

## Hypercatabolism of C3 and C4 in active and inactive systemic lupus erythematosus

J A CHARLESWORTH, P W PEAKE, J GOLDING, J D MACKIE, B A PUSSELL, V TIMMERMANS, AND D WAKEFIELD

*From the Division of Medicine, Prince Henry Hospital, Little Bay, New South Wales, Australia*

**SUMMARY** The metabolism of the complement proteins C3 and C4 was studied in patients with active and inactive systemic lupus erythematosus (SLE) using highly purified, functionally active preparations. Nine patients with active and eight with inactive SLE were examined and 11 control subjects. There was a significant difference in the level of double stranded DNA antibodies, immune complexes, and serum C4 between the patients with active and inactive disease. Seven of 16 patients had detectable C4 null alleles and four had low serum concentrations of complement inhibitors. Each subject received approximately 370 kBq [<sup>125</sup>I]C4 and 93 kBq [<sup>131</sup>I]C3. Both patient groups showed significant C4 hypercatabolism compared with control subjects, but there was no difference between patients with active and inactive disease. The fractional catabolic rate (FCR) of C4 was comparable in subjects with and without detectable C4 null alleles. C4 production rate was significantly lower in patients with active SLE than in control subjects. There was significant C3 hypercatabolism for both patient groups, but C3 production was normal. An inverse correlation was observed between serum concentration and FCR. There was a highly significant correlation between C4 FCR and C3 FCR for control subjects + patients with inactive disease but not for those with active SLE combined with either controls or the inactive group. We conclude that complement hypercatabolism occurs in SLE irrespective of disease activity and that accelerated turnover does not account completely for the low C4 concentration observed in patients with active disease. This low concentration also results from impaired plasma production, which could reflect a high incidence of C4 null alleles or (inhibitory) factors associated with pathological complement activation, or both. Low C4 production could affect generation of the C3 converting enzyme C4b, 2a and thus influence proceeding complement activation.

**Key words:** complement metabolism, immune complexes, null alleles.

Systemic lupus erythematosus (SLE) is associated with well documented abnormalities of the complement system.<sup>1</sup> Most patients show activation of the classical pathway and there is an increased prevalence of inherited defects of individual components. These inherited phenomena predominantly affect proteins of the classical pathway and include a high incidence of heterozygous and homozygous null alleles at one or both C4 genetic loci (C4A and C4B).<sup>2</sup> The coexistence of genetic and acquired

abnormalities complicates the interpretation of serum complement data. Although it is recognised that complement activation has an important role in the mediation of immunological injury, the significance of inherited defects remains uncertain. It has been suggested that they influence susceptibility to disease or modify the behaviour of immunoreactive proteins (such as immune complexes) *in vivo*, or both. Both of these proposals remain unproved. C4 null alleles may also limit formation of the C3 converting enzyme C4b, 2a and thus affect proceeding complement activation.

To clarify the basis for changes in serum complement levels we studied the metabolism of purified C4 and C3 in 17 patients with SLE, seven of whom

Accepted for publication 27 June 1988.

Correspondence to Professor J A Charlesworth, Department of Medicine, Clinical Sciences Building, Prince Henry Hospital, Little Bay, NSW 2036, Australia.

had one or more detectable C4 null alleles. Nine patients had both clinical and laboratory evidence of lupus activity. While there have been several reports of C3 turnover in this disease, there have been no detailed studies of C4 metabolism, even though this component may have an important role in pathogenesis. Ruddy and coworkers reported results in two patients,<sup>3</sup> although this work preceded the resolution of difficulties with purification and definition of C4A and C4B. The specific aim of the project was to examine the individual metabolic factors that contribute to abnormalities in plasma C3 and C4 concentrations and to determine whether C4 or C3 hypercatabolism, or both, could distinguish between patients with and without active lupus. The simultaneous investigation of both proteins also provided the opportunity to examine the effect of C4 turnover on downstream complement activation.

### Patients and methods

#### SUBJECTS

Seventeen patients with SLE and 11 healthy medical staff were studied. All of the SLE group satisfied the diagnostic criteria of the American Rheumatism Association. Nine of this group had active disease and were in hospital for diagnosis (two patients) or treatment, or both. Five patients had active involvement of two or more systems (including glomerulonephritis), three had predominant skin disease, and one patient had cerebral lupus. The other eight patients had clinically inactive disease, and five of these were investigated as outpatients; the remaining three were in hospital for conditions unrelated to lupus activity—for example, two patients for the treatment of hypertension. These patients showed a distribution of abnormality similar to those with active disease. The diagnosis of lupus glomerulonephritis had been supported in each case by renal biopsy. At the time of investigation only three patients were receiving significant doses of immunosuppressive drugs—that is, prednisolone >10 mg/day with or without a cytotoxic agent. None was undergoing plasma exchange treatment. The patients and control subjects were closely matched for age, but not for sex.

#### COMPLEMENT AND IMMUNE COMPLEX STUDIES

Serum samples were separated by centrifugation at 4°C and stored at -20°C until analysed. The serum concentration of C3, C4, and, in selected patients, C2 and factor B was measured by radial immunodiffusion<sup>4</sup> using monospecific antisera (Dakopatts), and total haemolytic activity was tested by a standard tube assay.<sup>5</sup> The complement

inhibitors C1-INH, C4-binding protein (bp), factors I and H were also measured by radial immunodiffusion.

The C4 allotype of each patient was examined on fresh plasma (in edetic acid) using the method described by Mauff and colleagues.<sup>6</sup> Briefly, neuraminidase treated plasma was subjected to agarose electrophoresis at 4°C before overlay with monospecific polyclonal anti-C4 (Silenus). A haemolytic overlay technique was used to define further the A and B loci, and two dimensional immunoelectrophoresis was performed to identify heterozygous null alleles at either locus.<sup>7</sup> Where possible, family studies were undertaken to resolve the isotypic pattern (four subjects) further.

Immune complexes were tested on the first day of the turnover study by a fluid phase C1q binding assay.<sup>8</sup>

#### PREPARATION OF COMPLEMENT COMPONENTS

Complement components were prepared from fresh plasma (in edetic acid) donated by volunteers who were negative for hepatitis B surface antigen and human immunodeficiency virus antibody. Human C4A3,B1 was prepared by the method of Bolotin and coworkers,<sup>9</sup> with modifications<sup>10</sup> and C3 by the method of Harrison and Lachmann.<sup>5</sup> Proteins were tested for immunochemical purity and functional activity by standard techniques.<sup>5,11</sup> These showed that preparations were essentially free from contaminants and that both C3 and C4 retained haemolytic activity comparable with that observed in an equal concentration of fresh normal serum.<sup>10</sup> C4 preparations showed deterioration during storage and were discarded once haemolytic activity fell below 70% of normal. Purified C4 was labelled with <sup>125</sup>I by the lactoperoxidase method<sup>12</sup> and C3 with <sup>131</sup>I by a modified chloramine T technique.<sup>13</sup> Radioiodination caused no significant change in haemolytic activity, and autoradiography of polyacrylamide gel electrophoresis in sodium dodecyl sulphate showed that there had been no significant production of radiolabelled contaminants or breakdown products. Proteins were sterilised by Millipore filtration, cultured for aerobic and anaerobic organisms, and pyrogen tested in rabbits before injection.

#### TURNOVER PROTOCOL

The turnover protocol has been described previously<sup>10,14</sup> and was approved by the hospital ethics committee. Informed consent was obtained from each subject. Briefly, one or more controls were studied simultaneously with groups of one, two, or three patients and each received approximately 370 kBq [<sup>125</sup>I]C4 and 93 kBq [<sup>131</sup>I]C3. (One

patient was studied with only [ $^{125}\text{I}$ ]C4 and one with only [ $^{131}\text{I}$ ]C3.) Thyroidal uptake of radioactivity was blocked by prior administration of oral potassium iodide for three days. Blood samples (in edetic acid) and 24 hour samples of urine were collected for the next 120–144 hours, or until there was less than 8% injected radioactivity remaining in the plasma. The serum concentration of C3 and C4 was measured at the beginning and end of each turnover to confirm a steady state.

#### ANALYSIS OF METABOLIC DATA

Metabolic data were analysed by three methods: (a) metabolic clearance<sup>15</sup>; (b) exponential analysis<sup>16</sup>; and (c) the integrated rate equations method.<sup>17</sup> The fractional catabolic rate (FCR) was calculated by each method except where urine collections were incomplete or there was significant iodide retention (such as in patients with renal failure). Plasma production rate (mg/kg/h) was derived from the formula:

$$\frac{\text{serum concentration (mg/ml)} \times \text{FCR (\%/h)} \times \text{plasma volume (ml)}}{\text{weight (kg)}}$$

The extravascular/intravascular distribution ratio was calculated by exponential analysis and the integrated rate equations method. This latter technique was also used to calculate the rate constant for extravascular catabolism. The final value for the FCR was calculated as the mean of results obtained by the three methods of analysis. Generally there was close agreement between the three methods, but where one value differed by more than 20% from the others only the other two data were used to calculate the mean FCR.

Differences between values for serum or metabolic parameters were examined by an unpaired Student's *t* test. Data for C4 were also tested by a Mann-Whitney U test. A non-parametric analysis was applied to C4 data because of the effect of null alleles on the distribution of serum C4 concentration. Correlations between individual parameters

were tested by linear regression analysis with calculation of the coefficient 'r'.

## Results

### IMMUNOCHEMICAL STUDIES

Tables 1 and 2 show the results of immunochemical studies. There were significant differences in the level of antibodies to double stranded (ds) DNA, immune complexes, and serum C4 between patients with active and inactive disease ( $p < 0.01$ ,  $p < 0.02$ , and  $p < 0.02$  respectively). The titre of antinuclear antibodies and the serum C3 concentration were comparable for the two groups ( $p > 0.05$ ). The serum concentration of all four complement inhibitor proteins was normal in 13 of the 17 patients with SLE. The remaining four patients (three with active disease) had significant reduction in three or more inhibitors, including factor I and C4bp in each case. The C4 FCR in these four subjects was 2.0, 2.76, 3.83, and 2.61 %/h, while values for C3 FCR were 2.80, 5.62, and 2.01 %/h. (The first patient did not have a C3 metabolic study.)

C4 null alleles were detected in four out of eight of the active group and three of these patients had two or more detectable null alleles. One patient could not be typed because of an extremely low serum C4 concentration and the unavailability of family members. Three of the inactive group had

Table 2 Complement inhibitors in patients with systemic lupus erythematosus (SLE). Values are given as mean (SD) of percentage pooled normal serum

Subjects	CI-INH	C4-bp*	Factor I	Factor H
Active SLE (n=9)	107 (26)	75 (24)	90 (48)	106 (31)
Inactive SLE (n=8)	107 (24)	106 (40)	122 (46)	107 (29)
Controls (n=50)	100 (16)	95 (35)	106 (16)	100 (15)

No significant differences were observed between groups.

\*bp=binding protein.

Table 1 Immunochemical data for patients with systemic lupus erythematosus (SLE). Values are given as mean (SD)

Subjects	Anti-dsDNA (%)	Immune complexes (%)	C3 (g/l)	C4 (g/l)	C4 Null (% subjects)
Active SLE (n=9)	54 (30)*	58 (29)*	0.65 (0.43)**	0.12 (0.09)*	50†
Inactive SLE (n=8)	24 (7)*	20 (23)	0.81 (0.34)	0.27 (0.16)	38
Normal	14 (7)‡	9 (4)‡	0.96 (0.15)‡	0.32 (0.07)‡	36§

Significance of difference compared with control subjects: \* $p < 0.001$ ; \*\* $p < 0.05$ .

†One patient not typed.

‡Values derived from healthy subjects (n=50).

§Refers to control subjects used for metabolic studies (n=11).

similar defects, one with two null alleles. A single null allele was found in four out of 11 control subjects.

METABOLIC STUDIES

C4 metabolism

Figures 1-3 and Table 3 show the C4 metabolic data for patients and controls. There was close agreement between Student's *t* test and the Mann-Whitney U test for all data. Fractional catabolism was significantly greater in both active and inactive patients than in control subjects ( $p < 0.001$  for each

pair), but there was no difference between the patient groups. Plasma production rate was significantly reduced in the patients with active disease compared with controls ( $p = 0.03$ ), but there was no difference between the inactive group and control subjects or between the two groups of patients. The extravascular/intravascular distribution ratio was significantly raised in the inactive group compared with control subjects ( $p < 0.02$ ); two patients with active SLE had a gross increase of this ratio (1.69

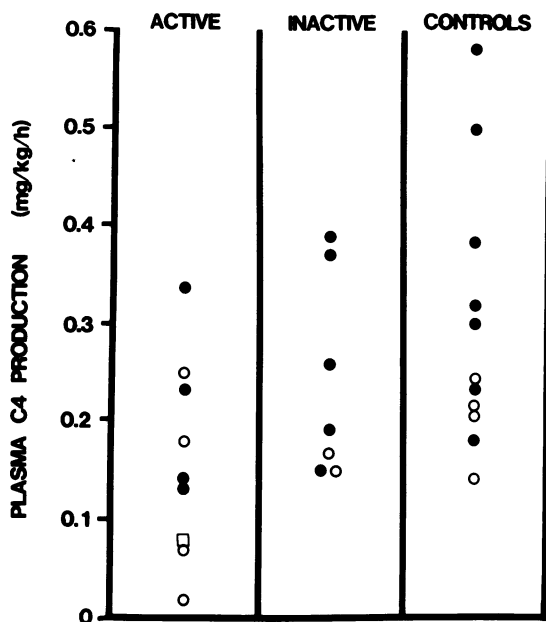


Fig. 1 Plasma C4 production rate for control subjects and patients with active and inactive systemic lupus erythematosus (SLE). ●=null alleles not detected; ○=detectable C4 null alleles; □=patient with undefined C4 allotype. Plasma C4 production in patients with active SLE was lower than in control subjects ( $p = 0.03$ ).

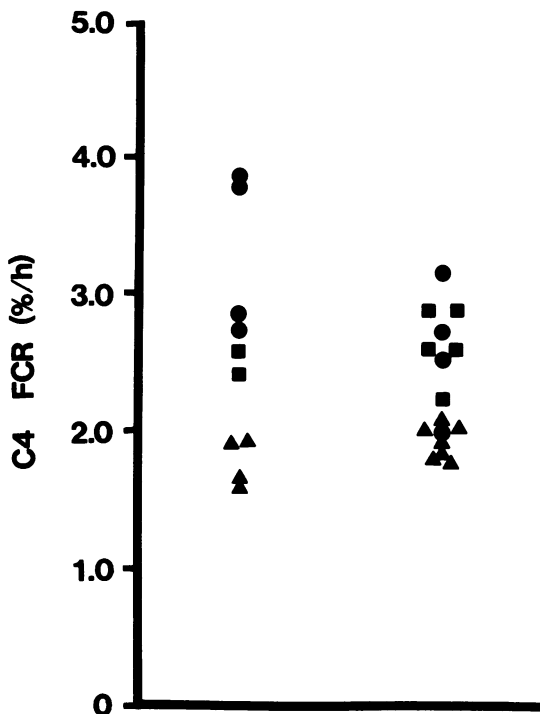


Fig. 2 A comparison of C4 fractional catabolic rate (FCR) in subjects with (left column) and without (right column) detectable C4 null alleles. ▲=control subjects; ●=active SLE; ■=inactive SLE. No significant difference was observed.

Table 3 C4 metabolic data in patients and control subjects. Values are given as mean (SD)

Subjects	$t_{1/2}$ (h)	FCR† (%/h)	Plasma C4 production (mg/kg/h)	EV/IV† distribution
Active SLE (n=9)	41 (8)*	3.19 (0.91)**	0.16 (0.10)***	0.92 (1.04)
Inactive SLE (n=7)	41 (5)*	2.56 (0.21)**	0.29 (0.17)	0.73 (0.27)**
Controls (n=11)	54 (8)	1.87 (0.17)	0.30 (0.14)	0.45 (0.13)

Significance of difference compared with control subjects: \* $p < 0.01$ ; \*\* $p < 0.001$ ; \*\*\* $p < 0.05$ .

†FCR=fractional catabolic rate; EV/IV=extravascular/intravascular.

and 3.46). Most patients and control subjects showed evidence of extravascular catabolism.

Figure 2 compares the C4 FCR of patients and controls with and without detectable C4 null alleles. There was no significant difference between the two groups.

### C3 metabolism

Table 4 and Fig. 3 show the C3 metabolic data. There was significant C3 hypercatabolism in both patient groups compared with controls (active:  $p < 0.01$ ; inactive:  $p < 0.01$ ), but no difference was observed between the two patient groups. Plasma C3 production was normal both in patients with

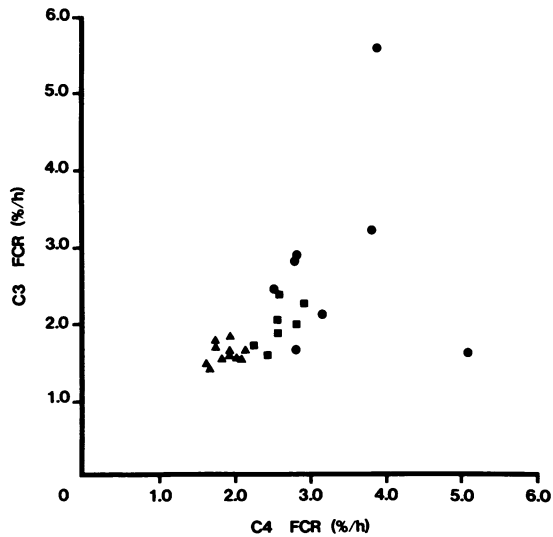


Fig. 3 C4 fractional catabolic rate (FCR) v C3 FCR in control subjects (▲) and patients with active (●) and inactive SLE (■). There was a significant correlation for combined patient and control data ( $r=0.52$ ;  $p < 0.01$ ) and for the inactive group + control subjects ( $r=0.78$ ;  $p < 0.001$ ). No correlation was observed when data for active patients was combined with that from control subjects or inactive patients.

active and inactive disease, though grossly reduced in one of the active group (0.14 mg/kg/h). The group with active lupus showed a significant increase in extravascular/intravascular distribution ( $p < 0.05$ ).

### INTERRELATIONS BETWEEN INDIVIDUAL METABOLIC PARAMETERS

There was a significant inverse correlation between the FCR and the serum concentration of C4 and C3 (C4:  $r = -0.513$ ,  $p < 0.05$ ; C3:  $r = -0.69$ ,  $p < 0.01$ ). No correlation was observed between the FCR and the concentration of individual complement inhibitors, and there was no relation between extravascular/intravascular distribution and other metabolic parameters. Regression analysis was not applied to the derived value for plasma production rate.

There was a significant correlation between C4 FCR and C3 FCR using combined data from control subjects and patients with SLE ( $n=26$ ,  $r=0.52$ ,  $p < 0.01$ ) (see Fig. 3). There was also a highly significant correlation between these two parameters in control subjects + patients with inactive disease ( $n=18$ ,  $r=0.78$ ,  $p < 0.001$ ). Moreover, these two groups showed a well defined ratio of C4 FCR:C3 FCR (range 0.76–0.99 and 0.67–0.88 respectively). In contrast, no correlation was observed when data for patients with active disease were combined with those of control subjects or inactive patients. The active group also showed wide variation in the ratio C4 FCR:C3 FCR (0.33–1.46) with six patients having a ratio below or above that observed in either of the other two groups. None of these subjects had homozygous C2 deficiency. Each of the three subjects with a low ratio had reduced C4 production (0.02, 0.08, and 0.13 mg/kg/h) and evidence of gross C4 hypercatabolism (2.80, 3.71, and 5.07 %/h). The C3 FCR was normal in each subject.

### Discussion

These data demonstrate significant hypercatabolism of the third and fourth components of complement

Table 4 C3 metabolic data in patients and control subjects. Values are given as mean (SD)

Subjects	$t_{1/2}$ (h)	FCR <sup>†</sup> (%/h)	Plasma C3 production (mg/kg/h)	EV/IV <sup>†</sup> distribution
Active SLE (n=8)	40 (11)*	2.78 (1.28)*	0.61 (0.25)	0.80 (0.35)**
Inactive SLE (n=8)	46 (6)*	2.02 (0.31)*	0.68 (0.29)	0.62 (0.34)
Controls (n=11)	56 (8)	1.61 (0.14)	0.55 (0.12)	0.39 (0.18)

Significance of difference compared with control subjects: \* $p < 0.01$ ; \*\* $p < 0.05$ .

<sup>†</sup>FCR=fractional catabolic rate; EV/IV=extravascular/intravascular.

in patients with both active and inactive SLE. There was no difference in the fractional catabolism of C3 and C4 between these two groups—that is,  $p > 0.05$ —even though the patients with inactive SLE had a significantly lower titre of dsDNA antibodies and a normal serum C4 concentration. Furthermore, immune complexes were not found in six out of eight of this group, whereas all of the patients with active disease had detectable complexes. Previous work shows that the C1q binding assay provides a high yield of detection in patients with SLE.<sup>18</sup> The data did not clarify whether the C3 and C4 hypercatabolism observed in the inactive group resulted from the persistence of pathological activators—that is, not identified by the C1q binding assay—or whether there were additional factors, such as relative failure of the inhibitor systems that control C3 and C4 turnover. Previous studies have shown reduction in the concentration of complement control proteins in patients with SLE,<sup>19</sup> and four of our subjects showed low concentrations of the inhibitors controlling C3 and C4 activation. Our study did not resolve the problem of whether these abnormalities contributed significantly to the accelerated turnover of C4 in these (and other) patients. Certainly, no outstanding difference in C4 FCR was observed between patients with and without abnormalities of inhibitor concentration.

It is unlikely that the C3 and C4 hypercatabolism observed in either patient group resulted from preparative denaturation. This phenomenon has hindered previous studies of complement turnover<sup>3 20</sup> and may invalidate the analysis of metabolic data. In this study the turnover characteristics of patients were compared directly with those of control subjects, and both proteins showed functional activity similar to that of fresh normal serum. Three patients had C4 FCR greater than 3.5 %/h, and two of these subjects had detectable C4 null alleles (the third patient could not be typed). Although this might suggest that the presence of null alleles is associated with higher rates of C4 catabolism, a comparison of all subjects with and without these defects showed no significant difference between the two groups (see Fig. 2). A study of the turnover of purified isotypes under normal conditions and after complement activation would be required to define metabolic differences between the products of the C4A and C4B loci.

The comparable level of C4 FCR in patients with active and inactive SLE suggests that other metabolic factors were responsible for the difference in C4 concentration between these two groups. Two patients with active lupus showed a gross increase of extravascular/intravascular distribution—that is, threefold greater than normal—and this could

account for the low serum C4 concentration observed in these two subjects. There was also significantly lower plasma production in patients with active disease than in control subjects. This could be explained, at least partially, by an increased incidence of C4 null alleles, which has been shown to influence serum C4 concentration.<sup>21</sup> Two observations suggest that other factors—that is, apart from C4A and C4B—influence the C4 production rate in SLE. Firstly, we observed little change in fractional catabolism during disease remission despite a significant rise in serum C4 concentration; the variation in C4 production, implicated in such a rise, could not be explained entirely in terms of C4A and C4B integrity. Secondly, other groups with C4 null alleles (including normal subjects) show significantly higher rates of production than patients with active SLE. For example, we have found normal or increased production in patients with rheumatoid arthritis despite a high frequency of C4 null alleles (unpublished observations). The relative failure of C4 production in patients with active lupus may reflect impairment of the acute phase response. Previous work has alluded to this impaired reactivity,<sup>22</sup> and such an abnormality would have direct relevance to complement proteins, many of which show acute phase behaviour.<sup>23</sup> Although it must be stressed that derived values for plasma production do not provide a direct measurement of protein synthesis, they quantify the release of protein into the plasma compartment where the functional activity of complement components is relevant to both the mediation and control of immunological inflammation.

A close relation was observed between the fractional catabolism of C4 and C3 in control subjects and patients with inactive SLE (see Fig. 3). This relation was less predictable in patients with active disease. Our data do not define the basis for this variation of C3 turnover in the active group. It is possible that low levels of C4 production—irrespective of molecular basis—restricted downstream complement activation by limiting the formation of the C3 converting enzyme C4b,2a. This is supported by our finding in three patients, where C3 catabolism was normal despite gross acceleration in C4 turnover; each of these subjects had subnormal C4 production. Alternatively, as mentioned above, variation in C3/C4 inhibition could influence interaction between these two components. Further experiments are required to define the factors that control proceeding complement activation in this disease.

physicians who permitted us to study their patients. The figures were reproduced by the Department of Medical Illustration, University of New South Wales and teaching hospitals.

#### References

- 1 Schur P H. Complement components in rheumatic diseases. In: Gupta S, Talal N, eds. *Immunology of rheumatic diseases*. London: Plenum Press, 1985: 563–80.
- 2 Rynes R I. Inherited complement deficiency status and SLE. *Clin Rheum Dis* 1982; **8**: 29–47.
- 3 Ruddy S, Carpenter C B, Chin K W, et al. Human complement metabolism: an analysis of 144 studies. *Medicine (Baltimore)* 1975; **54**: 165–78.
- 4 Mancini G, Carbonara A L, Heremans J F. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 1965; **2**: 235–54.
- 5 Harrison R A, Lachmann P J. Complement technology. In: Weir D M, Herzenberg L A, Blackwell C, eds. *Handbook of experimental immunology*. Vol 1. Oxford: Blackwell, 1986: 39.1–49.
- 6 Mauff G, Alper C A, Awdeh Z, et al. Statement on the nomenclature of human C4 allotypes. *Immunobiology* 1983; **164**: 184–91.
- 7 Awdeh Z L, Raum D, Alper C A. Genetic polymorphism of human complement C4 and detection of heterozygotes. *Nature* 1979; **282**: 205–7.
- 8 Zubler R H, Lange G, Lambert P H, Miescher P A. Detection of immune complexes in unheated sera by a modified <sup>125</sup>I.C1q binding test. *J Immunol* 1976; **116**: 232–5.
- 9 Bolotin C, Morris S, Tack B, Prah J. Purification and structural analysis of the fourth component of human complement. *Biochemistry* 1977; **16**: 2008–15.
- 10 Charlesworth J A, Timmermans V, Golding J, et al. The complement system in type 1 (insulin-dependent) diabetes. *Diabetologia* 1987; **30**: 372–9.
- 11 Gaither T A, Alling D W, Frank M M. A new one-step method for the functional assay of the fourth component (C4) of human and guinea pig complement. *J Immunol* 1974; **113**: 574–83.
- 12 Marchalonis J J. An enzymic method for trace iodination of immunoglobulins and other proteins. *Biochem J* 1969; **113**: 299–305.
- 13 McConahey P J, Dixon F J. A method of trace iodination of proteins for immunologic studies. *Int Arch Allergy Appl Immunol* 1966; **29**: 185–9.
- 14 Charlesworth J A, Gwyn Williams D, Sherington E, Peters D K. Metabolism of the third component of complement (C3) in normal human subjects. *Clin Sci* 1974; **46**: 223–9.
- 15 Berson S A, Yalow R S. Distribution and metabolism of <sup>131</sup>I-labelled proteins in man. *Fed Proc* 1957; **16**: 13–18s.
- 16 Matthews C M E. The theory of tracer experiments with <sup>131</sup>I-labelled plasma proteins. *Phys Med Biol* 1957; **2**: 36–53.
- 17 Nosslin B. Analysis of disappearance time curves after single injection of labelled proteins. In: *Protein turnover*. Amsterdam: Elsevier/North Holland, 1972: 113–20. (Ciba Foundation Symposium, No 9.)
- 18 Lambert P H, Dixon F J, Zubler R H, et al. A WHO collaborative study for the evaluation of eighteen methods for detecting immune complexes in serum. *J Clin Lab Immunol* 1978; **1**: 1–15.
- 19 Whaley K, Schur P H, Ruddy S. Relative importance of C3b inactivator and  $\beta$ 1H globulin in the modulation of the properdin amplification loop in systemic lupus erythematosus. *Clin Exp Immunol* 1979; **36**: 408–14.
- 20 Kaplan R A, Curd J G, Deheer D H, et al. Metabolism of C4 and factor B in rheumatoid arthritis—relation to rheumatoid factor. *Arthritis Rheum* 1980; **23**: 911–20.
- 21 Uko G, Christiansen F T, Dawkins R L, McCann V J. Reference ranges for serum C4 concentrations in subjects with and without C4 null alleles. *J Clin Pathol* 1986; **39**: 573–6.
- 22 Pereira Da Silva J A, Elkon K B, Hughes G R V, Dyck R F, Pepys M B. C-reactive protein levels in systemic lupus erythematosus: A classification criterion? *Arthritis Rheum* 1980; **23**: 770–1.
- 23 Schutte M, DiCamelli R, Murphy P, Sadove M, Gewurz H. Effects of anaesthesia, surgery and inflammation upon host defence mechanisms. 1. Effects upon the complement system. *Int Arch Allergy Appl Immunol* 1975; **48**: 706–20.