

Evidence for *In Vivo* Breakdown of β_{1C} -Globulin in Hypocomplementemic Glomerulonephritis *

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Summary. Evidence has been obtained for the presence *in vivo* of α_{2D} -globulin, a breakdown product of serum β_{1C} -globulin, in patients with acute and persistent hypocomplementemic glomerulonephritis. The protein has been identified by immunoelectrophoretic analysis, and the amounts present have been determined by direct measurement of specific antigenic determinants present on α_{2D} . β_{1A} -Globulin, another breakdown product of β_{1C} -globulin, may also be present *in vivo* in severely hypocomplementemic patients, but its levels are much lower than those of α_{2D} -globulin.

α_{2D} -globulin has been identified by immunoelectrophoretic analysis of fresh EDTA plasma from patients with hypocomplementemic nephritis as an arc in the α_2 region that shows a reaction of identity with the arc representing α_{2D} -globulin produced by aged normal serum. β_{1A} -Globulin was not seen in these patterns.

Measurement of specific antigenic determinants has been carried out in both fresh EDTA plasma and aged serum. In the fresh plasma, the concentration of D antigen, found on both β_{1C} - and α_{2D} -globulins, has been related to that of B antigen, found only on β_{1C} and taken as a measure of the concentration of this protein. In the hypocomplementemic patients, the concentration of D antigen, in comparison to that of B, was greater than in the normal subjects. Similarly, in aged serum, the level of α_{2D} was greater than would be expected from the amount of β_{1C} that had been broken down *in vitro*, measured by the concentration of β_{1A} .

Calculations indicated that the *in vivo* α_{2D} level in severely hypocomplementemic patients ranged from 7.5 to 18% of that which would be found in a pool of aged normal serum in which β_{1C} is completely broken down. The levels tended to be lower in less severely hypocomplementemic patients, and none could be detected in normal plasma.

Only small quantities of A and D antigens are detectable in the urine of patients with hypocomplementemic nephritis. The rate of excretion is about equal to that of the normal subject.

The study indicates that the low serum levels of β_{1C} -globulin that may be present over long periods in patients with persistent hypocomplementemic glomerulonephritis can be ascribed, in part, to *in vivo* breakdown of this pro-

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tein as a result of reaction with immune complexes. The contribution of β_{1C} deposition on immune complexes and of diminished synthesis to the depressed serum levels cannot be assessed by the present study.

Introduction

A type of persistent glomerulonephritis has recently been described (1-4) in which the serum β_{1C} -globulin concentration remains very low over periods of many months' duration. The persistently low levels of this protein, normally the most abundant of all of the complement components in serum, could be explained in several ways. The protein may be taken up by immune complexes responsible for the disease, and indeed, immunofluorescent techniques have shown deposition of β_{1C} -globulin as well as of IgG in the glomerular capillary walls (3). However, over the long periods of serum depletion observed, the amounts accumulating in this manner would become very great, making continued deposition as the complete explanation unlikely.

Alternatively, the low level could be explained by diminished synthesis of β_{1C} -globulin. Studies employing isotopically labeled β_{1C} have shown that the rate of disappearance of the label in hypocomplementemic subjects is identical to that in normal subjects (5). If this technique truly measures β_{1C} disappearance, it must be concluded that the rate of synthesis of the protein is diminished.

A third possible explanation for the persistently low levels is continued *in vivo* breakdown of the protein. Recent observations by Müller-Eberhard, Dalmasso, and Calcott (6) concerning the uptake and conversion of β_{1C} -globulin in immune hemolysis indicate that this explanation would be plausible, and the present paper reports studies bearing on this point. Müller-Eberhard and associates (6) found that one $C'_{4,2a}$ site on a red blood cell is capable of "activating" large numbers of β_{1C} molecules. The activated molecules may bind to the red cell membrane or can remain in free solution to become "inactive" C'_3 , designated as C'_{3i} . C'_{3i} is, by immunoelectrophoretic analysis, known as β_{1G} and has been found *in vitro* as the result of reaction of purified β_{1C} with EAC' $_{1,4,2a}$ cells. β_{1G} has not been demonstrated in serum.

The present paper gives evidence for the presence, *in vivo*, of another breakdown product of β_{1C} , known as α_{2D} (7), in patients with

hypocomplementemic nephritis. This breakdown product could originate from β_{1C} directly, or β_{1G} could be an intermediate step. Its presence has been demonstrated by immunoelectrophoretic analysis of fresh plasma from patients with hypocomplementemic nephritis and by measurement of the specific antigenic determinant group associated with the protein. This antigenic determinant was described in a recent study (7) in which three major antigenic determinants were found to be associated with β_{1C} -globulin. Each of the two major breakdown products of β_{1C} was found to have a distinctive determinant, and the third determinant was found only on the parent protein. Thus, one determinant, A, was found on the well-known breakdown product β_{1A} , whereas the other, D, allowed recognition of the second breakdown product, α_{2D} . Both of these determinants were also found on the parent protein, β_{1C} . The third determinant, B, was found only on β_{1C} and disappeared as serum aged or was reacted with immune precipitates or zymosan. Since reaction with immune precipitates also results in the formation of the two breakdown products, β_{1A} and α_{2D} , the presence of these products in hypocomplementemic patients would give evidence that β_{1C} participates in an immune reaction *in vivo* and that breakdown could account, at least in part, for the β_{1C} depletion.

Methods

Studies were done on serum and plasma specimens from 45 normal adults, 34 children and adults with hypocomplementemic acute glomerulonephritis, 10 children with hypocomplementemic persistent glomerulonephritis, and 5 children with normocomplementemic persistent glomerulonephritis. By renal biopsy, all of the patients with persistent normocomplementemic glomerulonephritis had focal glomerular lesions, but in none was there a history of anaphylactoid purpura. The renal lesions in these patients as well as in those with persistent hypocomplementemic nephritis have been recently described in detail (4).

Immunoelectrophoretic analysis. The micromethod of Scheidegger (8) was employed. Electrophoresis was carried out in 2% Nobel agar with a barbiturate buffer, pH 8.2, 0.030 M with respect to sodium barbitol and 0.011 M with respect to diethylbarbituric acid. For the visualiza-

tion of the α_{2D} arc in fresh plasma, the diameter of the antigen well was increased to 3.5 mm from the usual diameter of 2 mm.

Measurement of A, B, and D determinants in fresh EDTA plasma and in serum. Antigenic determinants on β_{1C} -globulin were measured by a modification of the immunoelectrophoretic precipitin method described previously (9). Specimens were obtained by introducing 3 ml of freshly drawn blood into a centrifuge tube containing 11.2 mg of EDTA (final EDTA concentration, 0.01 mole per L). The contents were mixed, the tubes centrifuged, plasma was removed, and within a 3-hour period the pipetting necessary for quantitation was completed. Other samples of the same blood were allowed to clot and the serum removed and incubated at 37° for 1 to 2 weeks. For preservation during incubation, the tubes contained enough dried merthiolate or NaF to produce concentrations of 1:10,000 and 0.001 M, respectively. Studies showed that neither preservative affected the A, B, or D antigen titration and that β_{1D} breakdown was as complete under these conditions after 1 week of incubation as after 2 weeks. In lieu of incubation at 37°, some specimens of serum were quantitated after storage for periods of 3 months or more at -10° without preservative. The results were the same as those obtained with incubated specimens. Both serum incubated at 37° and that stored in the deep-freeze are referred to in the text as aged serum.

The method of preparation of antiserum containing high titer of antibody to the A, B, and D determinant groups of β_{1C} -globulin was described previously (7). All antiserum was produced in goats. For immunoelectrophoretic determination of A antigen, the antiserum was diluted so that the addition of 44.6 μ g of β_{1A} to 1 ml of the diluted antiserum removed all but the small amount of antibody to A antigen required to produce the very faint end point precipitin arc (9). In this determination, the supernatants were reacted against β_{1A} contained in a dilute euglobulin precipitate of aged human serum (2). For the determination of A antigen in fresh serum, results were expressed in units (2), and in aged serum in milligrams of β_{1A} per 100 ml.

B determinants were measured only in fresh EDTA plasma. The standard antiserum, before use, was absorbed with aged serum to remove antibody to A and D determinants (7) and was standardized with fresh EDTA plasma from a normal individual. The dilution of antiserum was such that approximately 0.03 ml of the fresh plasma would remove virtually all antibody to B antigen from 1 ml of the standard antiserum to produce an end point. Supernatants were run against normal EDTA plasma diluted 1:4. This plasma was stored in samples at -70°, and, once thawed, was used immediately. That not used was discarded.

The antiserum for measurement of D determinants was adjusted in concentration so that values for D and A determinants, expressed in units per milliliter, in pools of aged serum were the same. For example, in a pool from normal subjects, the concentration of both β_{1A} and α_{2D} would be 33 U per ml (2). A unit of A determinant in aged serum was equivalent to 45.7 μ g of β_{1A} (2). Be-

cause α_{2D} has not been isolated in pure form, the milligram equivalence of a unit of this protein has not been determined. Supernatants were reacted against pooled aged serum diluted 1:4. Samples of the diluted pooled serum were stored at -10° and not refrozen for future use.

Measurement of A and D determinants in urine. A modification of the immunoelectrophoretic precipitin method (9) was used for measurement of A and D determinants in urine. Timed urine collections were made so that results could be expressed as excretion rates. From a given urine collection, samples corresponding in volume to the urine formed over periods of 10 to 120 minutes' duration were added to measured amounts of the standard antisera used for A and D determination. The mixtures were incubated for 30 minutes at 37° and for 2 days at 4°, after which they were centrifuged in the cold. The entire supernatant was then placed in dialysis tubing, and the contents were pervaporated in a current of air with one end of the sac immersed in dilute buffer. The concentrated material was then squeezed from the sac, the sac rinsed, and the material made to the volume that would be used in the immunoelectrophoretic precipitin method for the volume of standard antiserum employed. We then used the concentrated supernatants to develop the immunoelectrophoretic pattern in the same manner as supernatants of serum-antiserum mixtures in the immunoelectrophoretic precipitin method. The end point was determined in the same manner.

Recovery experiments indicated that this method has an inherent inaccuracy. To normal urine containing 0.036 U of D determinant per ml, sufficient aged serum was added to raise the level to 0.086 U per ml. The amount found, however, was 0.061 U per ml. In the case of β_{1A} , none was detectable in the original urine, and we added an amount of aged serum to produce a level of 2.5 μ g per ml. The amount found was 1.9 μ g per ml. At these low concentrations, the method therefore underestimates both proteins by 20 to 30%. At the urine concentrations observed, this error is of no consequence.

Results

A systematic search for *in vivo* breakdown products of β_{1C} -globulin was initiated as a result of the chance observation that serum from hypocomplementemic patients, when subjected to immunoelectrophoretic analysis, showed precipitin arcs representing α_{2D} -globulin with characteristics which suggested that the level of this protein was greater than would be predicted from the concentrations of β_{1C} - and β_{1A} -globulins. If true, this observation would suggest that this breakdown product circulates *in vivo* in these patients. However, such an observation per se means little, since α_{2D} forms rapidly from β_{1C} as serum stands at room temperature, and esti-

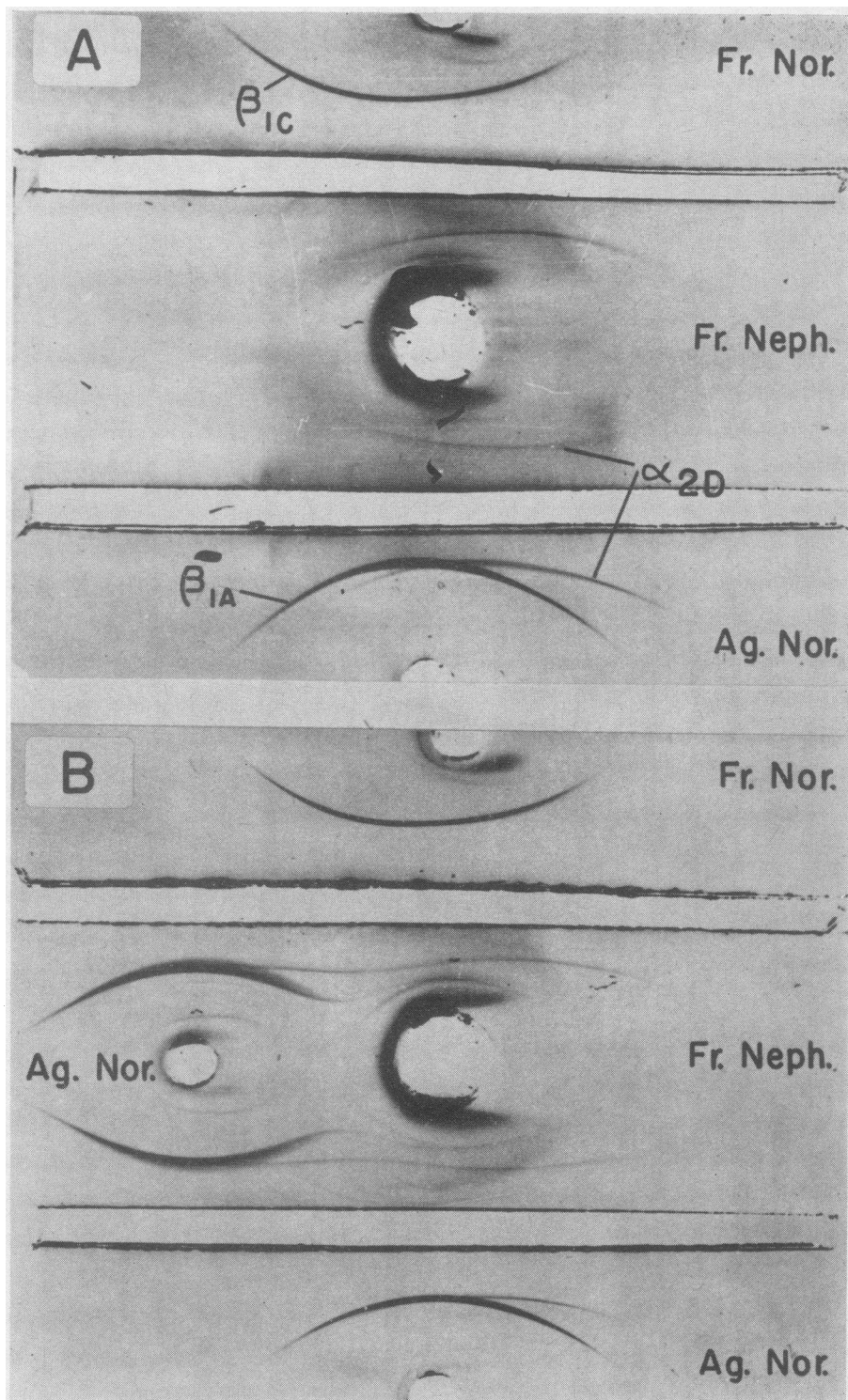


FIG. 1. A) IMMUNOELECTROPHORETIC PATTERNS OF FRESH EDTA PLASMA FROM NORMAL SUBJECT (FR. NOR.), FRESH EDTA PLASMA FROM A CHILD WITH ACUTE GLOMERULONEPHRITIS OF RECENT ONSET (FR. NEPH.), AND AGED NORMAL SERUM (AG. NOR.). An arc in the α_{2D} position is produced by the fresh plasma from the nephritic patient but not by the fresh normal plasma.

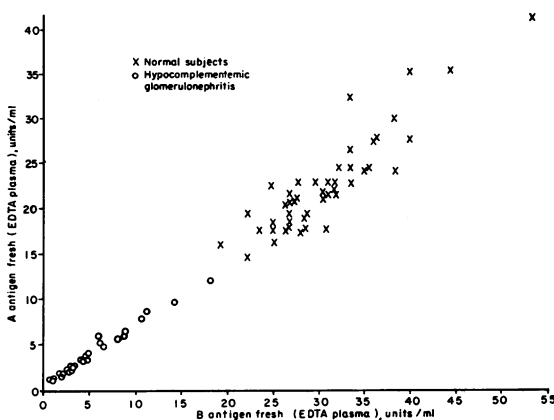
B) SAME AS A, WITH NORMAL AGED SERUM UNDERGOING ELECTROPHORESIS IN THE SAME AGAR STRIP AS THE NEPHRITIC PLASMA. See text for description.

mates of concentration based on the immunoelectrophoretic pattern may be deceiving.

To provide convincing evidence by immunoelectrophoretic analysis of the existence of α_{2D} *in vivo*, one must demonstrate the arc representing this protein in fresh plasma that has been drawn with precautions to prevent *in vitro* β_{1C} breakdown by the addition of EDTA. The demonstration of α_{2D} in fresh plasma from hypocomplementemic subjects was not possible by the routine immunoelectrophoretic method, apparently because it was present only at low concentrations. However, when the amount of serum subjected to immunoelectrophoresis was increased by enlarging the diameter of the antigen well from the usual 2 mm to 3.5 mm, an arc with the position and configuration of α_{2D} became readily visible. It is illustrated in Figure 1, A and B.

The antiserum used to produce the patterns in Figure 1 contained antibody to A and D antigens and hence allowed visualization of β_{1C} , β_{1A} , and α_{2D} (7). In the bottom panel of Figure 1, A, aged serum from a normal subject has been subjected to immunoelectrophoretic analysis, and a typical strong α_{2D} arc is seen. No comparable arc is seen in the upper panel in which fresh EDTA plasma from the same normal subject has been analyzed. In the center panel is shown the pattern produced by fresh EDTA plasma obtained from a child shortly after onset of acute glomerulonephritis. Arcs of the same character and mobility as α_{2D} are present, whereas no β_{1C} or β_{1A} arc can be identified with certainty.

Figure 1, B, is the same except that aged normal serum has undergone electrophoresis in the same agar strip with the fresh EDTA plasma from the patient with acute glomerulonephritis. The junction without spurring of the α_{2D} arc of the aged normal serum with the arc produced by the patient's plasma identifies the latter as α_{2D} . This observation constitutes strong evidence that α_{2D} was present in the circulation *in vivo*. The arc from the hypocomplementemic serum with which the β_{1A} arc of the aged serum identifies is probably β_{1C} ; in similar immunoelectrophoretic patterns of other patients less hypocomplementemic, a larger arc in this area that reacted with antibody to D antigen and hence interfered with the reaction of identity must of necessity have been β_{1C} -globulin.



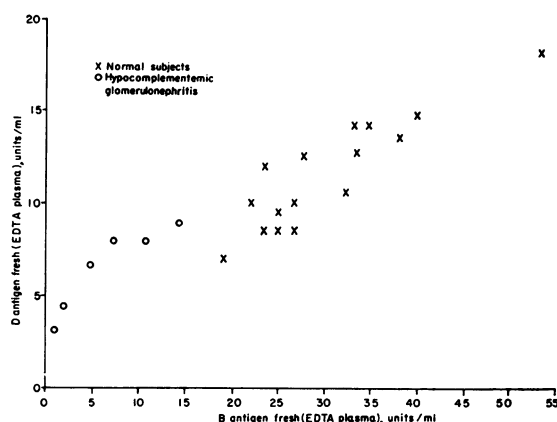


FIG. 3. CONSUMPTION OF ANTIBODY FOR THE D DETERMINANT PLOTTED AGAINST THAT FOR B, AS DETERMINED IN FRESH EDTA PLASMA.

B determinant, found only on intact β_{1C} (7), can be assumed to be a measure of β_{1C} -globulin. In plasma from hypocomplementemic patients, deviation in the relative amounts of B and D antigens from those in normal subjects would constitute further evidence for the presence *in vivo* of α_{2D} . Deviation in the relation of B to A would give evidence for B_{1A} .

In Figure 2, values for antibody consumption by A determinants are plotted vs. those for B, both expressed in units per milliliter of fresh EDTA plasma. The values are from 38 normal adults and from 30 patients with hypocomplementemic glomerulonephritis. Seven of the latter patients had persistent glomerulonephritis, and the remainder had acute glomerulonephritis. Considerable scatter can be seen in the data for the normal subjects. This would not appear to be the result of errors in the method, since determinations made on specimens obtained at different times from four of the subjects gave values that differed little. It is therefore tempting to ascribe this scatter to slight differences among individuals in the structure of their β_{1C} -globulin resulting in varying ability to bind antibody to A and B antigens. Despite the scatter it seems reasonable to conclude from this Figure that the relationship of A to B determinants in the hypocomplementemic patients differs little from that in normal subjects.

In contrast to the A determinants, the consumption of antibody to D determinants in relation to that of B is different in the plasma of the hypocomplementemic as compared to the normal sub-

jects. In Figure 3, this relationship is plotted for 16 normal subjects and 6 hypocomplementemic patients (3 with acute and 3 with persistent hypocomplementemic nephritis). The concentration of D antigen in the hypocomplementemic patients is greater than would be expected for the amount of B antigen present. Again, there is some scatter of the points for the normal subjects.

A more sensitive assessment of the relation of A and D to B antigens in hypocomplementemic patients is afforded by plotting the data as ratios. In Figure 4, the ratios of A:B and D:B are plotted against the concentration of B determinant. In the normal subjects, both ratios are relatively constant at all concentrations of β_{1C} . However, in hypocomplementemic patients, both ratios progressively increase as the concentration of B determinant falls. The relatively greater increase in D:B is readily apparent; A:B approximately doubled at low concentrations of B determinant, whereas D:B increased more than tenfold.

A and D determinants of aged serum. To obtain further evidence for the presence of α_{2D} *in vivo*, we have measured A and D antigens in serum aged by incubation at 37° for 1 week or longer or stored for long periods at -10° . Since we found β_{1C} to be completely broken down under these conditions, we could relate directly the D antigen concentration of these specimens to the concentration of α_{2D} , here expressed in units

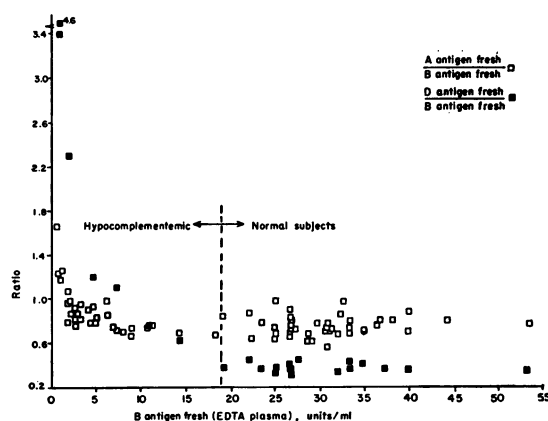


FIG. 4. DEMONSTRATION OF "EXCESS" A AND D ANTIGENS IN HYPOCOMPLEMENTEMIC PLASMA BY EXPRESSING THEIR CONCENTRATIONS IN TERMS OF RATIOS TO B ANTIGEN. The ratios are plotted on the ordinate, and the B antigen, denoting the concentration of β_{1C} , is plotted on the abscissa.

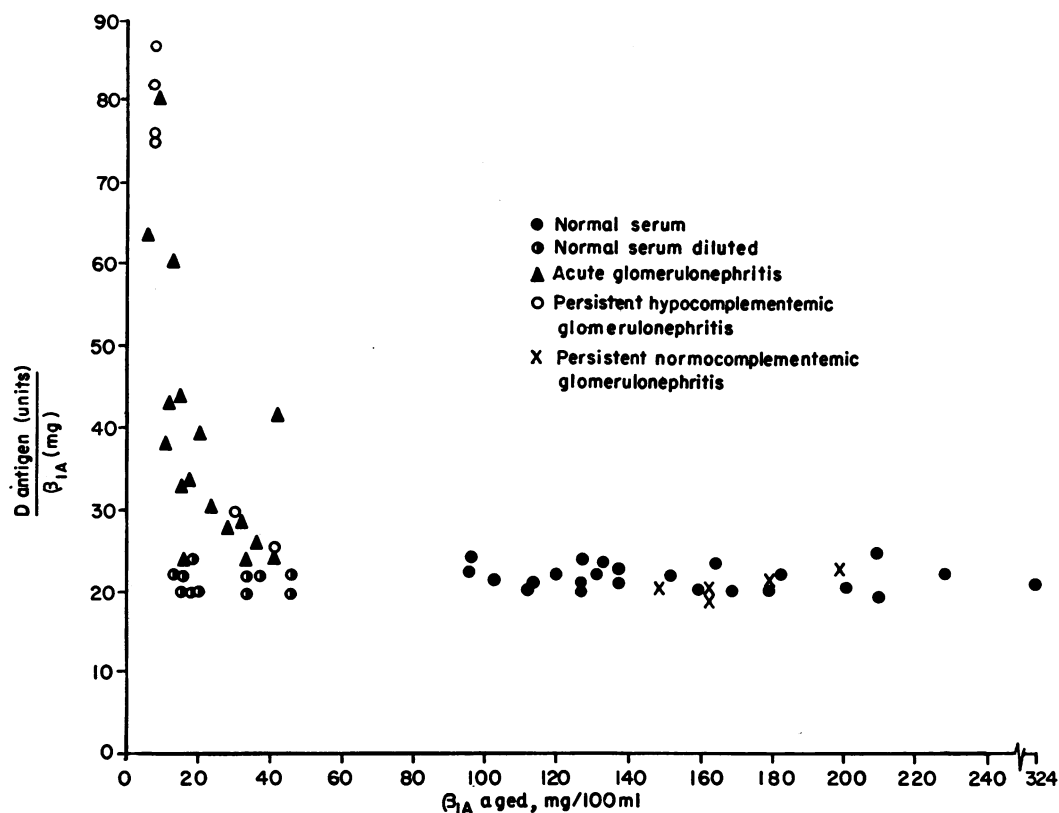


FIG. 5. ALPHA_{2D} CONTENT OF AGED SERUM RELATED TO β_{1A} CONTENT. The ratio of alpha_{2D} to β_{1A} is plotted on the ordinate and the β_{1A} concentration on the abscissa.

per 100 ml, and A antigen to the concentration of β_{1A} , here expressed in milligrams per 100 ml. The results are plotted in Figure 5, with the β_{1A} concentration on the abscissa and the ratio of alpha_{2D} to β_{1A} on the ordinate.

In the normal subjects, indicated by solid circles, the alpha_{2D} per milligram of β_{1A} was constant over a wide range of β_{1A} levels. The values averaged 21.4 U of alpha_{2D} per mg of β_{1A} . In the hypocomplementemic subjects with both acute and persistent glomerulonephritis, the ratio was greatly increased. In sera from the most markedly hypocomplementemic patients, alpha_{2D} was present in amounts four times as great as would be predicted from the amount of β_{1A} present. Evidence that this was not the result of a systematic error of measurement at low antigen levels is the fact that values for diluted normal serum, designated by the half-shaded circles, are in the same range as those for undiluted normal serum.

The disproportion between A and D concentra-

tions in hypocomplementemic serum would seem most logically ascribed to the presence of alpha_{2D} *in vivo*. Thus, the alpha_{2D} measured in aged serum from hypocomplementemic patients would not only be that formed *in vitro* by the breakdown of β_{1C} but also the alpha_{2D} produced by *in vivo* breakdown of β_{1C} and present at the time the blood was drawn. As an alternative explanation, it might be proposed that the disproportion is the result of a change in the character of β_{1C} -globulin with hypocomplementemia such that it breaks down *in vitro* to generate large amounts of alpha_{2D}. The results of immunoelectrophoretic analysis (Figure 1) make this explanation unlikely.

In Figure 5 are also shown the values for five patients with normocomplementemic persistent nephritis, indicated by crosses. The values are in the same range as for normal subjects. Blood specimens for these determinations were obtained when many manifestations of nephritis were present, 10 to 70 days after the presumed onset. In

TABLE I
Excretion of A and D determinants* of β_{1C} -globulin in the urine of normal and hypocomplementemic subjects

Subject	Age	Diagnosis†	Duration of disease	Serum levels (aged)			Excretion			
				β_{1A}	Alpha _{2D}	Alpha _{2D} / β_{1A}	Actual urine flow	Protein	β_{1A}	Alpha _{2D}
	<i>years</i>		<i>days</i>	<i>mg/100 ml</i>	<i>U/ml</i>	<i>U/mg</i>	<i>ml/min</i>	<i>mg/hour</i>	<i>μg/hour</i>	<i>U/hour</i>
P.T.	8	AGN	7	5.2	3.3	63.5	0.81	19.4	<34	0.75
			21	9.1	7.3	80.2	0.68	12.2	<23	0.5
Z.T.	9	AGN	8	41	17	41.4	0.32	10.9	<23	<0.5
R.W.	8	PHGN	660	7.5	5.7	76.0	0.28	0	<34	0.5
Normal adult				90-220‡	20-50‡	21.4§	0.35	0	<23	0.75

* Expressed as β_{1A} and alpha_{2D}-globulins, respectively.

† AGN = acute glomerulonephritis; PHGN = persistent hypocomplementemic glomerulonephritis.

‡ Ranges for β_{1A} in normal subjects determined previously (4), for alpha_{2D}, in the present study.

§ See Figure 5.

all five patients, biopsies gave evidence of focal inflammatory reactions in the glomeruli (4). The normal ratios of alpha_{2D} to β_{1A} in these patients indicate that if an immune reaction involving complement is occurring, it has no discernible effect on the serum content of either β_{1A} or alpha_{2D}.

In vivo concentration of alpha_{2D} in plasma. The plasma concentration of alpha_{2D} *in vivo* can be calculated from the measurements of B and D determinants in fresh plasma and of A and D determinants in aged serum. If we assume that alpha_{2D} is not present *in vivo* in normal subjects, the data allow an estimate of the amount of D determinant contributed by β_{1C} in hypocomplementemic plasma or serum. Subtracting this value from the total alpha_{2D} concentration gives the order of magnitude of the alpha_{2D} concentration *in vivo*.

From Figure 5 it can be seen that in aged serum the β_{1A} concentration in milligrams per 100 ml can be multiplied by 21.3 to estimate the units of alpha_{2D} per 100 ml derived from β_{1C} breakdown during aging. In hypocomplementemic serum, the amount in excess of this estimate would be preformed alpha_{2D}. Although there is considerable scatter of the data, there is a definite trend for the preformed alpha_{2D} present *in vivo* to be in higher concentration in specimens with the lowest β_{1A} concentrations. For example, at β_{1A} levels between 5 and 10 mg per 100 ml, preformed alpha_{2D} concentration averaged 4.2 U per ml (six patients), whereas between 30 and 45 mg per 100 ml the average was 2.1 U per ml (six patients). As suggested by the results of immunoelectrophoretic analysis then, the concentration of this break-

down product in hypocomplementemic patients tends to vary inversely with the level of β_{1C} .

A similar inverse relationship probably also obtains in fresh plasma. However, it could not be demonstrated by calculations with the data for D and B antigens given in Figures 3 and 4, probably because of the relatively small number of specimens available.

The data become more understandable if the concentration of alpha_{2D} in hypocomplementemic serum can be related to some readily available standard. Since this protein is not detectable in fresh plasma from normal subjects, the most appropriate comparison would be with aged normal serum. The calculation given above indicates that in the most hypocomplementemic patients, the alpha_{2D} level *in vivo* would range from 7.5 to 18% of the level in a pool of aged serum from normal subjects.

Urinary excretion of β_{1A} and alpha_{2D}. Accumulation of alpha_{2D} in the circulation of hypocomplementemic patients without evidence of *in vivo* β_{1A} accumulation might be the result of different rates of urinary excretion of the two products. Measurement was therefore made of the excretion rates of A and D antigens in the urine of hypocomplementemic patients. The results, expressed in terms of β_{1A} and alpha_{2D}, are given in Table I for two patients with acute glomerulonephritis, one with persistent hypocomplementemic nephritis, and a normal subject. The three patients with nephritis had amounts of alpha_{2D} in their aged serum considerably in excess of the normal value of 21.4 U per mg of β_{1A} (Figure 5). None had

marked proteinuria, even though in some the β_{1A} levels were extremely low.

In all instances, excretion of β_{1A} was too low to allow accurate measurement. Values were less than 34 μg per hour. In fact, there is reason to believe that there is virtually no excretion of this determinant, insofar as addition of increasing amounts of urine to the standard antiserum resulted in no detectable depletion of antibody, as evidenced by the lack of change in the intensity and character of the precipitin arc produced by the supernatants. The observation suggests that the urine was completely devoid of A determinants.

Alpha $_{2D}$ was present in measurable amounts in the urine of the normal subject and of two of the patients with nephritis. The normal adult had a rate of excretion equal to or greater than that of the patients with nephritis. In the first specimen from P.T., the rate of excretion of alpha $_{2D}$ of 0.75 U per hour gives a renal clearance of 0.23 ml of plasma per hour, assuming his alpha $_{2D}$ concentration *in vivo* was the same as that of his aged serum.

Although these excretion values may be erroneously low by a factor of 25 to 30% due to methodological error, it is nevertheless apparent that the excretion rates of β_{1C} -globulin and its breakdown products are almost negligible and would not account for the reduced serum levels.

Discussion

Two lines of evidence, immunoelectrophoretic analysis and titration of specific antigenic determinants, indicate that a small but significant amount of alpha $_{2D}$ is present *in vivo* in hypocomplementemic patients, presumably as a breakdown product of β_{1C} -globulin. There was no experimental evidence of its presence in normal subjects. In contrast, the data indicate that very little β_{1A} is present in hypocomplementemic patients. The only evidence for the presence of β_{1A} was the slightly increased ratio of A to B antigen in fresh plasma (Figure 4). This result is contrary to previous reports by Morse, Müller-Eberhard, and Kunkel (10) and by Lachmann (11). These workers demonstrated the presence of circulating β_{1A} -globulin by immunoelectrophoretic analysis of fresh serum or fresh EDTA plasma from patients with lupus nephritis. Their observations indicate a concentration of β_{1A} far greater

than observed in the present study. The reason for the different results is not known. Perhaps in lupus nephritis the reaction of β_{1C} -globulin or the disposition of its breakdown products is quantitatively different from that in other types of hypocomplementemic nephritis.

The readily demonstrable levels of alpha $_{2D}$ in the face of a very low concentration of β_{1A} , as found in the present study, cannot be explained at present. Studies by others (3) indicate that β_{1C} is not excreted in the urine, and the more detailed observations of the present paper indicate that neither β_{1A} nor alpha $_{2D}$ is excreted in significant amounts. The discrepancy in the *in vivo* levels of the two proteins will have to be explained by a difference in their rates of clearance by metabolic processes.

Definite evidence for *in vivo* presence of β_{1G} -globulin (C'_{31}) (6, 7) was not found. The small amount of A determinant, attributed above to β_{1A} , could conceivably be contributed by A determinants on β_{1G} . In this case, a moiety of the "excess" of D determinants should also be assigned to β_{1G} . It should, however, be noted that β_{1G} has only been produced from purified β_{1C} in the absence of whole serum; in serum, the condition that produces this transformation presumably has as its end products β_{1A} and alpha $_{2D}$.

Although alpha $_{2D}$ was the only breakdown product unequivocally found in the hypocomplementemic patients, its presence constitutes further evidence for the current concept that the reduced complement level in nephritis is the result of participation of complement in an *in vivo* immune reaction. One of the mechanisms by which the third component of this complex, β_{1C} , can remain reduced over long periods must therefore be *in vivo* breakdown. The roles of β_{1C} deposition and of diminished synthesis in keeping concentrations low cannot be defined by the present study.¹

The present observations coincide with the investigations of Müller-Eberhard and associates (6), who used radiolabeled β_{1C} in detailed studies

¹ *In vivo* breakdown of β_{1C} and persistence of breakdown products in the circulation could introduce error in the isotopic measurement of β_{1C} disappearance. If the portion of the molecule that yielded alpha $_{2D}$ were isotopically labeled, the breakdown product would contribute counts and the half-life calculated for β_{1C} would be erroneously long.

of the uptake and conversion of this complement component during immune hemolysis. Attachment of C'_3 (β_{1C}) to the red cell membrane was found to be the result of the reaction of β_{1C} with $C'_{1,4,2a}$ or $C'_{4,2a}$ sites on the red cell surface. Because one $C'_{4,2a}$ group could effect the binding of several hundred β_{1C} molecules, it was impossible to assume that the attachment was to the $C'_{4,2a}$ group. The point applicable to the present study was that, in addition to bound C'_3 , the reaction also caused conversion of large quantities of C'_3 to inactive C'_3 , designated C'_{3i} , or β_{1G} . In fact, $C'_{4,2a}$ groups in free solution were also capable of effecting conversion to C'_{3i} , and here none of the product was bound to cell membranes, even though red cells were suspended in the reaction medium. The results required those authors to postulate that $C'_{4,2a}$ groups are capable of converting, by enzymatic action, large numbers of C'_3 molecules to an "activated" form. This form is unstable and can either bind to cell membranes or convert to C'_{3i} . The half-life of activated C'_3 appears to be very short.

If we assume that $C'_{4,2a}$ groups are present in the glomerular capillary walls or on circulating complexes, the above observation can be applied to the events in hypocomplementemic nephritis. C'_3 , present in circulating plasma, is activated and, at least initially in the disease, is bound to surrounding glomerular structures or to complex. As the reaction proceeds, available receptors for attachment of C'_3 become saturated and the majority of the molecules convert to C'_{3i} . C'_{3i} is presumably in turn broken down by serum factors to β_{1A} and α_{2D} . The α_{2D} produced from C'_{3i} breakdown appears to circulate for a time, whereas β_{1A} must be quickly removed from the circulation. It should be noted that the enzymatic action of C'_{2a} groups would allow relatively few $C'_{4,2a}$ sites to be effective in maintaining a low circulating level of β_{1C} . It is possible that the rate of inactivation and breakdown of β_{1C} and hence its serum level is a function of the number of these sites available to circulating plasma.

For a time it was considered that the failure to find significant amounts of β_{1A} in the circulation as compared to the relatively large amounts of α_{2D} might be explained by attachment of β_{1A} to red cell membranes. If activated C'_3 binds with avidity to membranes, even, as shown by Müller-

Eberhard and associates (6), in the absence of antibody, it would seem plausible that the erythrocytes of hypocomplementemic patients, abundant in the glomerular capillary walls in sites where C'_3 is activated, might have attached to them large quantities of C'_3 . However, our initial attempts to demonstrate attached β_{1A} by hemagglutination techniques using antibody directed at A determinants have not been successful. Agglutination of cells from hypocomplementemic patients by such antiserum has been no greater than that of cells from normal subjects.

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