Clin. exp. Immunol. (1966) **1**, 13–23.

COMPLEMENT COMPONENTS IN THREE PATHOLOGICAL SERA: RELATION TO CLINICAL STATES

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(*Received* 29 *May* 1965; *accepted* 23 *June* 1965)

SUMMARY

Sera from three patients with systemic lupus erythematosus, hereditary angioneurotic oedema and erythema exudativum multiforme, respectively, have been investigated for total complement (C′), complement components (C′1, C′2, C′3, C′4), including β_{1C}-globulin, as well as inhibitory and enzymatic substances which are capable of inactivating C′ components. All three sera had a low total C′ activity. The serum from the patient with systemic lupus erythematosus had low levels of C′4, C′2 and β_{1C}-globulin, and the reduction of total C′ could possibly be attributed to an antigen–antibody reaction *in vivo*. It is known that the low total C′ activity and the pronounced lowering of C′1, C′4 and C′2 in serum from patients with hereditary angioneurotic oedema is due to the enzymatic inactivation of C′4 and C′2 by C′1 esterase. This mechanism has been confirmed in this investigation. Serum from the patient with erythema exudativum multiforme showed only slightly reduced C′1, C′2 and C′3 activities, whereas there was a pronounced reduction of β_{1C}-globulin and of C′4 activity as determined by the classical titration technique. However, C′4 activity was normal when determined with a stepwise technique. As the patient recovered, the C′ components returned to normal levels but the C′4 titre increased only moderately. Inhibitory or enzymatic substances could not be found in the erythema multiforme serum. The low total C′ activity in the erythema multiforme serum, therefore, is unlike that found in the systemic lupus erythematosus serum or the hereditary angioneurotic oedema serum. Obviously, different mechanisms account for the low total C′ activities in the sera from these patients.

Determination of the concentration of β_{1C}-globulin in sera from patients with various diseases (Lundh, 1966) revealed extremely low values in two sera. One of these sera originated from a patient with systemic lupus erythematosus (SLE) in an active phase; the other from a patient with erythema exudativum multiforme (EM). Both sera also had low total

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complement (C′) titres. Donaldson & Evans (1963) found that patients with hereditary angioneurotic oedema (HANE) have a profound disturbance in the C′ system. Therefore one such serum was included in this study which concerns estimation of β_{1C}-globulin and of the total C′ titre, the determination of the classical C′ components (C′1, C′2, C′3, C′4) and estimation of C′1 esterase and C′1 esterase inhibitor. These pathological sera varied in C′ component activity in a way that might indicate different mechanisms behind the low total C′ titres.

The findings with these sera may not necessarily be regarded as representative for all sera from patients with these diseases.

Patients and Subjects

The sera were from five healthy individuals and from three patients with erythema exudativum multiforme, systemic lupus erythematosus and hereditary angioneurotic oedema, respectively. The patients were treated at the Department of Internal Medicine, Malmö General Hospital, Malmö.

Case Reports

Case 1

A 42-year-old female. Diagnosis: Erythema exudativum multiforme (EM). At the age of 30 years she had an attack of rheumatic fever. Afterwards she had episodes of arthralgias.

The present disease started with a sore throat and fever, followed after 3 days by arthralgias in carpal, knee, and tarsal joints. After another 4 days, she developed a rash on the scalp, on the forehead, and on the dorsum of the hands and feet. The rash consisted of 10 mm papules with an erythematous peripheral zone and a bluish centre. Some of the papules developed vesicles which eventually converted to pustules. On admission, 9 days after onset, the patient stated that during the last month she had taken meprobamate and an analgesic containing ethylephedrin, phenacetin and salicylamide. Bedside examination revealed, in addition to the skin changes, vesicles in the oral mucous membranes. Temperature 38·5°C. ESR 132 mm/hr. Leucocyte count 12 000/mm^3 with 80% neutrophils and less than 1% eosinophils. Total eosinophil leucocyte count, 25/mm^3 (normal: 75–400). Paper electrophoresis of serum proteins revealed slight hypo-albuminaemia, markedly increased α-globulins, decreased β-globulins and moderately raised γ-globulins. The antistreptolysin titre was increased. The Wassermann reaction, the Waaler–Rose test, the cold agglutinin test, and the Paul–Bunnell reaction were negative. Plasma fibrinolytic activity was slightly increased. Throat swab culture demonstrated growth of group A haemolytic streptococci. Virus culture from vesicular contents was negative. Two blood cultures were negative. Chest X-ray showed a bronchopneumonic infiltrate in the right lower lobe. She had a moderate proteinuria (0·1–2·1 g/1000 ml). Urinary sediment showed 20–40 leucocytes per high power field. Endogenous creatinine clearance was 83 ml/min (normal: 96–148). The patient was treated with penicillin and sodium salicylate. The temperature and ESR declined, the arthralgias and the skin rash disappeared and her general condition improved. She was dismissed from hospital 7 weeks after admission, still with proteinuria and an abnormal urinary sediment.

Blood samples for the C′ investigations were drawn 2, 3, 5 and 6 weeks after admission. In Table 1(a) is shown the ESR, the temperature and the β_{1C}-globulin concentration on different occasions during the disease.

Case 2

A 26-year-old female. Diagnosis: Systemic lupus erythematosus (SLE). At the age of 22 she was treated with sulphonamide on a tentative diagnosis of pyelonephritis. The present disease

started September 1963, at the age of 24, with a rash on nose and cheeks and the dorsums of the hands, and also intermittent annular infiltrates on the arms and legs. On admission a high ESR and hypergammaglobulinemia was noted. Microscopic examination of a skin biopsy revealed a picture suggestive of systemic lupus erythematosus. LE-cells or antinuclear factors (fluorescence technique) were not demonstrable. During a short period of corticosteroid therapy the cutaneous changes disappeared. After a short time she relapsed with reappearance of the rash and, in addition, pain and swelling in carpal and phalangeal joints. Again the symptoms abated on a course of corticosteroid treatment.

Three months later she got fever and developed facial erythema and oedema. Continuous corticosteroid treatment was started. After an initial improvement her condition deteriorated progressively with attacks of fever, abdominal pain, diarrhoea and bilateral pleural effusions. At this stage typical LE-cells and antinuclear factors could be demonstrated. Massive proteinuria and a serum protein pattern compatible with a nephrotic syndrome developed. The steroid dosage was increased and a temporary improvement followed.

After another 4 months she was readmitted (January 1965) with fever, diarrhoea and vomiting. The clinical picture was dominated by a nephropathy with azotemia, proteinuria, and oedema. Haemoglobin varied between 11·0 and 5·1 g/100 ml, ESR between 11 and 90 mm/hr and leucocyte counts between 4700 and 14 200/mm^3. Serum creatinine increased from 0·65 to 3·45 mg/100 ml (normal: 0·5–1·2). Proteinuria 3–9 g/1000 ml. Paper electrophoresis of plasma proteins showed a nephrosis pattern, except for a normal γ-globulin level. Blood cultures negative. Her condition gradually deteriorated and she died 4 months after admission. Autopsy showed lesions in most of the renal glomeruli, with focal necrosis, haematoxylin bodies and occasional wire-loop lesions. In the spleen no definite perivascular lamellation could be demonstrated, but there were small degenerative changes with nuclear fragments.

The blood sample for the C′ investigation was drawn 1 month after the last admission. In Table 1(b) are shown the ESR, the temperature and the β_{1C}-globulin concentration during the last period of the disease.

TABLE 1. The ESR, temperature and β_{1C}-globulin concentration in a case of erythema exudativum multiforme (a) and in a case of systemic lupus erythematosus (b)

(a) CASE 1

	January				February		April
	13	15*	22	24*	6*	14*	7
ESR (mm/hr)	115	—	93	96	43	37	35
Fever	+	+	—	—	—	—	—
β_{1C}-globulin (arbitrary units)	12	0	11	10	43	50	98

(b) CASE 2

	17 January	14 February*	11 March
ESR (mm/hr)	47	42	90
Fever	—	—	+
β_{1C}-globulin (arbitrary units)	36	39	38

*Samples were taken for study of total C′ and C′ components.

Case 3

A 40-year-old male. Diagnosis: Hereditary angioneurotic oedema (HANE). A brother and his daughter suffer from chronic relapsing angioneurotic oedema affecting the skin, larynx and gastro-intestinal tract. The disease has been traced back two generations (Nilsson & Floderus, 1964). Since adolescence the patient suffers from frequent and rather regularly recurring attacks of migraine-like headache and peripheral oedema, often followed by abdominal pain, vomiting and diarrhoea. He has been investigated repeatedly since 1961. The attacks are not accompanied by fever but a moderate leucocytosis has been noted. No blood eosinophilia. Paper electrophoresis between attacks has shown a normal plasma protein pattern; during attacks a slight α_2-globulin increase has been present. Blood coagulation studies have shown on one occasion a somewhat lowered anti-haemophilic factor (AHF) activity, on another occasion a moderately increased fibrinolytic activity. When not otherwise stated the blood samples used in the present study were drawn in a symptom-free interval.

MATERIALS

Sensitized red sheep cells (EA) and *barbital buffer*, used in haemolytic C′ titrations, were prepared according to Kabat & Mayer (1961). *R reagents* (reagents deficient in one of the four classical C′ components) were used in titrations of individual C′ components. R1 and R2 were prepared according to Fjellström (1962); R3 and R4 were made according to Kabat & Mayer (1961).

β_{1C}-Globulin. The methods of Müller-Eberhard, Nilsson & Aronsson (1960) and of Lundh (1964) were used to prepare β_{1C}-globulin.

C′1 esterase was prepared according to Lepow *et al.* (1963) with the modification that EDTA was omitted in the fractionation procedure.

N-acetyl-L-tyrosine ethyl ester (ATEe), from Sigma Chemical Co., St. Louis, U.S.A., was used as substrate in the determination of C′1 esterase.

METHODS

Total C′ and C′ component titrations were performed according to Pillemer *et al.* (1956). The smallest amount of serum producing 50% haemolysis was taken as 1 unit. All sera were tested simultaneously and the tests were repeated at least twice. Differences in titre in the individual samples did not exceed one dilution step.

C′4 activity was also determined with the following technique. EAC′1 cells were formed by incubation of EA with a human R4 reagent diluted 1/10 in triethanolamine buffered saline (TBS) followed by washing in warm TBS (DeLooze & Leon, 1963). The cells were resuspended to 5×10^5 cells/mm^3. One millilitre of the EAC′1 suspension was added to prewarmed tubes. The sera to be tested were heated at 52°C for 30 min to destroy C′1 and C′2 activities, and 0·2 ml of doubling dilutions of the heated sera were added to the cells and thoroughly mixed. After 20 min at 37°C the tubes were centrifuged in prewarmed cups and the cells were washed twice with 1 ml warm TBS. The buffer was sucked off, and R4 and barbital buffer were added to a final volume of 1·2 ml. After incubation at 37°C for 30 min haemolysis was recorded.

C′2 activity was also determined according to Austen & Beer (1964) with the modification that EAC′14 cells were prepared with a human R3 according to Leon (1958).

β_{1C}-Globulin concentration was determined by an electrophoretic technique described by

Lundh (1964). The normal value was 124±56 AU (arbitrary units/100 ml serum) which corresponds to about 125±40 mg protein/100 ml serum.

C'1 esterase and serum inhibitor of C'1 esterase was determined as described by Laurell, Lundh & Malmquist (1965).

Test for C' inhibiting substances was performed by combined electrophoresis and immune-haemolysis (Siboo & Laurell, 1966).

RESULTS

Titres of total C'

The total C' titres of normal sera and of the three pathological sera are given in Table 2. All three pathological sera showed low titres of total C' (<20–24 units/ml). The titre of the EM serum became normal when the patient recovered (serum EM IV, Table 2).

TABLE 2. Titres of total C', C' components and the β_{1C}-globulin concentration in normal sera and in the sera from patients with erythema exudativum multiforme (EM), systemic lupus erythematosus (SLE) and hereditary angioneurotic oedema (HANE). EM I–IV refers to different sampling occasions (see case report 1)

Serum	Total C' (units/ml)	C'1	C'2	C'3	C'4	β_{1C}-globulin (arbitrary units)
		(Average of two determinations)				
Normal serum I	92	900	300	188	600	109
Normal serum II	92	900	233	233	500	126
Normal serum III	103	900	300	188	1100	104
Normal serum IV	103	900	233	267	900	135
Normal serum V	118	900	300	267	900	124
EM serum I	24	400	150	106	32	0
EM serum II	47	400	233	128	32	10
EM serum III	71	550	267	150	32	43
EM serum IV	83	750	300	217	44	50
SLE serum	22	225	27	183	57	39
HANE serum	<20	<50	<50	150	<5	95

Titres of C'1

The SLE serum and the EM serum taken in the acute phase of the illness had a moderate decrease of the C'1 titres. During convalescence C'1 returned to normal in the serum from the EM patient. No C'1 activity could be detected in the HANE serum (Table 2); however, with a stepwise technique C'1 activity was found to be present in a titre of 50 units/ml.

Titres of C'2

When determined with the classical technique, the C'2 titre of the SLE serum was sharply decreased, but in the EM serum taken in the acute phase of the illness only a moderate decrease was observed which rapidly returned to normal on convalescence. The HANE serum contained no detectable C'2 with the lowest dilution (1/10) tested (Table 2).

A good correlation was found between C'2 activity recorded with the classical technique and the technique described by Austen & Beer (1964) (see Methods). With the classical technique normal sera contained 1·8 times more C'2 activity than the EM serum and 10 times as much as the SLE serum. With the technique of Austen & Beer normal sera showed 1·5 times as much C'2 as EM serum and 4·4 times as much C'2 as the SLE serum. The HANE serum contained no C'2 activity with the technique of Austen & Beer when tested in a dilution of 1/25 (Table 3).

TABLE 3. C'2 activity determined by classical R2 technique and according to the technique of Austen & Beer (1964)

	Classical technique		Austen & Beer technique (per cent of normal)
	Units/ml	Per cent of normal	
Normal serum	273*	100	100
EM serum I	150	55	69
SLE serum	27	10	23
HANE serum	<50	<18	0

*Mean value of double determinations in five normal sera.

Titres of C'3

The C'3 titres of the SLE and the HANE sera were within normal limits. A slight decrease of the C'3 titre was observed in the EM serum which returned to normal on recovery of the patient (Table 2).

Concentration of β_{1C}-globulin (a C'3 component)

The concentration of β_{1C}-globulin was very low in the serum from the EM case in the acute stage, but as the symptoms regressed the concentration returned to a normal level (Table 1a). In the SLE case the concentration of β_{1C}-globulin in serum was low in all samples (Table 1b) in spite of intensive corticosteroid therapy. This patient was in a poor state during the whole observation period. The patient with HANE had a normal serum β_{1C}-globulin concentration both in symptom-free periods and during attacks.

No decrease of the β_{1C}-globulin concentration was seen in normal serum after incubation for 60 min at 37°C with an aliquot of EM serum or SLE serum. The samples of these sera had been stored at —20°C. Neither could any effect on the β_{1C}-globulin concentration be demonstrated if HANE serum, taken during an attack of the disease, was incubated for 60 min at 37°C.

On immunoelectrophoresis of the pathological sera using a specific rabbit anti-β_{1C}-globulin antiserum for development of the precipitation lines, β_{1C}-globulin had a normal location in the HANE serum. The EM serum formed a very weak β_{1C}-globulin precipitation line. The SLE serum was not investigated in this respect.

Titres of C′4

In all three pathological sera extremely low titres of C′4 were recorded when titrations were carried out with the classical technique. The C′4 titre in the EM serum remained low throughout the observation time as compared to C′1, C′2 and total C′ titres which returned to normal simultaneously with clinical recovery.

The R4 reagent used in the classical C′4 titration technique is known to be poor in β_{1C}-globulin (Müller-Eberhard, 1961). It could be argued that the low C′4 titre found in SLE and in erythema multiforme sera could be due to their low content of β_{1C}-globulin, i.e. β_{1C}-globulin and not C′4 being the limiting factor in the titration of C′4 in these special sera. To elucidate this problem purified preparations of β_{1C}-globulin were added to the EM and the SLE sera in a final concentration of 200 mg/100 ml serum. The test was performed with R4 and serial dilutions of these β_{1C}-globulin-enriched sera. Three different β_{1C}-globulin preparations were used. One preparation (according to Müller-Eberhard *et al.*, 1960) met the criteria established by Müller-Eberhard (1961) in that it prevented the decay of EAC′142 cells on incubation at 37°C for 90 min. Two other β_{1C}-globulin preparations (Lundh, 1964) lacked this property. Increase of the C′4 titres was not observed by enrichment of the EM serum with either of the β_{1C}-globulin preparations; a slight but not significant increase was observed with the SLE serum.

One normal serum and the SLE and EM sera were also examined for their C′4 content with EAC′1 cells after heating the sera at 52°C for 30 min (see Methods). By this technique the normal serum contained 533 units/ml. The EM serum contained 266 units/ml, that is, a surprisingly high titre compared to the titre obtained with the classical technique (32 units/ml). The C′4 titre of the SLE and HANE sera showed the same low level (<50 units/ml) as obtained with the classical C′4 titration technique.

Investigation for C′ inhibiting substances

The above experiments indicated that EM serum might contain a factor which inhibited the activity of C′4 or the later steps in immunohaemolysis. The following experiments were carried out to obtain information on this point. EM serum was incubated with normal serum or with a partly purified preparation of C′4 (Müller-Eberhard & Biro, 1963) at 37°C for 30 min and the mixture was then tested for C′4 activity with the classical technique. The same titre was found as in the control tubes. These results indicated that with the technique used no substances which inhibited C′4 were present in the erythema multiforme serum.

The three pathological sera were also investigated for C′ inhibitors by electrophoresis in agarose containing EA followed by addition to the slides of normal serum as a source of C′. No inhibitor of C′ activity was evident either in the SLE serum or in the EM serum. With the HANE serum inhibition of C′ lysis of the EA in the agarose supporting media was observed (Siboo & Laurell, 1965). This was due to the presence of active C′1 esterase in this serum.

Determination of C'1 esterase and serum C'1 esterase inhibitor

The SLE and the EM sera contained a normal content of C'1 esterase inhibitor and showed no C'1 esterase activity. C'1 esterase inhibitor was not detectable in the HANE serum, but ATEe esterase activity was pronounced, probably indicating the presence of active C'1 esterase.

DISCUSSION

Low serum haemolytic C' activity has been reported in some diseases, for instance, SLE, acute glomerulonephritis, and serum sickness (for review see Brückel, Schultze & Schwick, 1957; Osler, 1961). This feature has been taken as an indication that C' fixing antigen–antibody complexes are formed *in vivo*.

It is obvious that a low C' titre does not necessarily indicate a uniform lowering of all components of C', as a deficiency in only one component may give the same result in the estimation of total C'. Observations on the titres of C' components of some diseases have

TABLE 4. Pattern of total C', C' components, β_{1C}-globulin, C'1 esterase and C'1 esterase inhibitor in the sera from patients with erythema exudativum multiforme (EM), systemic lupus erythematosus (SLE) and hereditary angioneurotic oedema (HANE)

	EM serum I	SLE serum	HANE serum
Total C'	Low	Low	Low
C'1	Slightly reduced	Slightly reduced	Low
C'2	Slightly reduced	Low	Low
C'3	Slightly reduced	Slightly reduced	Normal
C'4 classical technique	Low	Low	Low
C'4 stepwise technique*	Normal	Low	Low
β_{1C}-globulin concentration	Low	Low	Normal
C'1 esterase	None	None	Active
C'1 esterase inhibitor	Normal	Normal	Absent

* See Methods.

previously been reported. In active SLE a reduction in the titre of C'4 and C'2 was observed (Morse, Müller-Eberhard & Kunkel, 1962). In rheumatoid arthritis, on the other hand, no abnormalities of the C' components were found (Laurell & Grubb, 1958). Sera from patients with HANE showed low titres of C'1 and often no detectable C'4 and C'2 (Donaldson & Evans, 1963).

To our knowledge no investigation on the levels of total C' or C' components in sera from EM patients has been reported.

The reduction of C' titres in sera of the above-mentioned diseases may be the result of different mechanisms. The reduction of C' components in SLE is probably due to a fixation of C' to antigen-antibody complexes formed *in vivo*. In HANE C' reduction seems to be the result of enzymatic inactivation by C'1 esterase.

In the sera studied different abnormal patterns of the C' components were found (Table 4).

With the classical C′4 titration technique both SLE and EM sera showed strongly decreased C′4 titres. As a return to normal of the C′4 titres was not observed in the SLE or the EM sera after addition of preparations containing β_{1C}-globulin (Lundh, 1964) or β_{1C}- and β_{1F}-globulin (Müller-Eberhard *et al.*, 1960; Nilsson & Müller-Eberhard, 1964) the decreased C′4 obtained with the classical technique cannot be due to a lack of these factors. The C′4 titre of the EM serum approached the normal range when a stepwise titration technique was used in contrast to the result with the classical titration technique. The SLE serum showed the same low level with both techniques. The difference found with the EM serum, therefore, might depend on some factor in this serum interfering after the C′4 fixation step. Experiments designed to give clearer information on this point were, however, unsuccessful. Inhibitor of C′ could not be found by investigation of this serum with combined electrophoresis and immune haemolysis, a technique capable of revealing the C′ destroying effect of active C′1 esterase in HANE sera (Siboo & Laurell, 1965). Furthermore no factor reducing the β_{1C}-globulin concentration could be demonstrated *in vitro*.

The results with the HANE serum are in accordance with the findings by earlier investigators (Donaldson & Evans, 1963; Donaldson & Rosen, 1964; Austen & Beer, 1964). In such sera, C′1 esterase inhibitor is lacking and active C′1 esterase is present. As C′1 esterase is known to destroy C′4 and C′2 in serum (Lepow, Ratnoff & Levy, 1958), the reason for the lack of C′4 and C′2 activity in HANE sera is obvious. The normal concentration and normal immunoelectrophoretic appearance of β_{1C}-globulin is in accordance with the results of Donaldson & Rosen (1964) and of Klemperer *et al.* (1965). These findings are at the moment contradictory to the results of Pondman & Peetom (1964). These authors showed that *in vitro* β_{1C}-globulin is converted to β_{1A}-globulin by C′1 esterase and a heat-labile serum factor, possibly C′2.

In diseases with a pathogenesis believed to involve autoimmunity or hypersensitivity it might be tempting to regard a depressed serum C′ level as an expression of a continuous C′ fixation to antigen–antibody complexes formed in the body. For instance, the observation of a low C′ titre in SLE during active phases of the disease and of fixation of β_{1C}-globulin to the tissues (Lachmann *et al.*, 1962) has been taken as evidence for an autoimmune pathogenesis.

The SLE patient studied in this investigation had a marked proteinuria but the possibility that the low serum C′ level could be due to urinary loss seems to have been ruled out by the studies of Lange & Wenk (1954).

There was a superficial similarity in the pattern of C′ components in the EM and SLE sera. This might suggest that the low total C′ titre was a result of C′ fixation to antigen–antibody complexes formed during an allergic reaction. However, the finding of a normal C′4 titre with one of the C′4 titration techniques used and of an only slightly decreased C′2 titre would seem incompatible with such a view. The possibility exists, however, that different antigen–antibody systems differ in their ability to fix C′ components. All types of complexes do not necessarily affect the C′ system in the well-known way seen in experimental *in vitro* immune haemolysis (Osler, 1961; Nishioka & Linscott, 1963).

A certain degree of fibrinolysis was found in the EM patient in the acute phase. Lepow *et al.* (1958) have claimed that plasmin converts C′1 to C′1 esterase which then inactivates C′4 and C′2. However, on re-investigation of this problem, Laurell *et al.* (1965) showed that

patients with streptokinase-induced extreme fibrinolysis did not show any decrease of total C′, C′1 or C′4 titres and only a slight reduction of the β_{1C}-globulin concentration. Accordingly the slight plasmin activation in the EM patient would not be the cause of the decreased total C′ titre and the low β_{1C}-globulin concentration.

The results of this investigation suggest that different mechanisms may be responsible for the lowering of C′ activity in the three sera. In the HANE serum an enzymatic destruction of C′4 and C′2 is responsible for the low C′ titre. The pattern of C′ components in the SLE serum indicates a classical C′ fixation mechanism. The existence of normal C′4 and slightly reduced C′2 titres together with a markedly reduced β_{1C}-globulin concentration in the EM serum probably is indicative of a C′-disturbing mechanism different from that found in the SLE serum. The possibility exists that the variation in the pattern of C′ components and of β_{1C}-globulin in the EM serum is due to C′ fixation in an allergic reaction, since it was not possible to demonstrate either enzymatic destruction of C′ components or inhibitors of C′ in this serum.

It is evident that reduced titres of haemolytic C′ in pathological sera may be based upon quite different types of derangements in the C′ systems and that a depressed titre does not in itself prove that an immunological pathogenesis is operating.

ACKNOWLEDGMENTS

This investigation was supported by grants from the Swedish Medical Research Council (16X-68·01), from the Alfred Österlund foundation and from the Medical Faculty, University of Lund.

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