

# Serum complement levels in active ulcerative colitis

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**EDITORIAL SYNOPSIS** The serum complement activity was measured in 15 patients with active ulcerative colitis. Normal or raised levels were found in all but one patient and the relevance of these findings to the possible autoimmune nature of ulcerative colitis is discussed.

The present study was originally stimulated by Long's (1963) observations on patients with burns. Many patients became ill with a pyrexia 10 to 14 days after burning and often this illness could not be explained by infection. Long found very low serum complement levels in these patients and produced a striking clinical improvement by transfusing fresh defibrinated blood which restored complement levels to normal or above. The existence of low complement levels in cases of severe burns has recently been confirmed by Fjellström and Arturson (1963) although their four patients with low levels all died within seven days. Ulcerative colitis and burns have in common extensive destruction of superficial tissue with systemic upset and it seemed possible that similar changes in complement might occur in the two conditions.

An investigation of serum complement levels in ulcerative colitis also seemed interesting because it has been suggested that ulcerative colitis is an autoimmune disease (Thayer and Spiro, 1963a) and in some of these diseases changes in the serum level of complement have been detected (Ellis and Felix Davies, 1959; Williams and Law, 1958; Nastuk, Plescia, and Osserman 1960).

## METHOD

The most convenient system for measuring serum complement utilizes its action of lysing sensitized red cells; the method of Walton and Ellis (1958) was used in this study. The 50% end-point was determined and the results expressed in arbitrary units by dividing the dilution of serum giving 50% haemolysis by 25, *i.e.*, 1/10 dilution = 0.4 units per ml. and 1/250 dilution = 10 units per ml.

The cells used were formalized sheep red cells (Burroughs Wellcome) which are unstable and last for one to two weeks only. Several different batches of cells had to be used and, as Walton and Ellis (1958) have shown, there is considerable variation in cells between batches, and this was corrected by using a reference standard complement. This is essential when comparing

measurements made on different dates but has been ignored in some published studies on complement.

In this study a reference standard was made by taking 0.5 ml. aliquots from a pool of serum from 10 normal subjects, Richardson's preserving solution (Richardson, 1941) was added, and the aliquots were freeze dried in convenient ampoules. To check the reproducibility of the method six of these ampoules were titrated against the same batch of cells and gave a mean result of 1.19 units/ml. with a standard deviation of 0.03 units/ml. To correct for the variation between batches of cells, the standard was titrated against 13 different batches of cells on different dates and the mean obtained was used as the complement concentration of the reference standard. All titrations of unknown sera were run in parallel with a titration of the reference standard and results were corrected using this value.

The sera were kept at  $-70^{\circ}\text{C}$ . in a  $\text{CO}_2$  ice box and the variations was less than 3% over three months; no serum was stored for longer than three months. This finding conformed with that of Fischel, Pauli, and Lesh (1949) on the stability of complement at this temperature.

## RESULTS

Complement levels were measured in 10 normal subjects aged 20-60, six men and four women. The results are shown in Fig. 1; the mean level was 1.69 units/ml. with a standard deviation of 0.25 units/ml. Walton and Ellis (1958), from measurements on 143 adults, found a mean of 1.45 units/ml. with a standard deviation of 0.27 units/ml. None of the present results for normals was significantly different from those of Walton and Ellis at the 5% level.

Complement levels were then measured in 15 patients with active ulcerative colitis, aged 18-65, the majority being young adults, seven men and eight women. All of these patients had a constitutional illness associated with pyrexia, and on sigmoidoscopy the rectal mucosa appeared inflamed and haemorrhagic; in most, but not all, the whole length of the colon appeared to be diseased on a

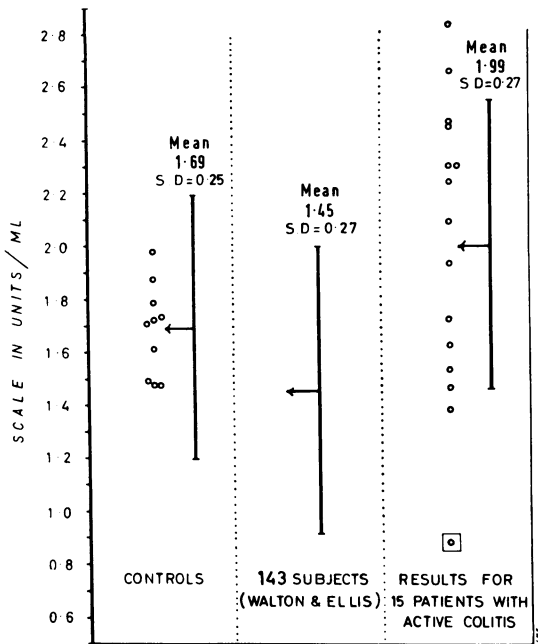


FIG. 1. Complement levels in controls, in 143 subjects from the series of Walton and Ellis (1958), and in 15 patients with active colitis.

barium enema examination. The results are shown in Figure 1. The mean complement level for the 15 patients was 1.99 units/ml. with a standard deviation of 0.27 units/ml. All but one (patient S.M.) of the results is within the range for normals or above it.

Sometimes a low serum complement level is due to anti-complementary activity of the serum. This was looked for in the one patient with a low complement level (patient S.M.). The complement was inactivated by heating at 56°C. for 10 minutes; dilutions of the heated serum were then added to a 1/20 dilution of the reference standard complement. The results (Fig. 2) show that anti-complementary activity was present. From the data it is possible to 'correct' the titration results of the active serum for anti-complementary activity at each dilution and this gives a 'corrected' complement level of 1.01 units/ml., still a low value. This patient was one of the illest in the series. When she had recovered the serum complement was measured again and had risen from 0.88 units/ml. to 1.27 units/ml., the latter figure being within the normal range.

In three patients serial measurements were made to see if steroids or A.C.T.H. affected the complement levels. The results are shown in Table I. In each case the complement levels rose, in two to above normal levels; there appeared to be no relationship to the activity of the disease.

TABLE I

SERIAL MEASUREMENTS OF COMPLEMENT LEVELS IN THREE CASES TREATED WITH STEROIDS		
Date	Serum Complement (units/ml.)	Clinical State
<i>Patient A.W.</i>		
24.5.63	1.53	Active colitis
31.5.63	1.53	Better on A.C.T.H.
7.6.63	2.33	Well, tailing off A.C.T.H.
<i>Patient M.C.</i>		
26.4.63	2.08	Active colitis
24.5.63	2.28	Colitis still active on prednisone or A.C.T.H. for 1 month
<i>Patient M.B.</i>		
15.7.63	1.93	Active colitis; no steroids
25.7.63	2.14	Colitis still active on A.C.T.H. for 10 days

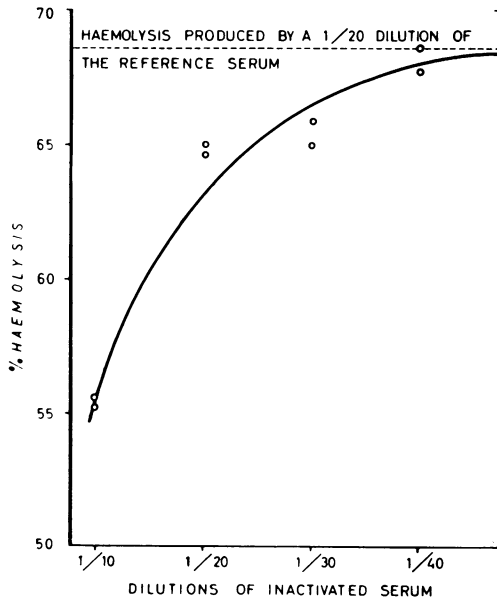


FIG. 2. Curve showing inhibition of complement by dilutions of inactivated serum.

Steroids or A.C.T.H. do not explain the normal or raised normal complement levels in the 15 patients reported here. None of these patients were receiving steroids at the time that serum was taken. Three patients had received steroids during previous relapses of the colitis but removing the results of these three patients makes no difference to the overall picture.

Finally, the complement level was re-estimated in one patient six weeks after total colectomy and it had not changed from its original high level of 2.64 units/ml.

#### DISCUSSION

Thayer and Spiro (1963b) have measured the level of serum complement in 15 patients with ulcerative colitis by the method of Kabat and Mayer (1961). The disease was in remission in 10 of their patients and the serum complement levels were normal. One patient in remission had a high serum complement level. Four other patients were acutely ill and receiving treatment and in these the complement levels were higher than normal. In the present study only patients who were systemically ill with active colitis were investigated and in this group the complement levels were normal or high in 14 of the 15 patients; this is in accordance with the findings in the four acutely ill patients of Thayer and Spiro (1963b).

There are many features of ulcerative colitis which suggest that it is an autoimmune disease. Broberger and Perlmann have supported this concept by showing that children with ulcerative colitis have circulating antibodies to specific colon antigens (Broberger and Perlmann, 1959; 1962). Recently they have been able to show that white blood cells from patients with colitis have a cytotoxic action upon colon cells in tissue culture and that this activity is increased by the presence of fresh serum, probably because of its complement content (Perlmann and Broberger, 1963).

One cause of a low serum complement level is a complement-fixing antibody-antigen reaction occurring *in vivo* (Stavitsky, Hackel, and Heymann (1954). A low level has been found in certain diseases where such reactions are thought to be important; this is so in systemic lupus erythematosus (Ellis and Felix Davies, 1959; Williams and Law, 1958), acute glomerulo-nephritis (Lange, Craig, Oberman, Slobody, Ogur, and LoCasto, 1951; Fischel and Gajdusek, 1952), and during the acute phase of myasthenia gravis (Nastuk *et al.*, 1960). Thus the finding of a low serum complement level suggests that such a reaction may be occurring. The present study reports normal and high levels of serum complement in patients with active ulcerative colitis. This does not suggest that complement is being fixed but, on the other hand, the finding of a high level of complement is not good evidence against an immunological mechanism for the following reasons. First, the antibody-antigen reaction may not fix complement; rheumatoid factor does not fix complement in the presence of altered gamma globulin and high serum

complement levels are found in active rheumatoid arthritis (Ellis and Felix-Davies, 1959; Williams and Law, 1958). Next, although antibody is reacting with an organ-specific antigen, the amount of complement fixation may be too small to influence the complement level in the serum. Finally, complement activity may rise when tissue is damaged and so counteract the effect of an immunological process. Fischel has shown in acute glomerulo-nephritis, a condition in which the serum complement is nearly always low, that an intercurrent inflammation, such as a dental abscess, will restore complement levels to normal (Fischel and Gajdusek, 1952).

High serum complement levels have been reported in rheumatic fever (Fischel *et al.*, 1949), erysipelas, scarlet fever, pneumonia, and following myocardial infarction (Boltax and Fischel, 1956). Boltax suggests that a high level is a non-specific acute phase phenomenon comparable to the E.S.R. and C-reactive protein changes (Boltax and Fischel, 1956).

Serial measurements on three patients in the present study showed a rise in the serum complement levels, in two to above normal, during treatment with steroids or A.C.T.H. Return of complement levels to normal following successful treatment with steroids of glomerulo-nephritis (Lange *et al.*, 1951; Ellis and Walton, 1958) (low serum complement) or rheumatoid arthritis (Williams and Law, 1958) (high serum complement) have been observed. A rise above normal with steroid therapy has not been reported.

Whatever the mechanism of the high levels of serum complement in active ulcerative colitis they contrast with the low levels reported in systemic lupus erythematosus (Ellis and Felix-Davies, 1959; Williams and Law, 1958). This is interesting as antinuclear factor has been found in both ulcerative colitis patients and their families (Calabresi, Thayer, and Spiro, 1961; Thayer and Spiro, 1963a).

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#### REFERENCES

- Boltax, A. J., and Fischel, E. E. (1956). Serologic tests for inflammation, serum complement, C-reactive protein and erythrocyte sedimentation rate in myocardial infarction. *Amer. J. Med.*, **20**, 418-427.
- Broberger, O., and Perlmann, P. (1959). Autoantibodies in human ulcerative colitis. *J. exper. Med.*, **110**, 657-674.
- , — (1962). Demonstration of an epithelial antigen in colon by means of fluorescent antibodies from children with ulcerative colitis. *Ibid.*, **115**, 13-26.
- Calabresi, P., Thayer, W. R., and Spiro, H. M. (1961). Demonstration of circulating antinuclear globulins in ulcerative colitis. *J. clin. Invest.*, **40**, 2126-2133.

- Ellis, H. A., and Felix-Davies, D. (1959). Serum complement, rheumatoid factor, and other serum proteins in rheumatoid disease and systemic lupus erythematosus. *Ann. rheum. Dis.*, **18**, 215-224.
- , and Walton, K. W. (1958). Variations in serum complement in the nephrotic syndrome and other forms of renal disease. *Immunology*, **1**, 234-250.
- Fischel, E. E., and Gajdusek, D. C. (1952). Serum complement in acute glomerulo-nephritis and other renal diseases. *Amer. J. Med.*, **12**, 190-196.
- , Pauli, R. H., and Lesh, J. (1949). Serological studies in rheumatic fever. II. Serum complement in the rheumatic state. *J. clin. Invest.*, **28**, 1172-1181.
- Fjellström, K. E., and Arturson, G. (1963). Changes in the human complement system following burn trauma. *Acta. path. microbiol. scand.*, **59**, 257-270.
- Kabat, E., and Mayer, M. M. (1961). In *Experimental Immunology*, 2nd ed., chap. 4. Thomas, Springfield, Illinois.
- Lange, K., Craig, F., Oberman, J., Slobody, L., Ogur, G., and LoCasto, F. (1951). Changes in serum complement during the course and treatment of glomerulonephritis. *Arch. intern. Med.*, **88**, 433-445.
- Long, D. A. (1963). Personal communication.
- Nastuk, W. J., Plescia, O. J., and Osserman, K. E. (1960). Changes in serum complement activity in patients with myasthenia gravis. *Proc. Soc. exp. Biol. (N.Y.)*, **105**, 177-184.
- Perlmann, P., and Broberger, O. (1963). *In vitro* studies of ulcerative colitis. II. Cytotoxic action of white blood cells from patients on human fetal colon cells. *J. exper. Med.*, **117**, 717-733.
- Richardson, G. M. (1941). Preservation of liquid complement serum. *Lancet*, **2**, 696-697.
- Stavitsky, A. B., Hackel, D. B., and Heymann, W. (1954). Reduction of serum complement following *in vivo* tissue antigen-antibody reactions. *Proc. Soc. exp. Biol. (N.Y.)*, **85**, 593-596.
- Thayer, W. R., and Spiro, H. M. (1963a). Protein abnormalities in ulcerative colitis patients and their families. *Gastroenterology*, **44**, 444-447.
- , — (1963b). Persistence of serum complement in sera of patients with ulcerative colitis. *J. Lab. clin. Med.*, **62**, 24-30.
- Walton, K. W., and Ellis, H. A. (1958). A method for serial determinations of serum complement. *Immunology*, **1**, 224-233.
- Williams, R. C., and Law, D. H. (1958). Serum complement in connective tissue disorders. *J. Lab. clin. Med.*, **52**, 273-281.

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