Annals of the Rheumatic Diseases 1989; **48:** 677–682

Low avidity antibodies to double stranded DNA in systemic lupus erythematosus: a longitudinal study of their clinical significance

J C NOSSENT,[1] V HUYSEN,[2] R J T SMEENK,[2] AND A J G SWAAK[1]

From the [1]Department of Rheumatology, The Dr Daniel den Hoed Clinic, Rotterdam, The Netherlands; and the [2]Department of Autoimmune Diseases, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands

SUMMARY In a longitudinal study the relevance of the detection of low avidity antibodies to double stranded DNA (dsDNA) as measured by the polyethylene glycol (PEG) assay in patients with systemic lupus erythematosus (SLE) was evaluated. It was found that 35 patients positive in the PEG assay only—that is, having only low avidity anti-dsDNA in their circulation, had a mild course of their SLE with absence of renal involvement. The PEG assay had little predictive value but a high specificity (90·2%) for clinical exacerbations; furthermore, a change in avidity of anti-dsDNA in such patients was seldom observed. In 14 patients positive in both Farr and PEG assays—that is, with a relative preponderance of high avidity anti-dsDNA, there was a clear correlation between rises in Farr assay and major exacerbations, while the PEG assay on its own was not helpful in predicting disease manifestations; disease manifestations were often heralded by a change in the Farr/PEG ratio, with renal and cerebral exacerbations associated with the greatest increase in the Farr/PEG ratio (more than 10 times the previous value).

Systemic lupus erythematosus (SLE) is considered to be the prototype autoimmune disease, in which immune complexes mediate inflammatory processes, though the mechanism by which they do so remains uncertain.[1–3] Antibodies to double stranded DNA (dsDNA) have been shown to be sensitive, though not specific markers for SLE.[4–6] Different properties of the anti-dsDNA antibody (specificity, avidity, complement binding capacity, immunoglobulin subclass) have been related to the various clinical manifestations of SLE.[7–10] The Farr assay is widely used for measurement of anti-dsDNA antibodies but detects mainly high avidity antibodies; the polyethylene glycol (PEG) assay, on the other hand, is thought to detect low avidity antibodies to dsDNA and uses a less powerful precipitation method, leading to less dissociation of DNA/anti-dsDNA complexes.[11 12] The clinical significance of a positive PEG assay has been studied transversally,[11] showing associations with autoimmune liver disease and myasthenia gravis and fair specificity for SLE; a longitudinal study showed a good correlation between Farr and PEG assays and clinical manifestations,[13] while a high Farr/PEG ratio (>5) was found to be associated with renal disease and a Farr/PEG ratio below five with cerebral disease in other studies.[14–16]

We studied the clinical significance of the PEG assay in two groups of patients with SLE. In serum samples of group I patients anti-dsDNA was detectable only with the PEG assay, whereas in the serum samples of group II patients anti-dsDNA was detectable by both the Farr and PEG assays. Our results indicate a fairly benign course of SLE for group I patients and a low predictive value of changes in anti-dsDNA levels, measured by the PEG assay, for disease exacerbations. Group II patients showed a good correlation between disease exacerbations and a rise in anti-dsDNA detected by the Farr assay: in this group an increase in the Farr/PEG ratio was also found to predict an approaching exacerbation.

Accepted for publication 28 December 1988.
Correspondence to Dr J C Nossent, Department of Rheumatology, The Dr Daniel den Hoed Clinic, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands.

Patients and methods

SERUM SAMPLES/PATIENTS

Group I consisted of 35 patients fulfilling at least four of the American Rheumatism Association criteria for SLE,[17] whose sera reacted positively in the *Crithidia luciliae* test (immunofluorescence test) and upon subsequent testing were positive (≥20 U/ml) for antibodies to dsDNA in the PEG assay, but negative (<20 U/l) in the Farr assay. Their attending physicians were asked to obtain serum samples every two months and to provide clinical information through questionnaires or record investigation, or both. In this way 491 serum samples were obtained.

Group II consisted of 14 patients fulfilling at least four of the American Rheumatism Association criteria for SLE, whose sera were positive (≥20 U/ml) in both the Farr and PEG assays for antibodies to dsDNA; the same follow up procedure was used as for group I patients. In this group 89 serum samples were obtained. Table 1 summarises characteristics of both groups of patients.

EXACERBATIONS

Disease manifestations were defined according to Lightfoot and Hughes[18] and Swaak *et al*,[19] distinguishing minor exacerbations (skin and joint symptoms, no increase in steroids of more than 15 mg required) from major exacerbations (renal: onset of proteinuria >0·5 mg/24 h or doubling of existing proteinuria with active urinary sediment; cerebral; onset of psychosis or epilepsy not otherwise explained; haematological: haemolytic anaemia, persistent leucopenia ($<3\times10^9$ cells/l) or thrombocytopenia ($<100\times10^9$ platelets/l), and serosa involvement, pleuritis, or pericarditis by history and physical examination or imaging technique).

CRITHIDIA LUCILIAE IMMUNOFLUORESCENCE TEST

Details of this immunofluorescence test have been published.[20] *Crithidia luciliae* were grown, harvested, and used as described. Serum samples were diluted 1:10 in phosphate buffered saline (PBS; 0·14 M NaCl, 0·01 M sodium phosphate, pH 7·4); 20 µl of this solution was incubated for 30 minutes at room temperature on a spot of *C luciliae*. After washing the slide for 30 minutes in PBS 0·5 ml of fluorescein isothiocyanate (FITC) conjugated anti-immunoglobulin (batch No SH 17-1-F9; dilution 1:50) was layered over the slide, followed by incubation for 30 minutes at room temperature. The slide was again washed with PBS and mounted, using a solution of 65% (w/v) sucrose in PBS pH 8·0, in which 0·5 µg/ml propidium iodide (Calbiochem) was dissolved. Fluorescence of the kinetoplasts is considered to indicate anti-dsDNA activity. The propidium iodide acts as a counterstain enabling localisation of the kinetoplasts. A KP-560-barrier filter was used to separate propidium iodide fluorescence from that of fluorescein isothiocyanate.

POLYETHYLENE GLYCOL ASSAY

Details of the PEG assay have been described.[21] To 50 µl of a dilution of serum was added 50 µl of a solution of 1·6 mg/ml normal human gammaglobulin, 50 µl of a solution of 0·2 mg/ml dextran sulphate (Pharmacia Fine Chemicals AB, Uppsala, Sweden; lot No 5259), 200 µl of 7% (w/v) polyethylene glycol (PEG, mol. wt 6000; Koch-Light Laboratories, Colnbrook, UK), and 50 µl of a solution of 2 µg/ml [^{3}H]-PM2-DNA (pseudomonal bacteriophage; specific activity $40–60\times10^3$ dpm/µg DNA), forming an incubation volume of 400 µl. All components were dissolved or diluted in PBS.

The incubation was carried out at 37°C for one hour, followed by a two hour incubation at 4°C. The mixture was then centrifuged for 15 minutes at 4000 rpm in a Hettich Rotanta/K centrifuge (2500 *g*). The radioactivity in 200 µl of the supernatant was measured after addition of 10 ml of NE-260$_{SP}$ (New England Nuclear Corp, USA). A '0% binding' control, in which serum was omitted, was included in each experiment. Anti-DNA activity was expressed in U/ml instead of percentage binding, using the definition of Aarden *et al*[20] for the Farr assay—that is, a sample contains one unit of anti-DNA if it binds 30% of 100 ng PM2-DNA under the conditions described above. A significant change in the PEG assay was arbitrarily defined as a change of 50% or more compared with the result obtained two months previously.

FARR ASSAY

The Farr assay was performed as described by Aarden *et al*.[20] To 100 µl of the appropriate serum dilution was added 50 µl of a solution of 16 mg/ml normal human gammaglobulin and 50 µl of a solution of 2 µg/ml [^{3}H]-PM2-DNA. All components were dissolved or diluted in PBS.

After incubation of the mixture for one hour at 37°C 5 ml of a cold 50% saturated ammonium sulphate solution was added. The precipitate was allowed to form for 30 minutes at 4°C, after which it was pelleted by centrifugation for 15 minutes at 3000 *g*. The precipitate was washed twice with 50% saturated ammonium sulphate and, finally, dissolved in 1 ml of Soluene-100 (Packard). After addition of 10 ml scintillation fluid (Instafluor 11, Packard) the radioactivity was measured. The mean (SD) binding of 150 normal control sera was 5 (3)%. Units are defined as stated under the PEG assay.

FARR/PEG RATIO

For group II patients we calculated a Farr/PEG ratio for each serum sample, using the results obtained with either assay in units per millilitre.

STATISTICS

Statistical significance was considered present when test results led to p values $\leq 0{\cdot}05$; both Student's t test and χ^2 with Yates's correction were used. Values are given as mean (SD).

Results

DEMOGRAPHY

Table 1 shows the characteristics of both groups of patients. When both groups were compared group I patients (positive in PEG assay only) were found to be older than group II patients (positive in both Farr and PEG assays) (41·0 *v* 28·4 years; $p<0{\cdot}02$) at the moment SLE was diagnosed; in group I there was one male patient, while group II patients were all female. Overall mortality during the study period was equal for both groups (3% *v* 7%; NS).

DISEASE EXACERBATIONS IN GROUP I

In group I we observed 26 manifestations of SLE activity (eight minor, 18 major exacerbations; see Table 2) in 17 of 35 patients (49%). When an arbitrarily chosen change of 50% or more in anti-dsDNA content measured with the PEG assay over a two month period was considered significant we found a very low sensitivity (5/26=19·2%) and a high specificity (419/465=90·2%) for changes detected with this assay and clinical disease activity. In five cases a clinical manifestation was associated with the development of a positive Farr assay. When these observations were left out of the analysed data we found a complete absence of renal disease, with cerebral involvement occurring in 3/30 patients (10%) and haematological disorders and serositis each occurring in 4/30 (13·3%) of all patients, while their sera showed less than 50% change in anti-dsDNA levels measured by the PEG assay (Table 2). When absolute changes in the anti-dsDNA levels measured by PEG assays (U/ml) were compared we found no difference between changes associated with minor exacerbations (mean (SD) 11 (15·5) U/ml) and those associated with major exacerbations (21 (16·9) U/ml; NS).

Table 1 *Characteristics of patients positive for antibodies to dsDNA in the polyethylene glycol (PEG) assay only (group I) and patients positive in both the PEG and Farr assays (group II)*

	Group I (n=35)	*Group II (n=14)*
Sex ratio (F/M)	34/1	14/0
Mean (SD) age at diagnosis of SLE (years)	41·0 (17·7)	28·4 (14·4)
Mean (SD) time period between diagnosis of SLE and study (months)	37·4 (46·8)	11·2 (16·2)
Mean (SD) period of study (months)	32 (13·3)	14·3 (5·5)
Number (%) who died during the study	1 (3)	1 (7)
Cause of death	Cerebral infarction	Renal insufficiency

DISEASE EXACERBATIONS IN GROUP II

Assay results of all patients in group II were expressed in U/ml, facilitating calculation of a ratio of Farr and PEG assay results. We observed 23 exacerbations (two minor, 21 major) in 13 out of 14 patients (93%); all major exacerbations were associated with high increases of anti-dsDNA in the Farr assay and relatively low increases of low avidity anti-dsDNA (Table 3). The lack of statistical significance for cerebral involvement and haematological exacerbations is probably due to the low numbers. When the calculated Farr/PEG ratio was taken into account an interesting order was found: no change in the ratio for minor exacerbations and increasing values of the Farr/PEG ratio for serosal, haematological, cerebral, and renal exacerbations in that order (Fig. 1). When an (arbitrarily chosen) increase in the Farr/PEG ratio of 100% was taken we found a 95% sensitivity (20 out of 21) and a 90% specificity (61 out of 68) for major exacerbations of SLE.

Table 2 *Exacerbation frequency in group I patients (PEG positive only)*

Disease features	*Total No (%) of exacerbations*	*Number of patients with:*		*Number of patients with:*	
		Δ PEG≥50%	*Positive Farr test*	*Δ PEG<50%*	*Positive Farr test*
Skin/joint involvement	8 (23)	1	0	7	1
Serositis	5 (14)	1	0	4	0
Haematological disorders	7 (20)	2	0	5	1
Cerebral involvement	5 (14)	0	0	5	2
Nephritis	1 (3)	1	1	0	0

Table 3 *Clinical disease exacerbations and changes in anti-dsDNA titres by Farr assay (U/ml), PEG assay (U/ml), and the Farr/PEG ratio in group II patients (PEG and Farr positive)*

Disease features	*Total number (%) of exacerbations*	*ΔFarr titre*		*Δ PEG titre*		*ΔFarr/PEG ratio*	
		Mean (SD) (U/ml)	*%*	*Mean (SD) (U/ml)*	*%*	*Mean*	*%*
Skin/joint involvement	2 (14)	143 (55)	381	38·5 (14·5)	58	1·0	100
Serositis	9 (64)	1092 (860)	1542	182 (248)	325*	7·7	679
Haematological disorders	4 (29)	1983 (1428)	1211	272 (323)	227	9·4	347
Cerebral involvement	2 (14)	1734 (1460)	953	48·0 (17)	118	11·5	1483
Nephritis	6 (43)	842 (340)	264	67·3 (63)	68*	13·6	1053

*p<0·02 for difference with Farr assay.

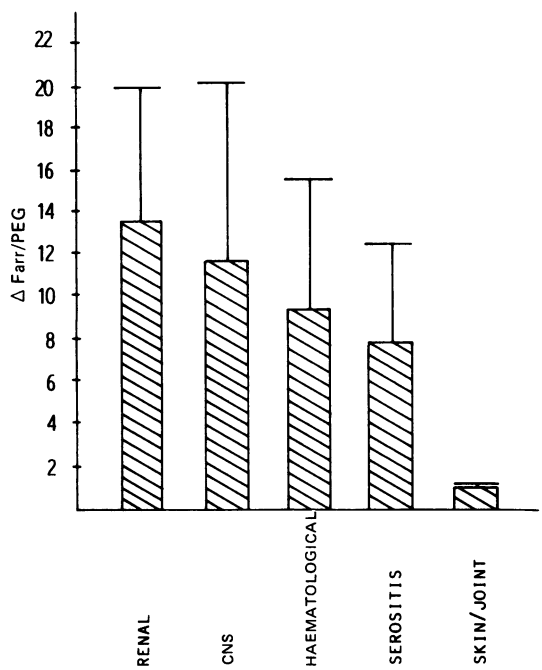

Fig. 1 *Changes in the Farr/polyethylene glycol (PEG) ratio associated with clinical manifestations of systemic lupus erythematosus. CNS=central nervous system.*

Discussion

Autoantibodies to dsDNA occur in most patients with SLE[4–6] and, although rises in anti-dsDNA titres are associated with disease manifestations,[19 22] their definite role in the pathogenesis of SLE remains to be established. The Farr assay for anti-dsDNA measures antibodies with high avidity, whereas the PEG assay also measures low avidity anti-dsDNA.[11 12] The PEG assay has been little used owing to uncertainty about its clinical usefulness. Early studies correlated low avidity anti-DNA with early and more severe disease manifestation in mice[23] and renal involvement in man,[24] but these findings were later contradicted.[25 26]

To determine the clinical significance of the PEG assay we followed up two groups of patients with SLE; one group, whose serum samples showed positive anti-dsDNA only by the PEG assay and a second group whose sera reacted positively in both Farr and PEG assays. Our results show that patients with SLE with anti-dsDNA only detectable by the PEG assay have a low incidence of disease manifestations with a complete absence of renal involvement; all patients were female and were relatively old when diagnosed as having SLE; the prevalence of other disease manifestations was lower than reported elsewhere, except for cerebral involvement, which was comparable with that of other groups of patients.[17 27] Changes in anti-dsDNA levels measured by the PEG assay were not helpful in predicting clinical disease, in contrast with results obtained with the Farr assay[19 22]; specificity on the other hand was high (90·2%). This means that small changes in anti-dsDNA levels detected by the PEG assay (less than 50%) are not associated with disease exacerbations; this is illustrated by the disease course of a patient shown in Fig. 2.

The second part of our study concerned patients with SLE whose sera contained anti-dsDNA detected by both Farr and PEG assays. We found a close correlation between increases in the Farr/PEG ratio and major disease manifestations, which confirms previous findings of the predictive value of rising anti-dsDNA titres in the Farr assay[19 22] and indicates the relatively minor role of low avidity antibodies here. No correlation could be found between absolute changes in Farr assay results and any specific disease manifestation; there was a clear trend, however, towards a higher Farr/PEG ratio associated with renal or cerebral involvement (Fig. 1). Figure 3 provides an illustration of this correlation, depicting the clinical course and anti-

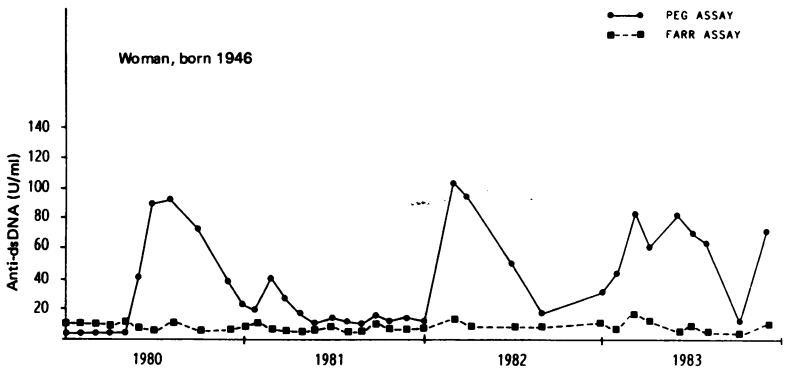

Fig. 2 *Course of serum anti-dsDNA levels in a young woman without clinical disease manifestations during follow up.*

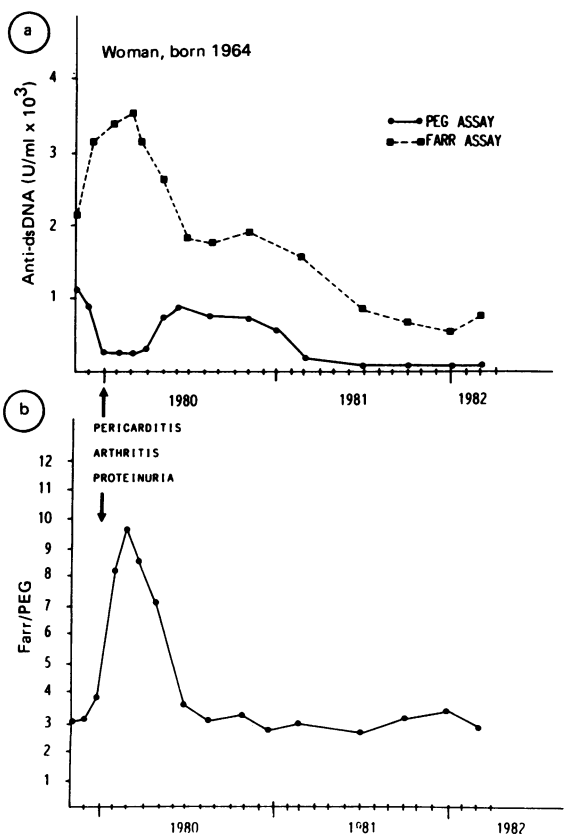

Fig. 3 *(a) Course of anti-dsDNA levels in the PEG and Farr assays and (b) course of the Farr/PEG ratio in a young woman experiencing pericarditis and proteinuria in January 1980, but remaining free of symptoms thereafter on treatment with corticosteroids and azathioprine.*

dsDNA level of a female patient. The raised Farr/PEG ratio for both renal and cerebral disease exacerbations in this study seemingly contrasts with previous findings,[14–16] which showed a Farr/PEG ratio above five to be specific for renal disease. In the current study, however, we looked at changes in the Farr/PEG ratio rather than at absolute values and therefore our findings of a rising ratio can be used as additional information for predicting SLE exacerbations next to the initial value of the Farr/PEG ratio.

In conclusion, we showed that a subset of patients with SLE exists, which consists of older women with only low avidity anti-dsDNA, who experience less exacerbations and do not develop renal disease. In these patients the PEG assay is not a sensitive marker for disease activity; about 17% of these patients develop high avidity anti-dsDNA detectable by the Farr assay, when experiencing an exacerbation. Furthermore, we showed that in patients with SLE positive in both Farr and PEG assays the PEG assay is of little prognostic value, whereas the Farr assay can be used to predict major exacerbations. A 100% increase in the Farr/PEG ratio over a two month period heralds a major exacerbation of SLE, with renal and cerebral disease associated with the highest increases.

References

1 Koffler D, Agnello V, Thoburn R, Kunkel H G. SLE-prototype of immune complex nephritis in man. *J Exp Med* 1971; **134:** 169–79.
2 Davis J S, Godfrey S M, Winfield J B. Direct evidence for circulating DNA-antiDNA complexes in SLE. *Arthritis Rheum* 1978; **21:** 17–22.
3 Bruneau Ch, Beneviste J. Circulating DNA-antiDNA complexes in SLE. *J Clin Invest* 1979; **64:** 191–8.
4 Stollar D B. Nucleic acid antigens. In: Sela M, ed. *The antigens.* Vol 1. New York: Academic Press, 1973: 1–85.
5 Tan E M, Schur P M, Carr R I, Kunkel H G. DNA and antibodies to DNA in serum of patients with SLE. *J Clin Invest* 1966; **45:** 1732–40.

6 Koffler D, Schur P M, Kunkel H G. Immunological studies concerning the nephritis of SLE. *J Exp Med* 1967; **126:** 607–23.
7 Gershwin M E, Steinberg A D. Qualitative characteristics of anti-DNA antibodies in lupus nephritis. *Arthritis Rheum* 1974; **17:** 947–54.
8 Griffiths I D, Holian J, Maini R N, Glass D N, Scott J T. Anti-DNA antibodies: quantitative and qualitative aspects of measurement using ^{125}I labelled DNA. *Ann Rheum Dis* 1977; **36** (suppl): 122–8.
9 Tron F, Bach J F. Relationship between antibodies to native DNA and glomerulonephritis in SLE. *Clin Exp Immunol* 1977; **28:** 426–32.
10 Sontheimer R D, Gilliam J N. DNA antibody class, subclass and complement fixation in SLE with and without nephritis. *Clin Immunol Immunopathol* 1978; **10:** 495–7.
11 Smeenk R J T, van der Lely G, Swaak A J G, Groenwold J, Aarden L. Specificity in SLE antibodies to dsDNA measured with the PEG-assay. *Arthritis Rheum* 1982; **25:** 631–8.
12 Riley R L, McGrath H, Taylor R P. Detection of low avidity antibodies to dsDNA in SLE. *Arthritis Rheum* 1979; **22:** 219–25.
13 McGrath H, Biundo J. A longitudinal study of high and low avidity antibodies to dsDNA in SLE. *Arthritis Rheum* 1985; **28:** 425–30.
14 Swaak A J G, Smeenk R. Clinical differences between SLE patients in relation to the avidity of their anti-dsDNA. In: Peeters H, ed. *Protides of the biological fluids*. Oxford: Pergamon, 1985: 317–20.
15 Smeenk R. Low avidity antibodies to dsDNA. Amsterdam University. 1982. (PhD thesis.)
16 Smeenk R, van Rooyen A, Swaak A J G. Dissociation studies of DNA anti-dsDNA complexes in relation to their avidity. *J Immunol Methods* 1988; **109:** 27–35.
17 Tan E M, Cohen A S, Fries J F, *et al*. The 1982 revised criteria for the classification of SLE. *Arthritis Rheum* 1982; **25:** 1271–7.
18 Lightfoot R W, Hughes G R V. Significance of persisting serologic abnormalities in SLE. *Arthritis Rheum* 1976; **19:** 837–43.
19 Swaak A J G, Groenwold J, Aarden L A, Statius v Eps L W, Feltkamp T E W. Prognostic value of anti-dsDNA in SLE. *Ann Rheum Dis* 1982; **41:** 388–95.
20 Aarden L, Smeenk R. Measurements of antibodies specific for DNA. In: Lefkovitz I, Pernis B, eds. *Immunological methods*. Vol II. New York: Academic Press, 1981: 75–82.
21 Maini R, Holborow E. Detection and measurement of circulating anti-DNA antibodies. *Ann Rheum Dis* 1977; **36** (suppl): 67–71.
22 Swaak A J G, Aarden L A, Statius v Eps L W, Feltkamp T E W. Anti-dsDNA and complement profiles as prognostic guides in SLE. *Arthritis Rheum* 1979; **22:** 226–35.
23 Steward M W, Katz F E, West N J. The role of low affinity antibody in immune complex disease. *Clin Exp Immunol* 1975; **21:** 121–30.
24 Steward M W, Glass D N, Maini R N, Scott J T. Role of low-avidity antibody to DNA in human and murine lupus syndrome. *J Rheumatol* 1974; **1:** 75–6.
25 Winfield J B, Faiferman I, Koffler D. Avidity of anti DNA in serum and glomerular eluates from patients with SLE. *J Clin Invest* 1977; **59:** 90–6.
26 Leon S A, Green A, Ehrlich G E, Poland M, Shapiro B. Avidity of antibodies in SLE: relation to severity of renal involvement. *Arthritis Rheum* 1977; **20:** 23–9.
27 Fries J, Holman H. *Systemic lupus erythematosus, a clinical analysis*. Philadelphia: Saunders, 1975.