

# Complement activation and complement control proteins in acute pancreatitis

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**SUMMARY** Serum levels of the complement proteins C3, C4, C1 inhibitor (C1 INH), factor I (C3b inactivator) and factor H (BIH) and plasma levels of cleavage products of C3 (C3c) and factor B were measured in 26 patients with acute pancreatitis. Breakdown of C3 occurred in 19 patients, as shown by a reduction in C3 level and the presence of C3c. C4 levels, however, did not fall and factor B breakdown products were not detected, thus suggesting that enzymatic cleavage of C3 occurred without significant involvement of either the early classical pathway or the alternative pathway. C1 INH and factor H both showed increases, presumably reflecting an acute phase response. Factor I showed an initial fall followed by a rise. There was no correlation between the presence or extent of C3 breakdown and the clinical condition of the patients. It is concluded that C3 cleavage in pancreatitis probably results from tryptic activity and that the measurement of complement components has no part to play in the management of the disease.

In acute pancreatitis activated proteases are released into the local glandular structure. Their role in the pathogenesis of the disease, however, is controversial. Normally, the presence of circulating protease inhibitors ensures that released proteolytic enzymes are quickly complexed but, in dogs with acute pancreatitis induced by a closed duodenal loop, when the inhibitors become saturated active trypsin becomes detectable in the ascitic fluid. This may then activate other proteases<sup>1 2</sup> such as plasmin, Hagemann factor, and complement, which mediate an inflammatory reaction.

Interest has centred on the complement system as the mediator in acute pancreatitis. Not only are its components, in particular C3 and C5, susceptible to attack by trypsin<sup>3-6</sup> but in human and experimental pancreatitis plasma C3 levels have been found to be depressed, sometimes associated with the presence of circulating C3 breakdown products.<sup>7-11</sup> It has also been suggested that the magnitude and duration of such depression in patients gives an indication of the prognosis.<sup>8</sup>

We have observed, however, that serum levels of

C3 may actually rise in some patients with acute pancreatitis, due to an acute phase response, and therefore we felt it necessary to examine in more detail the relationship between changes in complement components and other factors. In addition to studying C3 and C4 we have also studied other complement proteins, including the inhibitors which modulate complement turnover, and also the alternative pathway protease, factor B.

## Methods

### PATIENTS

Twenty-six patients admitted to the Bristol and Southmead Health Districts between December 1978 and August 1979 with acute pancreatitis were entered into the study. The clinical diagnosis was confirmed by serum amylase levels exceeding 1000 Sömogyi units or 2000 IU (Phadebas). Venous blood collected on admission and daily for the next three to four days was analysed for C3, C4, C3 conversion products, factor B conversion products, C1 inhibitor (C1INH), factor I, and factor H.

Blood for complement estimation was collected into ethylene diamine tetra-acetate (EDTA) tubes, (Labco, Marlow, UK) after discarding the first few

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millilitres of the sample. It was promptly transported to the laboratory for separation of the plasma, which was either examined immediately or stored at  $-70^{\circ}\text{C}$ . These precautions eliminate *in vitro* complement breakdown.<sup>12</sup>

C3 levels were measured by laser nephelometry using a Hyland PDQ laser nephelometer (Hyland, Costa Mesa, California, USA). C1INH, factor I, and factor H were measured by electroimmunodiffusion.<sup>11</sup> Antisera to C3, to factor B and to C1INH were obtained from Atlantic Antibodies (Westbrook, Maine, USA) and to factor I and factor H from Seward Laboratories (London, UK). C3 and factor B breakdown products were detected by immunofixation<sup>14</sup> and were later quantified by scanning with an automatic computing densitometer (Gelman Instrument Co, Ann Arbor, Michigan, USA). The C3 fragment identified in pancreatitis serum by this technique may be either C3bi or C3c. We think that it is C3c because it has the same electrophoretic mobility as the C3c generated in aged serum and by trypsinisation. We have also identified C3d in pancreatitis serum in concentrations that parallel the breakdown fragment detected by immunofixation. This technique has a sensitivity of 0.02 g/l for C3c and 0.05 g/l for factor B breakdown products.

Standardisation of assays for C3, C4, and C1INH was performed against Atlantic Antibodies Calibrator. In the absence of available standards, values for factor I and factor H were expressed as a percentage of a normal serum pool derived from 20 adults. Normal ranges for these assays are: C3, 0.55–1.20 g/l; C4, 0.20–0.50 g/l; C1INH, 0.15–0.33 g/l; factor I, 66–146%, and factor H, 56–164%.

## Results

Sequential measurements of complement components are presented in Figs. 1–6 both as concentrations and as the percentage change over the measurement on the day of admission, day 0. The Table shows the significance of the differences between the levels on days 1 to 4 and day 0 tested by a paired Student's *t* test.

### COMPLEMENT BREAKDOWN PRODUCTS

C3 breakdown was considered to be significant if C3c levels were greater than 10% of the total C3; levels below this may sometimes occur because of faulty blood sampling or prolonged storage of blood before separation. C3 breakdown was seen in 19 of the 26 patients. Breakdown was maximal on day 1 and showed a slight tendency to decrease over the four days (Fig. 1). Factor B breakdown was not encountered in any sample.

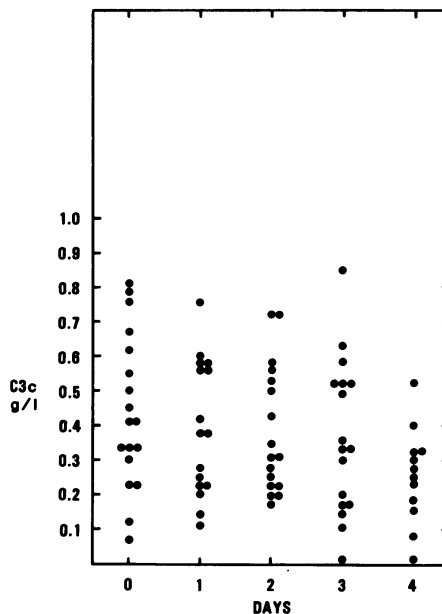


Fig. 1 C3c concentrations on the first four days after admission.

### COMPLEMENT COMPONENT LEVELS

Total C3 concentration fell during the first day in those patients with C3 breakdown products. In the remaining seven, C3 increased on day 3 (Fig. 2, Table). C4 showed a slight tendency to increase over the four days but these changes were not statistically significant (Fig. 3, Table).

### COMPLEMENT INHIBITOR LEVELS

C1INH and factor H both increased over the four days of the study. Factor I showed a slight fall followed by an increase (Figs. 4, 5, 6, Table).

### RELATIONSHIP OF COMPLEMENT CHANGES TO CLINICAL COURSE

We were unable to show any relationship between C3 breakdown or changes in other complement components and the clinical severity of the pancreatitis. Profound C3 breakdown occurred in some patients with mild symptoms, while at least one severely shocked patient showed insignificant levels of the breakdown product C3c.

## Discussion

The results confirm the findings of Goldstein *et al*<sup>7</sup> that C3 levels fall and C3 breakdown products are

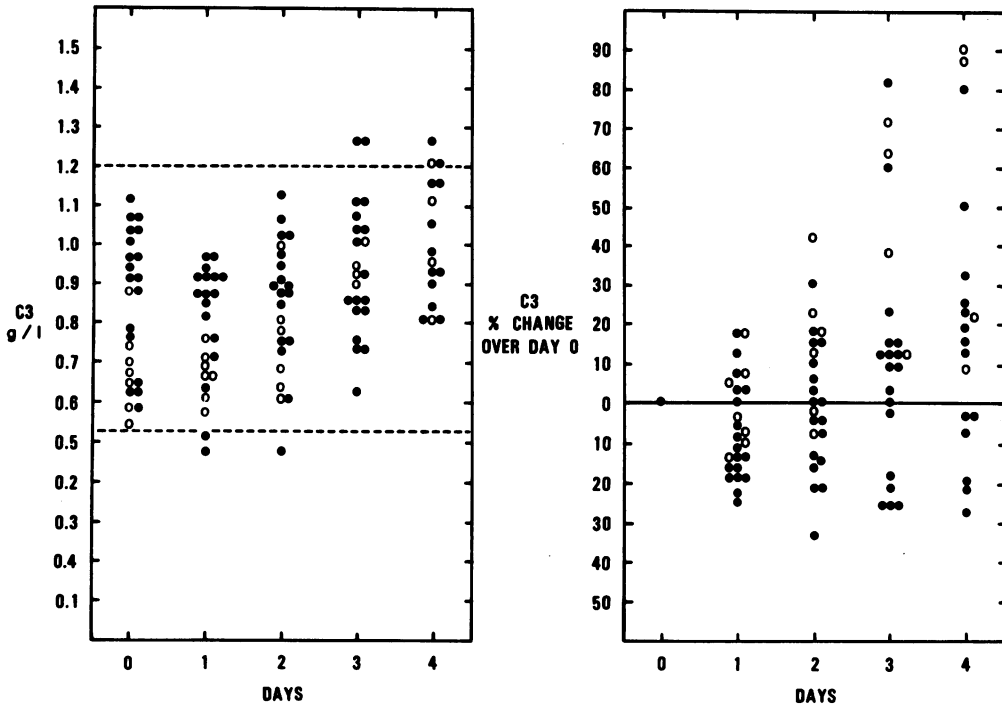


Fig. 2 C3 concentrations and percentage changes over the day of admission (day 0). Normal reference range is shown by interrupted lines. ● with complement activation. ○ without complement activation.

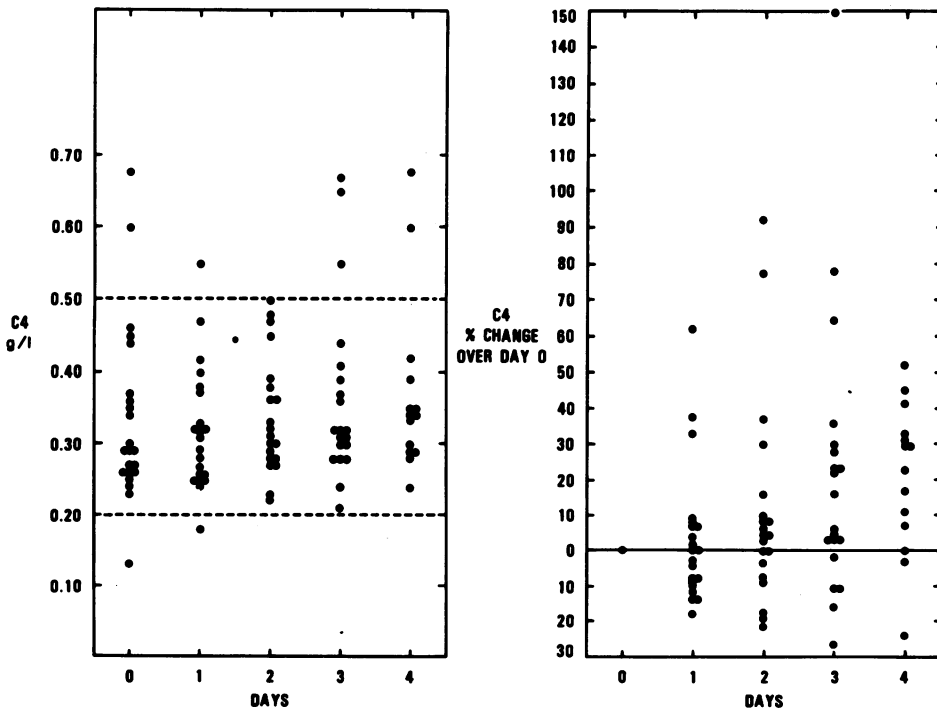


Fig. 3 C4 concentrations and percentage changes over the day of admission (day 0). --- Normal reference range.

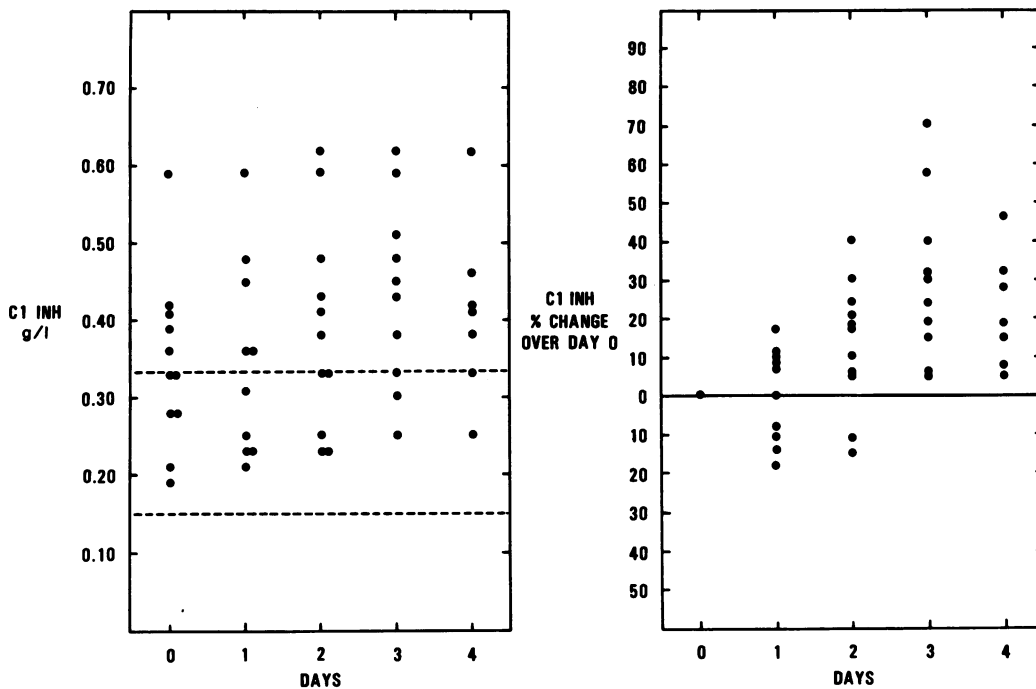


Fig. 4 C1INH concentrations and percentage changes over the day of admission (day 0). --- Normal reference range.

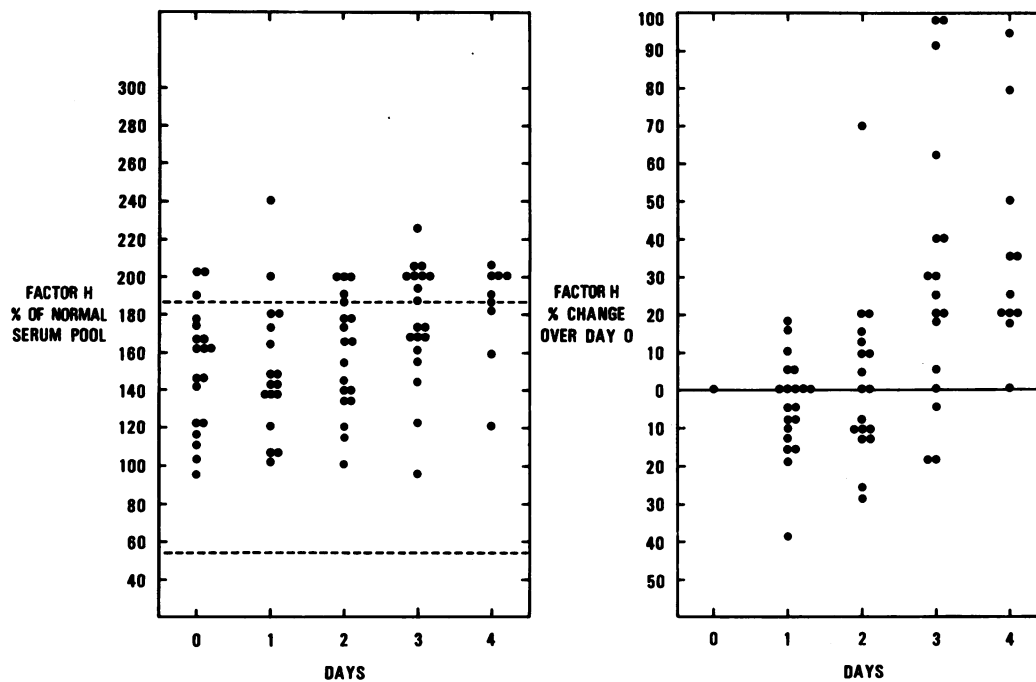


Fig. 5 Factor H concentrations and percentage changes over the day of admission (day 0). --- Normal reference range.

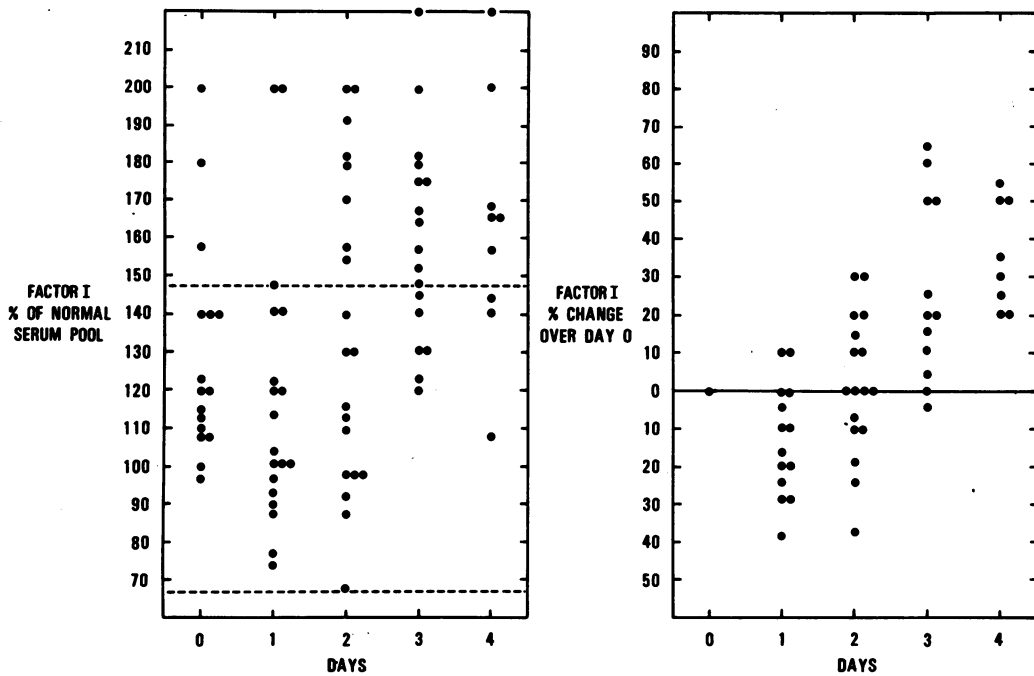


Fig. 6 Factor I concentrations and percentage changes over the day of admission (day 0). --- Normal reference range.

present in some patients with acute pancreatitis. In contrast we have shown no decrease in C4 levels and also an absence of factor B breakdown products.

There is good evidence that proteolytic enzymes – in particular, trypsin – cleave both C1 and C3.<sup>3-6</sup> The absence of a decrease in C4 levels in our patients suggests that cleavage of C3 is not mediated *via* activation of C1 but that it may be a direct effect of trypsin. The initial fall and subsequent rise in C3 concentration probably represents complement consumption as a result of breakdown followed by

the effects of an acute phase response in which complement levels are known to increase.<sup>12</sup> This interpretation is supported by the absence of an initial fall in C3 concentration in those patients who did not have C3 breakdown products in their plasma. The higher overall C3 levels in those patients with C3 breakdown suggests a more marked acute phase response in this group particularly on day 0. There was a tendency for those patients with higher initial C3 levels on day 0 also to have high factor I and factor H levels. It may thus be that the

Table Mean values ( $\bar{x}$ ) for complement components and significance of difference between values on day 0 and on subsequent days

Complement component	Days									
	0		1		2		3		4	
	$\bar{x}$	$p$	$\bar{x}$	$p$	$\bar{x}$	$p$	$\bar{x}$	$p$	$\bar{x}$	$p$
C3 (with breakdown)	0.89	0.01	0.81	0.01	0.87	NS	0.94	NS	1.0	NS
C3 (with no breakdown)	0.69	NS	0.67	NS	0.75	0.10	0.91	0.005	0.99	NS
C4	0.32	NS	0.32	NS	0.34	NS	0.35	NS	0.37	NS
C1INH	0.35	NS	0.35	NS	0.39	0.05	0.43	0.001	0.41	0.005
Factor I	127	0.05	117	0.05	135	NS	158	0.005	153	0.025
Factor H	140	NS	147	NS	160	NS	179	0.005	180	0.025

NS: Not significant.

inflammatory reaction was more severe in these patients, although this was not apparent clinically. The levels on day 0 are therefore in no way baseline values and reflect the effects of a disease process which has been proceeding for a variable period of time before admission to hospital.

The changes in concentrations of complement inhibitors support the suggestion that trypsin may be responsible for complement breakdown in pancreatitis. C1INH shows a significant increase in the acute phase in all patients regardless of the amount of C3 breakdown. There is thus no evidence of C1INH consumption as might be suspected if C1 activation were occurring. Both factor H and factor I showed a significant acute phase increase over the four days of the study. Factor I, however, showed a significant fall on day 1 suggesting the possibility of consumption by binding to C3b and subsequent rapid metabolism. This may occur because of the clearance of complexes from the circulation despite the fact that factor I is not consumed as a result of complement activation *in vitro*. The lack of a fall of factor H is interesting in view of the finding of Whaley *et al*<sup>15</sup> who saw a fall in the levels of both factor I and factor H in Gram negative endotoxic shock with C3 activation. Factor I enzymatically inactivates C3b to form C3bi. Factor H binds to C3b and potentiates the activity of factor I, but it also dissociates the alternative pathway convertases C3bBb and C3bBbP by displacing Bb from the complexes, thus allowing the conversion of the C3b. Significant alternative pathway activation with factor B breakdown occurs in Gram negative endotoxic shock possibly consuming factor H as a result of binding to factor B-containing complexes. The lack of fall in factor H in pancreatitis may thus be related to the absence of factor B activation.

It has been suggested that complement effector molecules may be responsible for some of the systemic pathological changes occurring in pancreatitis.<sup>8</sup> Lankisch *et al*,<sup>16</sup> measuring C3, C4, and total haemolytic complement activity, showed that the majority (24 out of 35) of the patients in their study had normal or raised levels. Eight patients who died of haemorrhagic pancreatitis, however, all had generally lowered or declining levels.

We believe that our findings suggest that C3 breakdown in pancreatitis is due to direct tryptic activation of C3 with rapid degradation of C3b to inactive C3c and C3d and a consequent lack of alternative pathway activation. Furthermore, such measurements appear to be of no clinical value in this condition. It is, however, important to appreciate that conclusions drawn from the measurement of complement component levels are at best tentative in acutely ill patients who may have

fluctuating plasma volumes. Turnover studies would be helpful in more clearly elucidating the changes which are occurring.

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