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THE COMPLEMENT SYSTEM IN HEREDITARY ANGIONEUROTIC OEDEMA—A NEW PERSPECTIVE

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SUMMARY

Fifteen members from a new family in which hereditary angioneurotic oedema exists have been studied with respect to serum concentrations of some components of the complement system. C'_{1q} levels were found to be normal in all family members studied. C'_1 haemolytic activity, previously reported to be decreased during attacks of oedema in some patients with this disease, was always within normal limits in our patients. Haemolytic C'_4 activity was found to be subnormal in diseased individuals both during and between acute episodes, an abnormality which is useful as a diagnostic tool. Serum C'_2 activity, previously used as a diagnostic indicator, was found to be normal in some affected individuals between attacks and only slightly decreased during attacks if assayed by a method which provides supplemental human C'_4. Immune adherence (I-A) titres were consistently very low in patients with hereditary angioneurotic oedema during clinically evident episodes of oedema or abdominal pain. This finding along with I-A studies in C'_2 deficient sera suggests that C'_4 is the limiting factor in development of the immune adherence reaction in human serum. In addition, I-A provides a simple effective screening test for hereditary angioneurotic oedema during episodes of oedema or abdominal pain.

INTRODUCTION

The potentially fatal outcome of an episode of laryngeal oedema in patients with hereditary angioneurotic oedema (HAO) makes it imperative to screen carefully all members of a family in which the disorder is suspected or is known to exist. The disease is characterized by apparently unprovoked attacks of relatively painless, non-pruritic, non-pitting oedema of any area of the body. If mucosae are involved, these may produce symptoms of intestinal obstruction or respiratory distress. The former may lead to unnecessary surgical procedures

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and the latter often results in fatal airway obstruction. The original serological abnormality which distinguished this disease entity from other forms of angioneurotic oedema was the deficiency, discovered by Donaldson & Evans (1963), of the normally occurring inhibitor of C'_1-esterase. It has been thought that because of the absence of this inhibitor, measurable esterase activity, which is not seen normally, appears in the serum. The lack of the C'_1-esterase inhibitor and the resulting free esterase activity can be detected directly with specially sensitive methods (Levy & Lepow, 1959; Donaldson & Evans, 1963). Since two of the components of the complement system (C'_4 and C'_2) are initially activated by C'_1-esterase and then rapidly lose activity in the fluid phase *in vitro* (Lepow *et al.*, 1956b; Müller-Eberhard & Lepow, 1965; Stroud, Austen & Mayer, 1965) measurement of one of these components might be useful in diagnosing this disease. Donaldson & Rosen (1964), Austen & Beer (1964), Austen & Sheffer (1965) and Ruddy & Austen (1967) have claimed that these methods provide a useful approach for detecting the disorder.

In this report we will present results of a study of several members of a family with HAO showing that haemolytic C'_1 and its subcomponent, C'_{1q}, are within the normal range in symptomatic and asymptomatic family members. This suggests that, in this disease, C'_{1s} may be converted to C'_1 esterase without participation of C'_{1q}. We found that diminished C'_4 haemolytic activity is a sensitive, reliable indicator for detection of affected individuals and that this relative lack of C'_4 can greatly influence the C'_2 titration. Immune adherence (I-A) titres were extremely low in affected individuals during attacks of oedema and this was related to the lack of haemolytic C'_4 rather than C'_2.

EXPLANATION OF ABBREVIATIONS

EAC'_{1a}: EA which has active C'_1 on the cell–antibody complex and is used to supply excess C'_1 in the C'_4 assay.

$EAC'_{1a,4}$: EA which has active C'_1 and C'_4 on the cell–antibody complex and is used to supply excess C'_1 and C'_4 in the C'_2 assay. These are referred to as 'cell intermediates'.

Superscript 'gp' or 'hu' indicates the species source of the components; i.e. guinea-pig or human, respectively.

MATERIALS AND METHODS

Blood was obtained from patients during and between attacks and from asymptomatic family members. The serum was separated immediately after clotting at 25–27°C for 1 hr and stored at −70°C until used.

The buffers used in all experiments were barbital buffered saline and barbital buffered saline–glucose both of which contained calcium and magnesium at optimum concentration and 0·1% gelatin (Mayer, 1961; Nelson, 1965).

$C'H_{50}$ activity

Total complement (C') activity was determined by the method of Osler, Strauss & Mayer (1952).

C'_1 determinations

The quantitations of C'_{1q} by precipitin and agglutination techniques and the haemolytic

activity of C'_1 were performed by methods published previously (Ewald & Schubart, 1966; Hanauer & Christian, 1967; Gewurz *et al.*, 1967, 1968a).

C'_4 haemolytic activity

The activity of C'_4 was determined (Mayer, 1961) using sheep erythrocytes optimally sensitized with rabbit antibody (EA) and converted to $EAC'_{1a}{}^{gp}$ by methods previously described (Gewurz *et al.*, 1967). To 0·5-ml dilutions of human serum, 0·5-ml aliquots of $EAC'_{1a}{}^{gp}$ at 1×10^9/ml were added. The resulting mixture was incubated at 37°C for 20 min; 0·5 ml (approximately 50 effective molecules per cell) of functionally pure guinea-pig C'_2 (Nelson, 1965 personal communication) was added to each tube and further incubation at 30°C was allowed for interaction of C'_2 with the $EAC'_{1a}{}^{gp}{}_{,4}{}^{hu}$ complex. The resulting

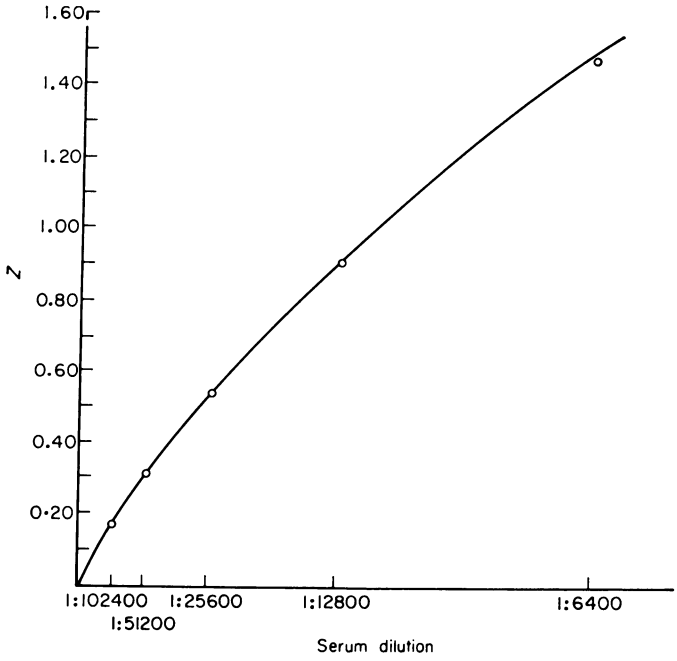

FIG. 1. C'_4 dose–response curve with normal human serum. $Z = -\ln(1-y)$ = Number of effective C'_4 molecules/cell.

$EAC'_{1a}{}^{gp}{}_{,4}{}^{hu}{}_{,2}{}^{gp}$ complex was converted to lysis with 1·0 ml of guinea-pig complement at 1:25 dilution in 0·04 M-ethylenediaminetetraacetic acid (C'-EDTA) and further incubated at 37°C for 90 min. At the end of this period 5·0 ml of normal saline was added to each tube and after centrifugation at 0°C the oxyhaemoglobin in the supernate was determined by spectrophotometry at a wavelength of 541 mμ (Gewurz *et al.*, 1967). This method yields a C'_4 dose–response curve with human serum which is slightly concave to the abscissa when plotted as a function of $Z[-\ln(1-y)]$ (Mayer, 1961) as shown in Fig. 1, indicating a stoichiometric interaction. Thus, in dilutions of serum or serum deficient in one or more components the relative decrease in concentration of the prerequisite components has no observable

effect on the measurement of C'_4. However, since we have previously expressed our C'_4 values as the reciprocal of the dilution which produces 50% haemolysis we will continue to do so here.

C'_2 haemolytic activity

The determination of C'_2 activity was carried out using a cell intermediate prepared with human components ($EAC'_{1a}{}^{hu},_4{}^{hu}$). These cells were made with C'_2 deficient human serum diluted (1:100) in barbital buffered saline–glucose and incubated at 30°C for 10–12 min with equal volumes of EA at 1×10^9/ml. The cells were chilled and centrifuged at 0°C,

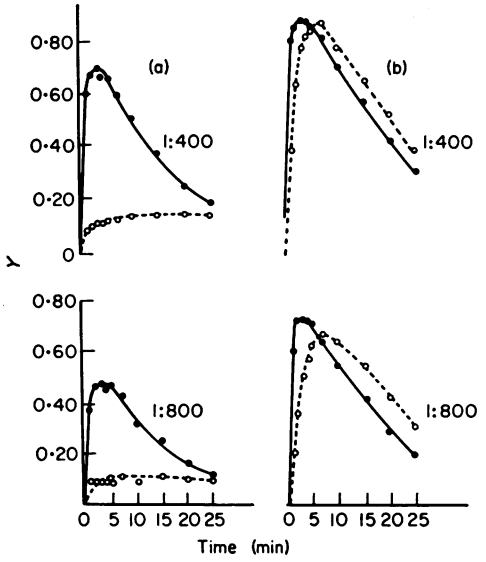

FIG. 2. The rate and maximum degree of formation of active human C'_2 sites by C'_4 deficient (a) and normal (b) human serum on human and guinea-pig intermediates. Since C'_2 sites are unstable and 'decay' spontaneously the point of maximum activity is followed by a steady decrease in the number of active C'_2 sites (Mayer, 1961). Y = Degree of lysis. ●, $EAC'_{1a}{}^{hu},_4{}^{hu}$; ○, $EAC'_{1a}{}^{gp},_4{}^{gp}$.

washed and decayed at 37°C for 2 hr (Mayer, 1961). They were then washed again and standardized in barbital buffered saline–glucose to $1{\cdot}5 \times 10^8$/ml. Since it is known that human C'_2 interacts poorly with guinea-pig C'_4 (Nelson, 1965; Nagaki, Fujikawa & Inai, 1967), we wished to determine whether the limited amount of C'_4 in the serum of patients with HAO would preclude accurate measurement of C'_2 in these sera. To study this we determined the rate and degree of interaction of C'_2 in normal serum and serum from patients with HAO using cell intermediates prepared with both human and guinea-pig complement components; the guinea-pig intermediate ($EAC'_{1a}{}^{gp},_4{}^{gp}$) was prepared according to established methods (Mayer, 1961). Both $EAC'_{1a}{}^{hu},_4{}^{hu}$ and $EAC'_{1a}{}^{gp},_4{}^{gp}$ reacted maximally with functionally purified guinea-pig C'_2 in 11–13 min indicating the presence of 15–30 C'_1 and C'_4 sites/cell (Mayer, 1961). Equal volumes of cell intermediates (at $1{\cdot}5 \times 10^8$/ml) and serum (1:400 or 1:800 dilution) were mixed at 30°C. Samples of 1·0 ml

were taken at intervals and delivered into 1·5 ml of ice cold C′-EDTA (1:37·5); the tubes were transferred immediately to a 37°C water bath. An incubation period of 90 min was allowed following the final sample; 5·0 ml of normal saline was added to each sample and, after centrifugation, the amount of oxyhaemoglobin in the supernate was determined by spectrophotometry at 412 mμ (Mayer, 1961). The degree of haemolysis (Y) was calculated and the results plotted as shown in Fig. 2.

Our C'_2 assay method is as follows: $EAC'_{1a,4}$ at 1×10^8/ml (0·5 ml) are added to serum dilutions (0·5 ml). The tubes containing the serum dilutions plus cells are incubated at 30°C for the time required for normal serum at 1:500 dilution to produce maximum $EAC'_{1a,4,2}$ reactive sites (this time is predetermined for each preparation of $EAC'_{1a,4}$). The tubes are

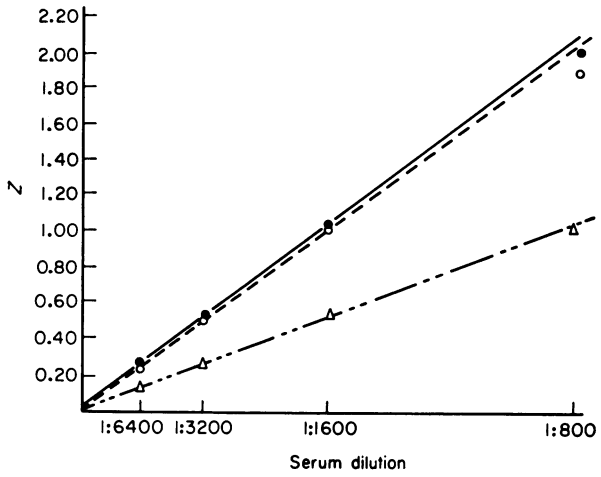

FIG. 3. This figure shows the $C'_2{}^{hu}$ dose–response curves in whole human serum using $EAC'_{1a}{}^{hu},_4{}^{hu}$ as a source of excess C'_1 and C'_4. ○, ●, Normals; △, patient K.L. Sr. (Table 2). $Z = -\ln(1-y)$ = Number of $SAC'_{1a,4,2a}$/cell.

then chilled to 0°C and 1·5 ml of C′-EDTA (1:37·5) is added. A 90-min incubation at 37°C is followed by addition of 5·0 ml of normal saline and spectrophotometric analysis as previously described. This assay system provides a stoichiometric dose response curve with $EAC'_{1a}{}^{hu},_4{}^{hu}$ which, when plotted as a function of $Z[-\ln(1-6)]$ (Mayer, 1961), is linear at Z values up to and slightly greater than 1·0 (Fig. 3). However, as with C'_4, we will continue to express our C'_2 values as the reciprocal of the serum dilution which produces 50% haemolysis rather than in terms of Z.

Immune adherence activity

The basic methods employed here were described previously (Gewurz *et al.*, 1966) and are based on those of Nishioka (1963) and Nelson (1965). Variations of these methods were designed to study the effects of providing additional C'_4 or C'_2 to the sera lacking these components.

EAC'_4 at 2×10^7/ml (prepared from human or guinea-pig serum and containing approximately 20 C'_4 sites/cell) were employed in order to provide additional C'_4. To 0·5 ml

of serum dilutions, 0·5 ml of EAC'_4 was added; after 30 min at each of 30° and 0°C, 0·1 ml of group O, Rh negative human erythrocytes (1×10^8/ml) was added to each tube. These were agitated for 10 min at 37°C then allowed to settle at 37°C for 50 min. Immune adherence haemagglutination patterns were read as previously described (Gewurz *et al.*, 1966).

Additional C'_2 (approximately 200 haemolytically effective C'_2 molecules/cell) was provided by adding functionally pure guinea-pig C'_2 (Nelson, 1965, personal communication) to dilutions of serum being assayed. In all other aspects including reaction volume and number of EA and human erythrocytes this assay was identical to the basic methods previously described (Gewurz *et al.*, 1966).

RESULTS

Measurement of haemolytic C'_1 and the C'_{1q} subcomponent

Values for haemolytic C'_1 activity in the serum of fifteen members of a family with HAO are shown in Table 1 along with our normal values based on the study of twenty-nine normal children and twelve normal adults (Gewurz *et al.*, 1966, 1967). Of the family members studied, four have had clinical symptoms classical for this disease while eleven have had no symptoms. Of the four symptomatic members, two were studied (C.S. and K.L.Sr.), but neither was found to have detectable C'_1-esterase inhibitor by biochemical

TABLE 1. Total haemolytic complement, C'_1 and C'_4 activity in several members of a family with hereditary angioneurotic oedema

Subject	Age	Sample No.	$C'H_{50}$	C'_1	C'_4
K.L.	21	1	38		1,000
K.L.*	21	2	15	30,000	320
S.L.	4	1	32		1,000
S.L.*	4	2	10	29,300	350
T.L.*	3	1	40	40,000	680
C.S.*	23	2	12	16,000	240
S.E.	8		61	36,000	65,000
B.E.	7		57	27,000	33,000
L.E.	13		55	20,000	42,000
V.L.	6		49	30,000	15,000
V.L.	8		49	29,000	37,500
R.S.	5		43	21,000	2,800
R.L.	2		57	24,000	13,000
O.L.	26		49	17,600	4,500
V.L.	2		70	22,000	42,500
J.L.	17		49	22,000	34,000
C.L.	5		46		3,500
Normal children			49 ± 8 (33–65)	24,500 ± 4,000 (16,500–32,500)	11,600 ± 3,500 (4,600–18,600)
Normal adults			61 ± 10 (41–81)	27,500 ± 6,000 (15,500–39,500)	16,900 ± 3,000 (10,900–22,400)

* Samples obtained during clinically active disease.

assay. These determinations were kindly performed by Dr Virginia Donaldson. Since other investigators (Donaldson & Rosen, 1964; Laurell *et al.*, 1966) have found haemolytic C'_1 to be below normal in patients during an attack of oedema, we were surprised to find the haemolytic C'_1 values, as presented here, to be within the normal range. In support of these haemolytic C'_1 findings, as shown in Table 2, the C'_{1q} levels by agglutinating function and precipitin assay were found to be normal or very slightly below the normal range in all symptomatic and asymptomatic family members studied.

TABLE 2. C'_{1q}, C'_1 and C'_4 in individuals from a family with hereditary angioneurotic oedema

	Age (years)	C'_{1q}† (μg N/ml)	C'_{1q}‡ agglutination	C'_1 (50% haemolytic units/ml)	C'_4 (50% haemolytic units/ml)
(A) Clinically affected					
C.S.*	23	23	128	22,500	300
C.S.*	23	17	128	16,000	240
K.L. Sr.*	20	18	128	30,000	320
(B) Clinically unaffected					
R.L.	2	21	128	24,000	13,000
R.B.S.	2	18	128	19,000	15,000
R.E.	5	18	256	35,000	17,000
E.W.	10	17	128	30,000	26,500
R.W.	13	22	256	28,000	46,000
H.L.	4	26	128	—	30,000
Normal children		17–28	64–256	16,500–32,500	4,600–18,600
Normal adults				15,500–39,500	10,900–22,400

* Blood obtained during an acute episode of oedema.

† Immunodiffusion assays by Dr C. L. Christian.

‡ Titre expressed is the reciprocal of the highest dilution of serum which agglutinates IgG coated latex particles.

Haemolytic C'_4 activity

Low haemolytic C'_4 values have been reported by others (Donaldson & Rosen, 1964; Laurell *et al.*, 1966; Ruddy & Austen, 1967). Our C'_4 values presented in Table 1 show that the four symptomatic members had unequivocally reduced activity of this component between attacks of oedema and that during an acute episode a significant additional drop in activity occurred in each of two patients. In addition, of the eleven members without clinically apparent disease, three (R.S., C.L. and O.L.) also had unequivocally reduced C'_4 activity indicating that they carry the genetic trait and have latent or occult disease.

Effect of decreased C'_4 activity on measurement of C'_2

Since Nelson (1965) has indicated and Nagaki *et al.* (1967) have shown that human C'_2 reacts poorly with guinea-pig C'_4 it would seem necessary to provide human C'_4 in order to measure C'_2 in human serum; therefore, if one uses guinea-pig intermediates, the human C'_4 must be supplied by the serum being studied before C'_2 can be measured. The results of our studies to determine the effect of a limited amount of C'_4 in human serum on the

detection of C'_2 activity in that same serum are shown in Fig. 2. Though the degree of C'_2 activity in *normal* human serum, as reflected by the degree of lysis (Y), is approximately the same at these serum dilutions with and without additional human C'_4 ($EAC'_{1a}{}^{hu},{}_4{}^{hu}$ and $EAC'_{1a}{}^{sp},{}_4{}^{sp}$, respectively), the time required to reach maximum C'_2 activity (T-max) is almost twice as long when no supplemental human C'_4 is provided. This delay observed in the absence of supplemental human C'_4 likely reflects the time required for this component in the serum to form human C'_4 sites on the guinea-pig intermediate before human C'_2 can interact in the haemolytic sequence.

When serum deficient in C'_4 (as in a patient with HAO) is used as a source of C'_2, the significance of this requirement for exogenous human C'_4 becomes manifest. Fig. 2 demonstrates this phenomenon by showing the discrepancy in apparent C'_2 activity with and without supplemental human C'_4. In addition to the delay, also observed above, the apparent C'_2 activity in the absence of supplemental human C'_4 is strikingly lower than the C'_2 activity detectable when additional human C'_4 is provided. This has been recognized (Nelson, 1965; Austen & Russell, 1966; Nagaki *et al.*, 1967) as a possible deficiency in the assay of C'_2 in human serum using guinea-pig intermediates and, as our studies show, can be misleading in evaluation of C'_2 in this disease.

Haemolytic C'_2 activity

The results of a study of C'_2 activity in human serum from two normal adults, two pools of adult human serum and four patients with HAO are shown in Table 3. Here we compare

TABLE 3. Comparison of $C'_2{}^{hu}$ titres using $EAC'_{1a}{}^{sp},{}_4{}^{sp}$ and $EAC'_{1a}{}^{hu},{}_4{}^{hu}$

Patient	Sample No.	Diagnosis	$C'H_{50}$/ml	C'_2 titre with $EAC'_{1a}{}^{sp},{}_4{}^{sp}$	C'_2 titre with $EAC'_{1a}{}^{hu},{}_4{}^{hu}$
K.L.	1	HAO	38	252	1830
K.L.	2	HAO*	15	96	1260
S.L.	1	HAO	32	161	1430
S.L.	2	HAO*	10	202	2200
T.L.	1	HAO	40	125	1680
C.S.	1	HAO	47	198	1570
D.W.		Normal	58	1400	2100
D.P.		Normal	55	1500	2180
P.Q.		Normal	50	1480	2000
P.W.		Normal	52	1550	2200

HAO, Hereditary angioneurotic oedema.

* During an episode of oedema.

the C'_2 titres obtained with guinea-pig and human intermediates. Among the values presented here the lowest C'_2 titre obtained in the presence of supplemental human C'_4 is approximately 50–60% of the normal. In contrast, the apparent C'_2 activity in the same serum may be less than 10% of normal when additional human C'_4 is not provided in the assay.

Immune adherence activity

In previous studies of I-A in human serum (Gewurz *et al.*, 1966; Rapp & Borsos, 1966) we showed that this complement mediated function is normal in a serum so deficient in haemolytic C'_2 that the total haemolytic complement titre is less than 3 $C'H_{50}$ units/ml (normal 49 ± 8 $C'H_{50}$ units/ml). We were surprised, therefore, to find that the I-A titre in the serum of patients with C'_4 deficiency (i.e. HAO) is extremely low (Table 4). Table 4 compares the I-A titres of sera from normals, patients with HAO and an individual with congenital C'_2 deficiency; relates these values to haemolytic titres of total complement, C'_4 and C'_2; and shows the effect of providing additional C'_4 or C'_2 on I-A activity of these

TABLE 4. Immune adherence complement activity of normal human serum, hereditary angioneurotic oedema serum and C'_2 deficient human serum

Patient	$C'H_{50}$ units/ml	C'_4	C'_2	I-A			
				EA	EA+ C'_2	*$EAC'_4{}^{hu}$	*$EAC'_4{}^{gp}$
C.S.	12	290	500	50	50	6,400	50
P.E.	38	3,700	655	1,200	1,200	3,200	1,200
R.E.	48	17,000	1,000	3,200		4,800	4,800
G.J.	60	50,000	1,200	4,800	9,600	9,600	6,400
Pool 1	52	20,000	1,000	3,200		6,400	6,400
Pool 2	53	21,000	1,100	3,200	6,400	6,400	4,800
C'_2 deficient	< 2	18,000	9	3,200	6,400	4,800	4,800

This table relates the total complement, C'_4 and C'_2 haemolytic activities to I-A complement activity with and without additional $C'_4{}^{hu}$, $C'_4{}^{gp}$ and $C'_2{}^{gp}$.

C.S. and P.E. are individuals with hereditary angioneurotic oedema. The serum from C.S. was obtained during an episode of oedema.

* EAC'_4: Prepared from parent $EAC'_{1a,4}$ which contained approximately 20 $SAC'_{1a,4}$/cell.

sera. It is of interest that the C'_2 deficient serum has virtually undetectable total haemolytic complement activity but normal I-A complement titre while HAO serum with total haemolytic complement activity of approximately $\frac{1}{4}$ to $\frac{1}{3}$ of normal has an extremely low I-A titre. The addition of C'_2 (or human C'_4), does not appreciably alter the I-A activity of the C'_2 deficient serum. In striking contrast, provision of human C'_4 (as $EAC'_4{}^{hu}$) increases the I-A titre of the C'_4 deficient HAO serum to normal. The use of guinea-pig C'_4 ($EAC'_4{}^{gp}$) known to interact poorly with human C'_2, on the other hand, failed to produce any increase in the I-A activity of the HAO serum.

DISCUSSION

Though a number of abnormalities of the complement system have been observed in the serum of patients with HAO direct evidence of the relationship of these findings to the clinical syndrome is lacking. Limited understanding of the normal *in vivo* and *in vitro* interactions of the components involved, i.e. C'_1-esterase inhibitor, C'_1 (C'_{1q}, C'_{1r} and

C'_{1s}), C'_4 and C'_2, complicates interpretation of the deviation of these components from normal in this disease. It seems important, therefore, to establish which of these components is consistently abnormal and to attempt to understand the mechanism underlying the abnormality in order to relate the complement system to the basic disturbance in HAO.

Previous reports (Donaldson & Rosen, 1964; Laurell *et al.*, 1966) indicated that, using methods known to have important limitations (Mayer, 1961), for example, R-reagents (Bier *et al.*, 1945), the haemolytic activity of C'_1 seemed to be extremely low in some patients during attacks of oedema. In contrast, using improved methods we have found C'_1 haemolytic activity to be within normal range in our patients even during attacks. An explanation for the apparent low C'_1 function in haemolysis found by others may lie in the peculiarity of the R-reagent method in which the excess of all components other than the one being measured is presumed to be supplied in the fluid phase. Under these conditions, unless 0°C temperature is maintained prior to addition of EA, C'_4 and C'_2 in the R-reagent could be inactivated by the high level of C'_1-esterase present in the patients' sera (Lepow *et al.*, 1956b; Donaldson & Rosen, 1964). By supplying C'_4 on the sensitized sheep erythrocyte we avoid fluid phase interaction between C'_1-esterase and C'_4.

In view of the conflicting haemolytic C'_1 results it is important to attempt to understand the C'_1 complex and its related components. It therefore seems appropriate to compare some of the complement findings in systemic lupus with those in HAO. In the former, generally considered an immune complex disease, C'_{1q} concentration (Hanauer & Christian, 1967) and haemolytic activity of C'_1, C'_4 and C'_2 (Gewurz *et al.*, 1966, 1967, 1968b) are far below normal, presumably due to depletion as a result of extensive immune complex formation. These observations are consistent with the concept of Lepow, Ratnoff & Pillemer (1956a) and Lepow, Ratnoff & Levy (1958) that immune complexes in serum combine with the subcomponents of C'_1, effectively isolating them from the influence of the normal C'_1-esterase inhibitor, thus permitting autocatalytic conversion of C'_{1s}, in the presence of C'_{1q} and C'_{1r}, to C'_1-esterase and subsequent reaction of this esterase with its substrates, especially C'_4 and C'_2. In contrast, we have shown that serum from patients with HAO contains a normal concentration of C'_{1q} and has normal haemolytic C'_1 activity while it is extremely deficient in C'_4 and moderately deficient in C'_2. The depletion of C'_{1q} in systemic lupus can be attributed to immune complex interaction while the lack of detectable involvement of C'_{1q} and C'_1 in HAO strongly suggests that conversion of C'_{1s} to C'_1-esterase may be enhanced without participation of C'_{1q} in this disease. In the absence of C'_1-esterase inhibitor this could lead to extensive inactivation of substrates C'_4 and C'_2. Indeed, Ratnoff & Naff (1967) have shown that enzymes otherwise unrelated to the complement system such as plasmin and trypsin, activated by enzymes such as streptokinase, can convert C'_{1s} to C'_1-esterase in the absence of C'_{1q} and C'_{1r}. It seems possible that a similar mechanism is operative in HAO and that intermittent exposure to agents which activate plasmin or trypsin-like substances might be responsible for the complement alterations observed during episodes of oedema. In addition these related mechanisms may be more directly responsible for the clinical manifestations of the syndrome than the complement system (Becker & Kagen, 1964; Stroud, 1967).

We also observed that the activity of C'_4 may be greatly diminished both between and during attacks and that this deficiency of C'_4 can impair detection of C'_2 in these sera unless additional human C'_4 is provided in the assay. During an attack C'_4 activity in the serum of these patients was as low as 5% of normal while C'_2 activity in the same serum sample

was approximately 50% of normal. Haemolytic C'_4 was below normal in all patients studied during and between attacks with a significant decrease occurring during the acute phase while C'_2 was essentially normal in two patients between attacks. Thus the C'_4 assay appears to be more reliable than C'_2 titration for diagnosing this disease in the absence of clinical activity.

It has been shown by Nishioka (1963) and Nelson (1965) that maximum I-A activity and phagocytosis enhancement is produced by antibody plus the first four components of the haemolytic sequence of guinea-pig complement, C'_1, C'_4, C'_2 and C'_3 (C'_{3c}). In earlier studies (Gewurz *et al.*, 1966) we showed that human serum extremely deficient in haemolytic C'_2 activity is capable of producing normal I-A titres. This indicated that a very small amount of haemolytic C'_2 can activate a sufficient number of C'_3 molecules (Müller-Eberhard, Dalmasso & Calcott, 1966) to produce normal I-A activity. In contrast, the data presented here show that serum deficient in C'_4 is extremely poor in I-A production. This finding and the additional observation that supplemental human C'_4 can completely restore the I-A activity of C'_4 deficient serum suggest that this component of complement, together with activated C'_3, is responsible for establishing the site which ultimately combines with the I-A receptor on the primate erythrocyte. Therefore, in I-A activity and, by inference, complement enhancement of phagocytosis by human serum, C'_4 rather than C'_2 appears to be the limiting component. Though these findings might suggest that in human serum C'_1 and C'_4 alone can produce maximum I-A activity the fact that our $EAC'_{1a,4}$ cells prepared from human serum are I-A negative rules out this possibility.

Previously, among a number of diseases studied (Gewurz *et al.*, 1966; Rapp & Borsos, 1966) immune adherence levels were found to be very low only in systemic lupus erythematosus and plasma cell hepatitis. Our findings presented here indicate that this simple I-A assay, which can be carried out in any laboratory, is the most useful diagnostic test for HAO in individuals during transient acute episodes of oedema or abdominal pain. Systemic lupus and plasma cell hepatitis must be ruled out by other methods in the face of extremely low I-A titres.

While investigating this disorder further it will be important to keep in mind that the actual cause of the recurrent oedema in clinically affected individuals is still unknown. The observations presented here suggest the need for continued re-investigation with newer techniques and re-evaluation of concepts of the role of complement and other related systems in this disease in the light of new findings. In addition our observations provide a simple diagnostic aid and indicate again the importance of employing sera deficient in certain complement components as tools for evaluating the relative requirement of components in complement dependent functions.

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