1			1		9	7
26–45	6		10		6	1	6	12
46–60	10		8			1	4	3
61+	9	1	4				2	1

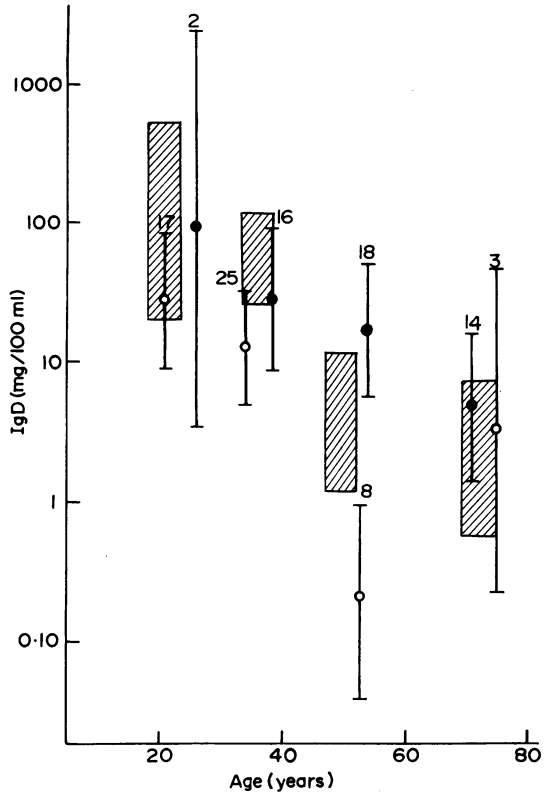

FIG. 1. The relation between mean serum IgD concentrations in healthy subjects and patients with tuberculosis (●) and sarcoidosis(○). Bars represent the domain of the mean and standard error of the mean of controls at mean age within each age span. Circles and vertical lines represent the mean and standard error of the mean of patient groups at mean age within each age span. Note the log scale for IgD concentrations and the age-dependent differences between tuberculosis and sarcoidosis patients.

levels was evaluated separately in healthy controls ($P<0{\cdot}0001$), tuberculosis patients ($P=0{\cdot}0467$), and sarcoidosis patients ($P<0{\cdot}0001$.)

Table 4 presents the age dependence of the disease related changes observed. Graphic comparisons of age grouped patients' IgD are also presented in Fig. 1. Tuberculosis patients below age 45 years had less IgD than controls, but the differences were small. Serum IgD increased in tuberculosis patients older than age 46 years. This hyperglobulinaemia became highly significant in patients older than 60 years ($P<0{\cdot}0001$). Table 4 also suggests that

TABLE 4. Comparison of patients and age, race and sex matched controls within age groups

Disease comparison	Age (years)	Patients No.	Patients Geometric mean IgD (mg/100 ml)	Controls No.	Controls Geometric mean IgD (mg/100 ml)	P
Tuberculosis	14–25	2	93·5	2	119·3	0·9928
	26–45	16	28·2	14	46·3	0·5672
	46–60	18	17·0	19	5·1	0·1106
	61+	14	4·8	15	0·1	0·0001*
Sarcoidosis	14–25	17	28·1	15	84·5	0·1718
	26–45	25	12·6	29	62·3	0·0118*
	46–60	8	0·2	5	2·6	0·0489*
	61+	3	3·3	4	43·6	0·1418

* Statistically significant difference.

TABLE 5. Correlation between IgD serum levels and serum concentrations of IgG, IgA and IgM

	Correlation coefficients, r		
	IgG	IgA	IgM
IgA	0·4511		
IgM	0·3679	0·1918	
IgD	0·0809	0·0591	−0·1944

$r>0{\cdot}1651$; $P<0{\cdot}0500$.

the decreased levels of serum IgD in sarcoidosis patients become more severe with advancing age. The hypoglobulinaemia observed prior to age 25 years was not significant. Differences in serum IgD became significant between age 26 and 60 years, where the significance of the differences observed seemed related to the number of patients in each age span (Tables 3 and 4).

The relationship between serum levels of IgD and the three major serum immunoglobulins are presented in Table 5. The estimates of the statistical significance of the correlation coefficients presented have been adjusted for inferences based on four simultaneous estimates. Significant positive correlations exist between IgG and IgA, IgG and IgM, and IgA

and IgM. In contrast to these positive correlations between the three major serum immunoglobulins, a negative correlation was detected between IgD and IgM ($r=0{\cdot}1944$, $P=0{\cdot}0170$). IgD levels were not systematically related to the other major serum immunoglobulin concentrations. This suggests that low levels of IgD were often associated with high levels of IgM.

DISCUSSION

Although adequate evidence supports the inclusion of IgD within the immunoglobulin family (Rowe & Fahey, 1965a, b; Pernis, Chiappino & Rowe, 1966; Crabbe & Heremans, 1966; Rowe *et al.*, 1968a), its biological activity is not known. Recent studies have demonstrated antibody activity in the IgD class of immunoglobulins (Levine & Redmond, 1967; Ritchie, 1968; Gleich, Bieger & Stankievic, 1969; Kantor, van Herle & Barnett, 1970; Devy *et al.*, 1970). However, IgD does not fix complement nor increase the vascular permeability of guinea-pig skin (Henny *et al.*, 1969; Ovary, 1969). Uncertainty exists about the precise protective role of IgD.

The serums analysed and presented in this report were collected over several years' time and maintained at $-20°C$ with minimum handling until the time of analysis. While serum sample deterioration could have contributed to the low IgD levels observed in sarcoidosis, under the conditions of collection and analysis, similar changes would be expected in serums from tuberculosis patients and controls. This effect would be expected to diminish the magnitude and significance of the experimental differences observed. Moreover, the levels of IgD measured in healthy subjects in these studies compare favourably with those reported by other investigators (Kohler & Farr. 1967; Rowe *et al.*, 1968b; Onodera *et al.*, 1968). This suggests that the experimental conditions and sampling used for these studies were optimal and that the differences presented are valid.

The influences of normal aging on serum IgD values suggests abiotrophy similar to that previously observed with IgG and IgM (Buckley & Dorsey, 1970b). A similar age related trend in IgD levels may be seen in studies of a much larger population reported by Rowe *et al.* (1968b) and McGregor *et al.* (1970). It was of interest that patients with a chronic infection, tuberculosis, exhibited less age related decrease in IgD than controls, suggesting that continued stimulation may reverse the immunoglobulin changes observed in healthy controls. Extreme hyperglobulinaemia was observed in four apparently healthy black control subjects in the seventh and eighth decades of life. A tendency toward hyperglobulinaemia at the extreme of the life span of man has been observed with IgG, IgA and IgM (Buckley & Dorsey, 1970b). Whether or not this finding represents prolonged survival of a hyperglobulinaemic elite portion of the population, or a change in anticipation of death, remains to be determined.

These changes establish a relative failure of patients with sarcoidosis to maintain normal serum levels of IgD. In contrast, older patients with tuberculosis have increased serum concentrations of IgD. This hyperglobulinaemia occurs at a time of life when the clinical diagnosis of active tuberculosis is most often difficult. The catabolic destruction of IgD is approximately twice as rapid as IgM and IgA and more than eight times as rapid as IgG (Rogentine *et al.*, 1966). The identification of antibody activity within the IgD class might reasonably be expected to bear a more proximate relationship to recent infection than IgG, IgA and IgM. The information represented in this report suggest the possible importance

of evaluation of antibody activity to tuberculoprotein within the IgD class as a serologic test for active tuberculosis.

The association between low levels of IgD and delayed cutaneous anergy in sarcoidosis is provocative. The concurrence of low levels of IgD and increased levels of IgG and IgM suggests subtle imbalances in humoral immunity may be important in immunologic diseases in older patients. Alterations in the balance of the immunoglobulin composition of serum can be clearly related to many of the immunologic diseases which occur early in life. The very frequent occurrence of hypoglobulinaemia involving one or more immunoglobulin and frequent infection leads conveniently to the interpretation of compromised immunity. Hyperglobulinaemia is more characteristic of individuals who present with immunologic diseases late in life. Sarcoidosis represents an example of this phenomenon. The information presented in this report suggests that the overt hyperglobulinaemia in adults with sarcoidosis may occur in association with covert hypoglobulinaemia involving IgD. It seems reasonable to suggest that the hyperglobulinaemia of sarcoidosis may represent compensatory changes secondary to the more subtle deficiency in the humoral immune system presented in this report. Studies of IgD antibodies to mycobacteriophage will be of interest. Impaired ability to maintain normal levels of serum IgD and cellular immunity coexist in sarcoidosis and may represent the compromised responsive mechanisms responsible for the more obvious clinical attributes of the disease.

ACKNOWLEDGMENTS

This investigation was supported by the United States–Japan Cooperative Medical Science Program administered by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, Department of Health, Education, and Welfare (AI-07499), the North Carolina Tuberculosis and Respiratory Disease Association, and USPHS Grant No. HD00668.

REFERENCES

BECKER, W. (1969) Determination of antisera titres using the single radial immunodiffusion method. *Immunochemistry*, **6**, 539.

BOWMAN, B.U. (1968) Neutralization of mycobacteriophages by sera of patients with and without sarcoidosis. *Proc. Soc. exp. Biol. (N.Y.)*, **129**, 696.

BUCKLEY, C.E., III & DORSEY, F.C. (1970a) A comparison of serum immunoglobulin concentrations in sarcoidosis and tuberculosis. *Ann. intern. Med.* **72**, 37.

BUCKLEY, C.E., III & DORSEY, F.C. (1970b) The effect of aging on human serum immunoglobulin concentrations. *J. Immunol.* **105**, 964.

BUCKLEY, C.E., III, NAGAYA, H. & SIEKER, H.O. (1966) Altered immunologic activity in sarcoidosis. *Ann. intern. Med.* **64**, 508.

CRABBE, P.A. & HEREMANS, J.F. (1966) Presence of large numbers of plasma cells containing IgD in the rectal mucosa of a patient with ulcerative colitis. *Acta clin. belg.* **21**, 73.

DEVY, M., CARTER, D., SANDERSON, C.J. & COOMBS, R.R.A. (1970) IgD antibody to insulin. *Lancet*, **ii**, 1280.

GLEICH, G.J., BIEGER, R.C. & STANKIEVIC, R. (1969) Antigen combining activity associated with immunoglobulin D. *Science*, **165**, 606.

HENNEY, C.S., WELSCHER, H.D., TERRY, W.D. & ROWE, D.S. (1969) Studies on human IgD. II. The lack of skin sensitizing and complement fixing activities of immunoglobulin D. *Immunochemistry*, **6**, 445.

KANTOR, G.L., VAN HERLE, A.J. & BARNETT, E.V. (1970) Autoantibodies of the IgD class. *Clin. exp. Immunol.* **6**, 951.

KENT, D.C. & SCHWARTZ, R. (1967) Active pulmonary tuberculosis with negative tuberculin skin reactions. *Amer. Rev. resp. Dis.* **95,** 411.

KOHLER, P.F. & FARR, R.S. (1967) Quantitative comparison of immunoglobulins in atopic (reaginic) and nonatopic (nonreaginic) individuals. Higher gamma-D levels in atopic sera. *J. Allergy*, **39,** 311.

LEVINE, B. & REDMOND, A. (1967) Immunochemical mechanisms of penicillin induced Coombs positivity and hemolytic anemia in man. *Int. Arch. Allerg.* **21,** 594.

MANKIEWICZ, E. (1964) The relationship of sarcoidosis to anonymous bacteria. *Acta med. scand.* (Suppl.) **196,** 68.

MANKIEWICZ, E. & VAN WALBECK, M. (1962) Mycobacteriophages. Their role in tuberculosis and sarcoidosis. *Arch. environm. Hlth*, **5,** 122.

MCGREGOR, I.A., ROWE, D.S., WILSON, M.E. & BILLEWICZ, W.Z. (1970) Plasma immunoglobulin concentrations in an African (Gambian) community in relation to season, malaria and other infections and pregnancy. *Clin. exp. Immunol.* **7,** 51.

ONODERA, S., SHIBATA, A., MIURA, A.B., SUZUKI, A., SAKAMOTO, C., SUZUKI, C. & ITOH, C. (1968) Quantitative determination of serum immunoglobulin D in gammopathy. *Tohoku J. exp. Med.* **95,** 145.

OVARY, Z. (1969) Lack of skin-sensitizing property of gamma-D myeloma protein in guinea pigs. *J. Immunol.* **102,** 790.

PERNIS, B., CHIAPPINO, G. & ROWE, D.S. (1966) Cells producing IgD immunoglobulins in human spleen. *Nature* (*Lond.*), **211,** 424.

PINNER, M. (1946) Editorial: On the etiology of sarcoidosis. *Amer. Rev. Tuberc.* **54,** 582.

RITCHIE, R.F. (1968) IgD antinuclear antibodies. *Arthr. and Rheum.* **11,** 506.

ROGENTINE, G.N., ROWE, D.S., BRADLEY, J., WALDMANN, T.A. & FAHEY, J.L. (1966) Metabolism of human immunoglobulin D (IgD). *J. clin. Invest.* **45,** 1467.

ROWE, D.S., CRABBE, P.A. & TURNER, M.W. (1968a) Immunoglobulin D in serum, body fluids and lymphoid tissues. *Clin. exp. Immunol.* **3,** 477.

ROWE, D.S., DOLDER, F. & WELSCHER, H.D. (1969) Studies on human IgD-I. Molecular weight and sedimentation coefficient. *Immunochemistry*, **6,** 437.

ROWE, D.S. & FAHEY, J.L. (1965a) A new class of human immunoglobulins. I. A unique myeloma protein. *J. exp. Med.* **121,** 171.

ROWE, D.S. & FAHEY, J.L. (1965b) A new class of human immunoglobulins. II. Normal serum IgD. *J. exp. Med.* **121,** 185.

ROWE, D.S., MCGREGOR, I.A., SMITH, S.J., HALL, P. & WILLIAMS, K. (1968b) Plasma immunoglobulin concentrations in a West African (Gambian) community and in a group of healthy British adults. *Clin. exp. Immunol.* **3,** 63.

SIEGEL, S. (1956) *Nonparametric Statistics for the Behavioral Sciences.* McGraw-Hill, New York.

STARMER, C.F. & GRIZZLE, J.E. (1968) A computer program for analysis of data by general linear models. Institute of Statistics Mimeo Series No. 560. University of North Carolina, Chapel Hill, N.C.

VANEK, J. & SCHWARTZ, J. (1970) Demonstration of acid-fast rods in sarcoidosis. *Amer. Rev. resp. Dis.* **101,** 395.