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Combined genetic deficiency of C6 and C7 in man

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SUMMARY

By routine screening of sera, a subject was discovered who showed a sub-total deficiency of C6 and C7. No clinical disease was associated with this deficiency which was transmitted through the subject's family as a single genetic characteristic, the C6 deficiency being associated with a silent allele at the structural locus. The propositus was found to have low quantities of an abnormal C6 which was both antigenically deficient and smaller in size than normal C6 (110,000 daltons compared with 140,000 daltons) and small quantities of apparently normal C7. It is concluded that the most likely explanation for this defect is that the subject has a structural mutation in his C6 gene which produces hyopsynthesis not only of C6 but also of the closely linked gene for C7. These findings suggest the possibility that C6 and C7 may function as a single genetic unit and that the primary transcript copied from the genome includes information for both proteins.

INTRODUCTION

Deficiencies of isolated complement components in man have been described with increasing frequency since the original description of the C2 deficient subject by Silverstein in 1960. The field has recently been reviewed in detail (Rosen & Lachmann, 1978), and the following generalizations can tentatively be made. Isolated genetic deficiencies have been discovered for all components of the classical complement pathway with the exception of Clq and C9 and of these deficiencies, C2 deficiency is much more common than any of the others. On the other hand, no isolated deficiencies of the factors peculiar to the alternative pathway, factors B, D and properdin have yet been described. The complement profile of the deficient subjects is in general normal for all the other components when the subjects are healthy. There is, however, an increased incidence of immune complex disease associated with deficiencies, particularly of the components of the classical pathway C3 convertase, and in such patients there may be changes in complement components due to excessive complement consumption. Despite this reservation, it does seem to be the case that all hitherto described complement deficiencies essentially affect a single component.

In this paper we describe a complement component deficiency of a type that has not previously been encountered: namely, one that apparently involved two complement components, C6 and C7. Our subject was found to show very low levels of both C6 and C7 activity and investigations have therefore been carried out to try to ascertain the nature of the genetic defect which he carries.

C6 and C7 are closely related complement components. They act sequentially in the complement sequence. Both C6 (Hobart, Lachmann & Alper, 1975) and C7 (Hobart, Joysey & Lachmann, 1978) show genetic polymorphisms and it has further been shown (Hobart *et al.*, 1978) that the loci for these two components are closely linked to one another. Furthermore, there are close physiochemical similarities

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between the two proteins (Podack, Kolb & Müller-Eberhard, 1976). For this reason it is wholly feasible to regard C6 and C7 as products of tandemly duplicated genes.

In this case a number of possible mechanisms might be put forward by which a genetic defect could give rise to a deficiency involving both components. For example, there could be a sizeable depletion involving the genetic region including the structural genes for both proteins, in which case no protein of either kind would be made. Alternatively there could be a smaller deletion overlapping the genes for the two proteins. In this case a number of possible outcomes might be envisaged. Abnormal proteins of both types could be made (an abnormal C6 and an abnormal C7), either or both of which could show some function; or one protein might not be made at all and the other be abnormal; or a 'Lepore' protein might be made comprising the N-terminal part of one protein and the C-terminal part of the other which might show some function of either or both of the parent molecules. Alternatively, the primary defect could be in the structural gene for one protein and the effect on the other protein be secondary, due either to an effect on its synthesis or possibly to hypercatabolism due to reaction with the abnormal protein. In this case one protein should be abnormal and the other normal in structure. Yet again, the genetic lesion could be in a control gene affecting the synthesis of both proteins. In this case both proteins should be structurally normal. Finally, the possibility that the observed deficiencies could be due to an inactivator of one or both components has also to be considered.

MATERIALS AND METHODS

Antisera. Anti-C6 was raised in a C6-deficient rabbit by immunizing with partially purified rabbit C6 as described by Lachmann (1970).

Anti-C7 was raised in a rabbit by immunizing with purified human C7. The antiserum was absorbed with C7 deficient human serum. Two separate sera were used. These were generously supplied by Dr A. Peltier and Dr H. Gewurz. Antisera to WFH C6 and WFH C7 were made by immunizing rabbits with precipitates made with the above two antisera and a concentrate of WFH serum.

Staphylococcal Protein A was obtained from Pharmacia and coupled to Sepharose using the Cynaogen Bromide method as described by Cuatrecasas (1970).

Measurement of complement components aud complement activity. The methods used for these purposes were performed exactly as described by Lachmann & Hobart (1978).

Polacrylamide gel electrophoresis. 10% acrylamide gels were employed using the discontinuous buffer system as described by Laemmli (1970). The precipitates were dissolved in 25μl Tris pH 10 + 25μl 2% SDS/8M urea 0·1M Tris pH 8 in the presence of 50mM dithiothreitol. The solutions were heated at 100°C for 2 min and applied to the gel. The gel was electrophoresed vertically overnight at 6mA, fixed in 25% TCA for 45 min and then stained with Coomassie Blue.

CLINICAL HISTORY

The propositus, WFH came to our notice on the basis of the routine screening of blood taken from patients attending the Outpatients' Clinics at Addenbrooke's Hospital in July 1976.

He is a white male born in January 1909, by occupation a plasterer although he retired from full-time work in 1974.

In his past history he had measles at the age of three and possibly again at the age of four. He had pneumonia at the age of five and an appendectomy was performed at the age of twenty-one. He was first seen at Addenbrooke's Hospital in 1952 complaining of an epigastric pain related to meals which, at that time, he had already had on and off for ten years. He was thought to have a duodenal ulcer although barium meal studies were negative. In 1954 he was seen again for similar symptoms. In 1955 he was seen in the orthopaedic clinic having dislocated his shoulder following a fall from scaffolding. In 1971 and again in 1973 he was seen in the Otology clinic with symptoms of Meniere's disease on the left side.This gradually improved on conservative treatment.

In 1976 he was again seen in Medical Outpatients with recurrence of his abdominal pain. It was on this occasion that his blood was first screened for complement. In 1976 barium meal and gastroscopy showed hiatus hernia with quite severe oesophagitis. Conservative treatment failed to provide relief and in October 1976 surgical repair of the hiatus hernia was undertaken with good results.

On the various occasions on which he has had blood taken since July 1976 he has always appeared in good health. There is no suggestion even on direct questioning of any undue liability to infections, of any arthritis or of any skin troubles.

Investigations

Blood count was normal. Coagulation screen was also normal. Serum immunoglobulins were: IgG 12·5g/l; IgA 2·0g/l; IgM 1·1g/l. All these values are within normal limits.

Chromosome studies were performed by Dr D. J. Bartlett. These showed WFH had a normal male chromosome complement.

Complement studies

His complement profile is shown in Table 1. It can be seen that all complement components measured were present in normal amounts except for C6 and C7 which were present in very low quantities. It is particularly noteworthy that his C5 levels were normal both functionally and antigenically. There was no detectable $C\overline{56}$ activity in his serum, nor could any $C\overline{56}$ be produced by activation with yeast.

TABLE 1. WFH Complement Profile

Component	Functional	Antigenic
C1	68%	
C2	92%	
C3		0·85mg/ml
C4	76%	
C5	80%	>100%
C6	1–5%	Very low (protein abnormal; accurate quantification not possible).
C7	3–9%	Low (approx. 5% of NHS)
C8+C9	360%	
$C\overline{1}$ Inh.	>100%	150%
KAF		126%
β1H		150%
Factor B	80%	
Factor D	80%	

Percentages are given as those of a normal human serum pool. The values for C6 and C7 were measured in a number of different samples.

Genetic history

The propositus is the youngest and only surviving of the fourteen children of JTH who died in 1923, aged 66 and IW who died in 1945, aged 82. He believed his parents to have been first cousins. His father, JTH, had six siblings all of whom he believes lived a normal life span. His mother IW had about eight siblings all of whom, as far as he knows, lived their normal life span. Of his thirteen siblings, one was killed in the first world war and the rest also lived normal life spans. WFH himself has been married twice (see Family Tree). By his first wife he had five children and by his second wife one further child. His children and his grandchildren are all alive and well. The Family Tree is shown in Fig. 1.

In the propositus no C6 allotype can be detected and levels of C6 and C7 are close to zero. All his six children have approximately half normal levels both of C6 and of C7 compatible with a heterozygous deficiency state. C6 alloytpes of the five children of the first marriage are all C6A and it is presumed that this was the phenotype of his first wife. The one child of the second marriage is C6B, as is her mother. These findings suggest hemizygosity at the C6 locus for the children. That this is the case is confirmed by consideration of the grandchildren. Here it can be seen that the six children fall into two groups: those who carry only the paternal C6 allele (C6B) and who have approximately half normal levels of both C6

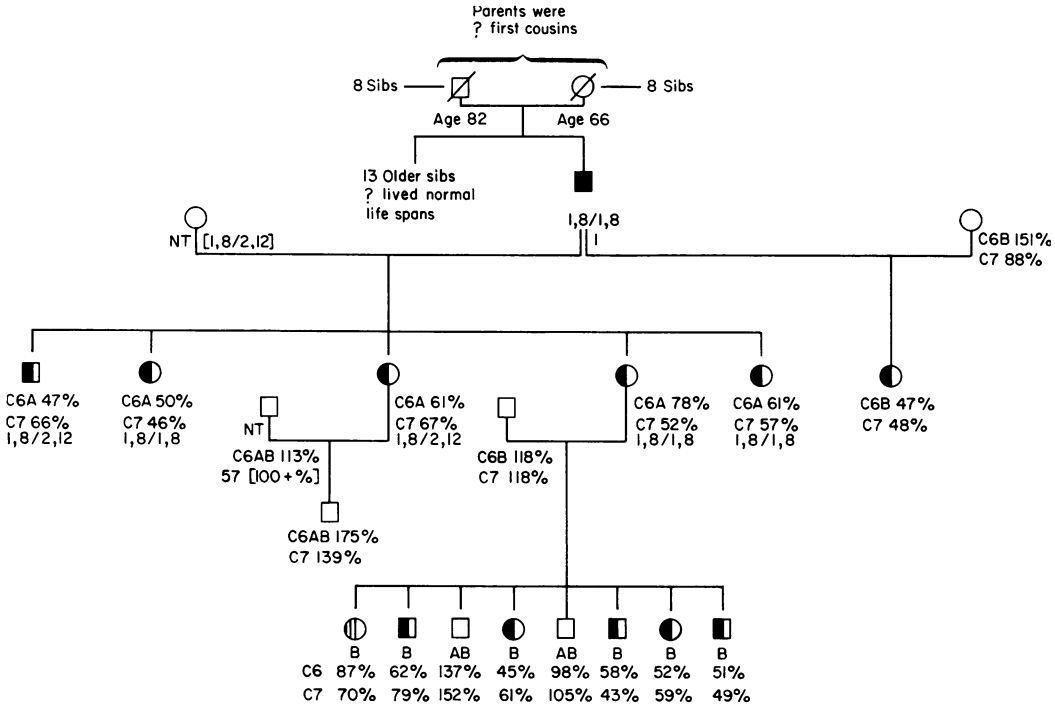

FIG. 1. Family tree of WFH. Levels of allotypes of C6 are shown. Levels of C7 are shown. No allotype other than $C7^1$ was found in the family. HLA types are shown where they were obtained. They are not informative in this family.

and C7; and those who are phenotypically C6AB and who have normal levels. The observation that a C6A mother can produce C6B children establishes the hemizygosity in both mother and offspring. The consideration of the grandchildren also confirms that the abnormality in C6 and C7 is transmitted as a single genetic character.

STUDIES OF THE C6 AND C7 IN WFH SERUM

The absence of inhibitory factors towards normal C6 or C7 in the serum

No inhibition of either C6 or C7 could be detected when WFH serum was mixed with either normal serum or, in the case of C7, with a purified component. Details of the experiments are given in Tables 2 and 3. Table 2 shows an experiment carried out in the cuvette of a continuously recording spectrophotometer maintained at 37°C. The technique used is to measure the time taken to achieve the 50% lysis as described by Lachmann & Hobart (1978). The test reagent was 0·5ml of 0·1% EAC43 (Antrypol) containing 5% C6 deficient rabbit serum. To this, 0·5ml of the test reagent was added and the mixture put into the spectrophotometer. Loss of turbidity at 600nm accompanies haemolysis and typical sigmoid curves are

TABLE 2. To test whether WFH serum inactivates C6

Test reagent	50% Lysis time
Normal human serum 1/50	5·0 min
WFH serum 1/5	(15% lysis at 30 min)
WFH serum 1/5 in NHS 1/50 (pre-incubated for 15 min at 37°C)	5·0 min

TABLE 3. To test whether WFH serum inactivates C7

Time of incubation	0	30 min	60 min	120 min
WFH serum undiluted	81%	110%	128%	128%
WFH serum undiluted. (Heated at 56°C for 30 min.)	95%	128%	110%	102%

C7 preparation added to 4 vol. of WFH serum and after incubation as shown tested on C7 plate.

Figures are percentage of activity of C7 preparation (dil 1/5 in diluent).

The figures shown are C7 activity measured as a percentage of the activity of the purified C7 preparation when diluted 1/5.

obtained with time. This allows the time taken to achieve 50% lysis to be measured accurately. In a further experiment 1 vol. of a purified C7 preparation was added to 4 vol. of WFH serum and incubated as shown (Table 3). The samples were then tested on a standard C7 plate as described by Lachmann & Hobart (1978).

The fractionation of C6 and C7 from WFH serum

In view of the very low concentrations of these factors present in WFH serum and the limited amount of serum available, no attempt at complete isolation was made.

Salt fractionation. The great majority of the activity of both C6 and C7 could be precipitated with 20% sodium sulphate (the addition of 20g sodium sulphate/100ml solution), or by 40% saturation with ammonium sulphate. The latter preparation is somewhat less contaminated with other proteins. When these salt fractions were dialysed against 20mM phosphate buffer at pH 5·4 the bulk of both the C6 and the C7 activity appeared in the euglobulin.

The salt fractionation, particularly with ammonium sulphate, was a convenient way of concentrating the material sufficiently for antigenic and functional analysis and most of these analyses were carried out with a preparation of the ammonium sulphate precipitate concentrated to about 1/10 vol. of the original serum.

Gel filtration on G-200 Sephadex. About 2·0ml of a salt fraction of WFH serum was fractionated on a 2·5 cm × 100 cm column of G200 Sephadex in phosphate buffered saline pH 7·2, containing 0·5M NaCl and 0·01M sodium azide.

The elution pattern is shown in Fig. 2. Only two peaks were seen after albumin was removed by the salt fractionation. Pools were made as shown and tested for protein and for C6 and C7 in haemolytic diffusion plates. It can be seen that C6 and C7 activities both peak on the descending slope of the '7S' peak, their usual position. There is no suggestion of any larger molecule carrying either activity.

DEAE fractionation. A euglobulin precipitate of 20% sodium sulphate fraction of WFH serum was fractionated by ion exchange chromatography on DEAE. The starting buffer was 10mM phosphate pH 7·0 and the excluded peak contained neither C6 nor C7 activity and was discarded. Elution was carried out with a gradient to 200mM sodium chloride in the starting buffer. The starting material was dialysed against the starting buffer and applied to the column. A concave gradient of the eluting buffer was applied. The pools were then tested for their protein content (Folin reaction) and their antigenic level of C3, and C6 activity by tube titration and C7 activity in a haemolytic diffusion plate were measured. The results are shown in Table 4. It can be seen that C6 activity appears abnormally early, there being a considerable amount of C6 before any C3 appears. In our hands C6 normally elutes at a conductivity of between 9 and 11K. The C7 peaks at approximately its normal value but it may trail rather further than is usually found. However, the extent of trailing varies from column to column and whether this is a genuine difference from normal is not clear. It is, however, clear that C6 and C7 are not completely superimposable, although the separation that can be achieved between them is less than is usual in this type of DEAE fractionation.

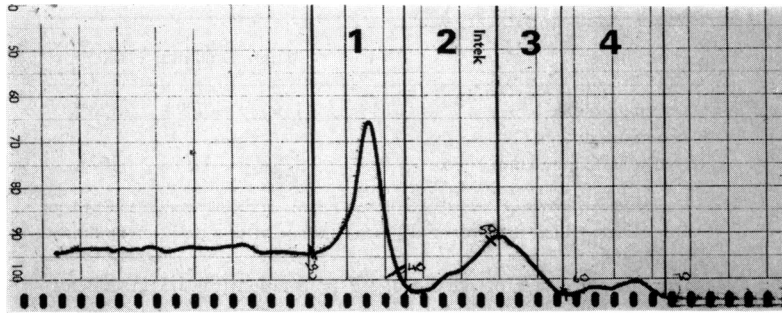

FIG. 2. G-200 Sephadex Gel Filtration of a salt fraction of WFH serum. WFH serum was precipitated with 20g/100ml of Na_2SO_4. The precipitate was dissolved in minimum water and applied to a 2·5cm × 100cm G200 Sephadex column equilibrated with 0·5M NaCl, 0·01M phosphate buffer pH 7·2, 0·01M Na Azide and 0·01M EDTA. Pools were made as shown on the optical density trace and concentrated to the volume of the original serum by 20% Na_2SO_4 precipitation. Pool 1 is the exclusion peak; Pool 2 is the ascending slope of the '7S' peak; pool 3 is the descending slope of the '7S' peak and pool 4 is the 'albumin' peak. This is small because the albumin has been left in the supernatant of the salt precipitate.

Pool	1	2	3	4	
(Protein) mgm/ml	0·62	1·6	2·8	0·34	
C6	0	Tr.	6%	0	percentage of normal
C7	0	4%	8%	3%	human serum

TABLE 4. Fractionation of WFH C6 and C7 on DEAE cellulose

Pool	Conductivity micromhos	(P) mg/ml	C3 antigenically % NHS	C6 (titre)	C7 plate approx. % NHS
1	3·7–4·9	0·17	0	0	9
2	5·0–6·3	0·29	0	4	19
3	6·4–7·1	0·70	0	16	21
4	7·2–9·6	5·0	650	16	19
5	10–15·8	5·5	330	16	11
6	16–19·5	2·7	54	1	5

C6 activity appears abnormally early, i.e. well before C3 (normal C6 elutes maximally between 9 and 11K).

C7 trails rather more usual, i.e. well into C3 (normal C7 has almost all eluted before C3 peak).

C6 and C7 activities are not superimposable.

Antigenic analysis

Ouchterlony plates are shown in Figs 3 and 4. It can be seen that the C6 activity in WFH is antigenically deficient compared to normal C6 (Fig. 3b) whereas the C7 appears to be antigenically entirely normal (Fig. 3a). Antisera were raised against a C6 anti-C6 precipitate made using WFH serum as antigen, and similarly to an anti-C7 precipitate. Neither of these antisera detected any antigenic material in WFH serum that was not also present in normal human serum (not shown).

Considerable efforts were made to see whether WFH serum showed any evidence of antigenic material

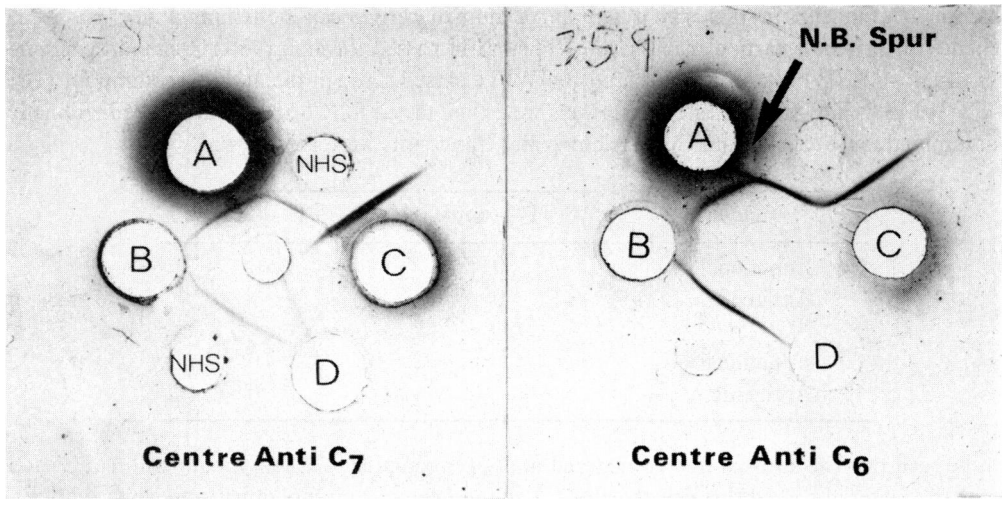

FIG. 3. Antigenic analysis of WFH C6 and C7 on Ouchterlony plates. (A) WFH 0–37·5% saturated amonium sulphate; (B) WFH 37·5–50% saturated ammonium sulphate; (C) WFH + anti-C6 0–40% saturated ammonium sulphate and (D) WFH + anti-C6 40–50% saturated ammonium sulphate.

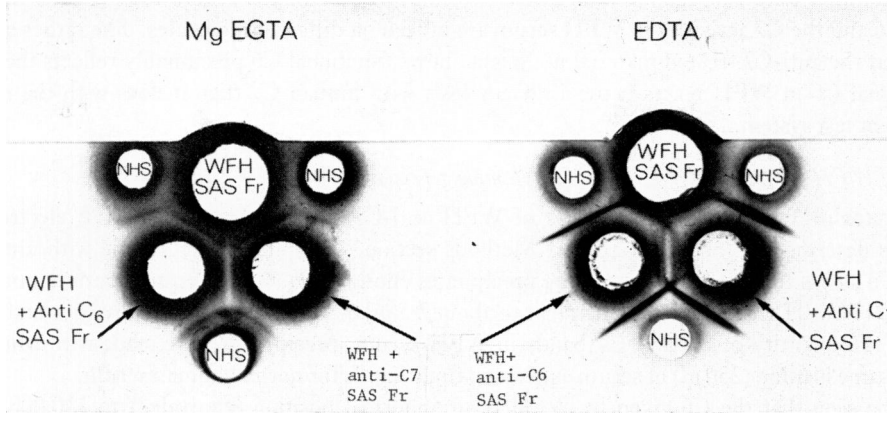

FIG. 4. Antigenic analysis of WFH C6 and C7 on Ouchterlony plates. The left hand plate is made up in 10mM EGTA and 7mM magnesium to allow activation of the alternative pathway by the whole human sera. The ammonium sulphate fractions do not contain factor B and therefore cannot activate the alternative pathway even in the presence of magnesium EGTA.The right hand plate is made up in 10mM EDTA in the usual way. SAS fraction is a fraction precipitated by 40% saturation with ammonium sulphate. Note: there are two lines between the WFH containing anti-C7 and the WFH containing anti-C6, and the C6 and C7 lines given with normal human serum cross in the EDTA plate but fuse in the EGTA plate, where $C\overline{567}$ has been formed.

reacting both with anti-C6 and anti-C7. In normal human serum C6 and C7 show no antigenic cross-reactivity providing that the Ouchterlony plates are run in EDTA. When the plates are run in magnesium EGTA (allowing the activation of complement to form $C\overline{567}$) then fusion between the C6 and C7 lines can be observed (see Fig. 4).

In order to study this cross-reaction with WFH serum some further reagents were used. Two 1·0 ml sepharose protein A columns were prepared.

5·0 ml of rabbit anti-human C6 antiserum were applied to one column and the column then washed until no further protein eluted. A second similar column was treated in the same way with anti-C7. 5·0 ml of the WFH serum was then slowly filtered and the eluted material collected and concentrated by precipitation with 40% ammonium sulphate. The precipitate formed in the eluted WFH serum was removed by ultracentrifugation. The IgG in the human serum competes for the IgG bound to the

Sepharose column and in this way a sample of human serum can be obtained that is specifically enriched with IgG of a particular antiserum. The WFH passed through the IgG column contained an excess of anti-C6, (WFH + anti-C6), and the WFH passed through the anti-C7 column an excess of anti-C7 (WFH + anti-C7). These preparations were then tested for C6 and C7 activity functionally and were examined for reaction on an Ouchterlony plate. The results were as follows:

		WFH + anti-C6	WFH + anti-C7
C6	Functional	0	0
	Antigenic	0	+
C7	Functional	+	0
	Antigenic	+	0

It can be seen that the anti-C6 treated material no longer contained C6 activity but still had C7 activity, whereas the material passed through the anti-C7 column showed no functional activity for either C6 or C7. On the Ouchterlony plates it can be seen that the material passed through the anti-C6 column contained anti-C6 giving a line of precipitation with C6 in normal human serum as well as with WFH serum and that similarly, material passed through the anti-C7 column contained anti-C7. When the two preparations were run against each other two lines of precipitation were found. This must show that the C6 and the C7 activities in WFH serum are indeed on different molecules. The rather surprising finding that the anti-C7 treated material no longer shows functional C6 presumably reflects the fact that the abnormal C6 in WFH reacts better in haemolysis with human C7 than it does with the rabbit C7 in a different test system.

Analysis of WFH C6 and WFH C7 by polyacylamide gel electrophoresis

Precipitates of WFH with anti-C6 and of WFH anti-C7 were analysed by PAGE electrophoresis (Fig. 5) as described in the Materials and Methods section. Precipitates were made with anti-C6 and anti-C7. To assess the bands present in the precipitates enough of a 40% saturated ammonium sulphate precipitate of WFH serum (250 μl equivalent to about 2·5 ml of serum) was used to give optimal banding. To assess the quantity of C6 and C7 bands in WFH serum, precipitates were made with whole serum using the same loading (250 μl) of serum as gives optimal bands for normal human serum.

It can be seen that the C6 given by WFH is an abnormal position (equivalent to 110,000 daltons), being smaller than normal C6 (about 140,000 daltons) and close to, but nevertheless distinct from, the normal C7 bands. On the other hand, C7 in WFH serum appears in the entirely normal position (about 110,000 daltons). A contaminating band can be seen coming up in both antisera, but this is also present in normal serum and in C7 deficient serum and its nature has not so far been studied further. It is, however, too large to be one of the chains of Clq, which might have been expected to bind to the precipitates.

Isoelectric focusing with functional techniques

The salt fractions of WFH serum were subjected to isoelectric focusing on polyacrylamide gels according to the techniques described by Lachmann & Hobart (1978). The plates were developed for C6 and for C7. The bands were extremely difficult to obtain and somewhat difficult to see and for this reason Fig. 6 shows a drawing rather than a photographic representation. It can be seen that C6 and C7 bands given by WFH are in a rather similar position to each other, a position which is distinct from normal C6 banding of any of the known alleles and also somewhat distinct from the normal $C7^1$ pattern. The C7 bands may, however, represent a C7 allele other than $C7^1$.

When the serum was reacted with anti-C6, the C6 bands disappeared but the C7 bands persisted. However, when the serum was treated with anti-C7 both sets of bands disappeared. This is the same observation made in the experiments described above using Sepharose-protein A bound anti-C6 and

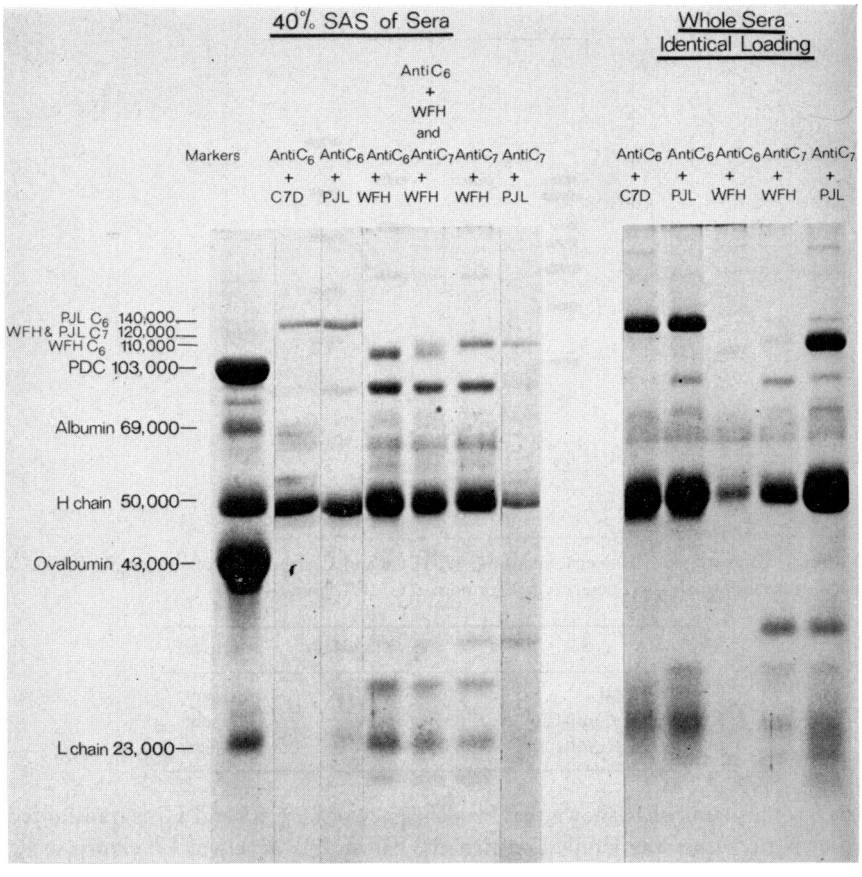

FIG. 5. 10% slab SDS-polyacrylamide gels of precipitates made as shown on top of each column; WFH being the propositus with C6 + C7 deficiency; PJL being the normal control, and C7D being serum from a patient with an isolated deficiency of C7 (and a normal level of C6) kindly donated by Dr H. Gewurz. PDC is the E_1 component of pyruvate decarboxylase of the pyruvate dehydrogenase multi-enzyme from *E. coli* kindly donated by Dr R.A. Harrison. In the left hand section 40% SAS precipitates were used in such a quantity as to give optimal proportion precipitation. In the right hand section whole serum was used and WFH was used in a similar loading to the normal serum. The unlabelled band seen with WFH at about 85,000 on the left hand section is not peculiar to this serum but is seen well because of the very high protein loading.

anti-C7. It seems likely that the abnormal C6 does not react sufficiently well with rabbit C7 to give bands on isolectric focusing and the C6 bands are therefore formed only in the C6-7 overlap regions on the gel. For the same reason anti-C7, by inhibiting the human C7, prevents the formation of bands with C6 altogether.

It can be concluded from the fractionation data and the antigenic and PAGE analysis that C6 in WFH is present in very low amounts; i.e. smaller in size than normal C6 by about 30,000 daltons, and that it is antigenically deficient. C7 is also present in low amounts, but it is normal in molecular weight and antigenicity. On isoelectric focusing, however, the C7 band pattern is out of pattern with the common $C7^1$ allele.

DISCUSSION

WFH was discovered on the routine screening of outpatient blood to be deficient in haemolytic complement. Investigation of his defect revealed that he has a hitherto undescribed form of genetic complement deficiency, one affecting two components. The deficiency for both components is less than total.

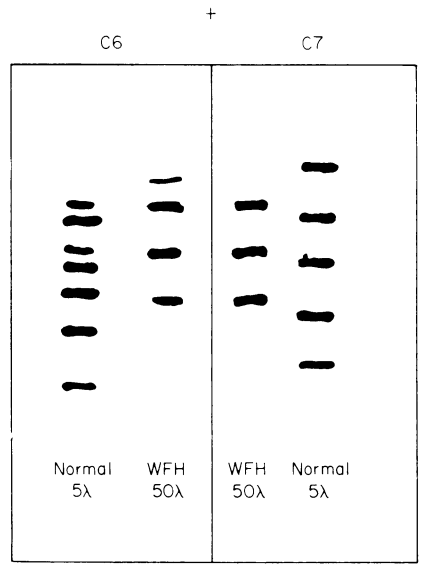

FIG. 6. C6 and C7 patterns by isolectric focusing. WFH C6 and C7 bands are of similar pI. WFH C6 bands are not the same as normal ($C6^{AB}$ heterozygote) or normal C7 ($C7^{1}$ homozygote).

	C6 bands	C7 bands
WFH 50μl	Present	Present
WFH Tr. anti-C6	Absent	Present
WFH Tr. anti-C7	Absent	Absent.

Genetic analysis of his family has shown that his deficiency of both C6 and C7 is transmitted as a single genetic characteristic within the family and that the half normal levels of C6 shown in heterozygotes are accompanied by hemizygosity for the C6 allele. This proves conclusively that the effects on C6 must be due to hyposynthesis and not hypercatabolism. Unfortunately, the family shows no polymorphism at the C7 locus so that it is not possible to ascertain whether the heterozygotes are hemizygous for the C7 structural marker. Since we have so far not felt it justified to subject WFH to metabolism studies we cannot be certain that the low levels of C7 are due to hyposynthesis, but the transmission of half normal levels to the heterozygotes makes this very likely.

Analysis of the C6 and C7 activities in WFH serum have shown that the C6 is an abnormal protein being both deficient antigenically and smaller in size than a normal C6. The C7, on the other hand, is normal, both antigenically and in size. It is therefore clear that there is a defect in the structural gene for C6 since an abnormal C6 protein is being produced, albeit at a very low level. There is however no evidence of any defect in the structural gene for C7 to account for its low level. These findings exclude most of the explorations mentioned in the introduction. Thus, no evidence has been found for a Lepore protein or for a deletion involving any part of the C7 structural gene. Nor is a control gene the site of the primary defect.

The possibility that the abnormal C6 binds to C7 and causes its inactivation can be excluded as far as *in vitro* studies are concerned, although in the absence of metabolism studies it is still not possible to exclude finally the possibility that the abnormal C6 may cause abnormally rapid *in vivo* catabolism of itself and of C7. It seems much more likely to us, however, that the C7 deficiency is due to a defect in synthesis and that this is occurring in consequence of the structural mutation at the C6 locus. It is known from studies of C7 polymorphism (Hobart *et al.*, 1978) that C6 and C7 are closely linked loci. In WFH, therefore, we seem to have a situation where a structural mutation in one gene causes hyposynthesis not only of its own abnormal product but also of the apparently normal product of a closely linked gene. However, isolated deficiencies of C6 have been reported which do not show C7 deficiency

and vice versa (see Rosen & Lachmann 1978), so hyposynthesis of C7 is clearly not an invariable accompaniment of the absence of C6.

The mechanism by which a structural mutation brings about the hyposynthesis of the product of a neighbouring cistron in eukaryotes is not definitely known. However, in view of the new information on the immunoglobulin genes, for example, showing that the primary RNA transcript copied from the nuclear DNA is larger than the messenger RNA found on the polysomes (Gilmore-Herbert & Wall, 1978), one may speculate that the same phenomenon may occur for these complement components and that the primary transcript of the C6-C7 region of DNA may contain message for both these cistrons. In this case it would not be surprising that a structural mutation, which might interfere with transcription or post-transcriptional events at the C6 locus, could have similar effects upon the C7 locus. If this explanation is correct it might also be suggested that the C6 gene should lie on the 'N terminal' or 5′ side of the C7 gene.

Although exactly this type of defect has, to the best of our knowledge, not been described previously in man, it has been reported (e.g. by Sussman *et al.*, 1973) that a subject homozygous for C2 deficiency had an apparently isolated low factor B level. Since factor B is closely linked to C2, a similar explanation might apply—although in this case hypercatabolism of factor B due to excess use of the alternative pathway would be more plausible than in the case of C7. Among C4 deficient guinea-pigs a number have low C2 levels (Ellman, Green & Frank, 1970) which could again reflect a similar mechanism since C4 and C2 are closely linked. However, the low C1 levels also sometimes found in these animals must have a different explanation since the C1 genes are not known to be linked to C2 or factor B.

It is also of interest that WFH has enjoyed good health throughout his life. He is the youngest of many siblings, all of whom apparently lived their normal life span and in whom neither immune complex disease nor neisserial infections appear to have been troublesome.

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REFERENCES

CUATRECASAS, P. (1970). Protein purification by chromatography. *J. Biol. Chem.* **245**, 3059.

ELLMAN, L., GREEN, I. & FRANK, M. (1970). Genetically controlled total deficiency of the fourth component of complement in the guinea pig. *Science*, **170**, 74.

GILMORE-HERBERT, M. & WALL, R. (1978). Immunoglobulin light chain messenger RNA is processed from large nuclear RNA. *Proc. Nat. Acad. Sci.* **75**, 342.

HOBART, M.J., JOYSEY, V. & LACHMANN, P.J. (1978). Inherited structural variation and linkage relationships of C7. *Immunogenetics*, **5**, (in press).

HOBART, M.J., LACHMANN, P.J. & ALPER, C.A. (1975). Polymorphism of human C6. *Protides of the Biological Fluids. 22 Colloquium* (ed. by H. Peeters), Pergamon Press.

LACHMANN, P.J. (1970). C6 deficient in rabbits. *Protides of the Biological Fluids. 17 Colloquium* (ed. by H. Peeters), Pergamon Press.

LACHMANN, P.J. & HOBART, M.J. (1978) Complement technology. *Handbook of Experimental Immunology* (ed. by D.M. Weir). Chap. 5A. Blackwell Scientific Publications, Oxford.

LAEMMLI, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*, **227**, 681.

PODACK, E.R., KOLB, W.P. & MÜLLER-EBERHARD, H.J. (1976). Purification of the sixth and seventh components of human complement without a loss of haemolytic activity. *J. Immunol.* **116**, 263.

ROSEN, F.S. & LACHMANN, P.J. (1978). Complement disorders. *Seminars in Immunopathology. Immune Deficiency.* (Ed. by H. Müller-Eberhard and P. Miescher) Springer International, (in press).

SILVERSTEIN A.M. (1960). Essential hypocomplementaemia. Report of a case. *Blood*, **16**, 1338.

SUSSMAN, M., JONES, H., ALMEIDA, J.D. & LACHMANN, P.J. (1973). Deficiency of the second component of complement associated with an anaphylactoid purpura-like rash. *Clin. exp. Immunol.* **14** 531.