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J Clin Invest. 1969;48(8):1495-1505. <https://doi.org/10.1172/JCI106116>.

Research Article

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Complement Metabolism in Man: Hypercatabolism of the Fourth (C4) and Third (C3) Components in Patients with Renal Allograft Rejection and Hereditary Angioedema (HAE)

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ABSTRACT Highly purified and radioiodinated human C4 and (or) C3 were administered to patients with renal allografts in rejection, with hereditary angioedema (HAE), with chronic glomerulonephritis, and to control subjects. The latter group included normal individuals, anephric patients before transplantation, and stable renal allograft recipients. The catabolic rates of these complement proteins were determined by analysis of the disappearance of plasma protein-bound radioactivity (k_m), and by direct measurement of urinary excretion of radioactivity (k_u). The correlation coefficient between these two methods was 0.96. The mean ± 2 SD for catabolic rates in the control subjects was 0.9–2.7% plasma pool/hr for C4 and 0.9–2.0% plasma pool/hr for C3. Patients experiencing renal allograft rejection had unstable levels of C4 and C3, and exhibited moderate hypercatabolism of both proteins. One patient with chronic glomerulonephritis had hypercatabolism of C4 and C3 in the presence of stable normal serum levels. In patients with HAE who had extremely low levels of C4, catabolic rates for C4 were markedly elevated (3.7, 5.8, 7.0 and 8.8%/hr). Analysis of plasma curves in HAE revealed a three component disappearance curve instead of the two component curve in control subjects receiving the same preparation. Even though C3 levels were normal, moderate hypercatabolism of C3 was also

present in HAE (2.6, 2.8, 2.8, and 3.2% of pool/hr). The marked hypercatabolism of C4 in HAE constitutes the first direct evidence for the in vivo destruction by uninhibited C1 esterase of its natural substrate C4. The moderate hypercatabolism of C3 is consistent with the in vivo formation of C3-convertase.

INTRODUCTION

The degree to which activation of the complement system participates in disease processes in man may not be apparent from measurements of complement or complement-component activities in serum. Rapid synthesis of component proteins could compensate for increased complement utilization, and serum levels might be maintained within the normal range in spite of hypercatabolism. Decreased synthesis could account for depressions of complement levels in the absence of activation. The use of radiolabeled C3 ($\beta 1C$ globulin) to determine catabolic and synthetic rates in patients with diseases such as glomerulonephritis, lupus erythematosus, hereditary angioedema (1), and hemolytic anemia (2) has already provided some insight into the dynamics of complement production and utilization.¹ Even greater sensitivity might be expected from studies with C4 ($\beta 1E$

Dr. Carpenter is a recipient of an NIH Career Research Development Award. Dr. Ruddy and Dr. Merrill are Investigators of the Howard Hughes Medical Institute. Dr. Shehadeh is a Fellow of the Commonwealth Fund.

Received for publication 14 February 1969.

¹The nomenclature used conforms to that found in 1968 *Bull. World Health Organ.* 39: 935. Components of complement (C) react in the sequence C1, 4, 2, 3, 5, 6, 7, 8, and 9. The suffix "i" indicates the form of the component which has been inactivated as a result of participation in the reaction sequence, e.g. C4i.

globulin), since this protein is the substrate for the C1 enzyme which initiates the complement sequence, and is exquisitely sensitive to a very few molecules of C1 (3). Furthermore, the action of C1 on C4 and C2 results in the formation of C3-convertase which determines the rate at which C3 is utilized (4).

The present investigations of complement metabolism utilizing radioiodinated and highly purified C4 and C3 include the first such studies of C4 in man; marked hypercatabolism was observed in patients with hereditary angioedema (HAE), and moderate hypercatabolism in patients with rejecting renal allografts. The patients with HAE provide an opportunity to observe the effects of the uninhibited C1 enzyme on its natural substrate, C4, while the allograft recipients were studied to see if C4 and C3 were involved in the rejection reaction.

METHODS

Subjects

Control group. The control group for C4 consisted of two normal volunteers, one anephric patient before transplantation, and eight stable patients with renal allografts. The control group for C3 also included two normal volunteers, one anephric patient, and eight stable renal allograft recipients. Simultaneous studies with C4 and C3 were performed in only four of these individuals. The control patients with renal allografts were studied at a time when renal function was normal or stable, and when no clinically apparent rejection episode was in progress (5); namely, there was no fever, oliguria, hypertension, renal enlargement, proteinuria, or abnormalities of urine sediment attributable to a rejection process. Fever per se was present in R. L. No. 1 as a result of a wound infection, but renal function and blood pressure were normal. Patients in the allograft control group were studied at varying times after transplantation, and as early as 2 days after the allograft procedure (F. F.). All subjects in the control group had stable C4 (β 1E globulin) and C3 (β 1C globulin) levels in serum which were within the normal range or occasionally elevated.

Experimental group. Two patients were having acute, though mild renal allograft rejection episodes 4 days (M. J.) and 128 days (W. S.) after transplantation. M. J.'s study began at a time when renal function was improving. During the week of the study, however, her blood pressure rose from 118/76 to 150/100, and renal function deteriorated slightly as evidenced by a rise in the serum creatinine from 0.9 to 1.2 mg/100 ml. Therapy subsequently was intensified and rejection was reversed. This episode was not marked by fever, renal enlargement, or proteinuria. W. S. had been subject to recurrent mild rejection reactions during the 4 months before study, all of which were characterized by deterioration of renal function, reversible by increasing prednisone dosage. At the time of study he had been admitted because of the fourth such episode: the serum creatinine had risen from 1.0 to 1.4 mg/100 ml during the previous week. He had no other manifestations of rejection. There was no evidence for urinary infection or obstruction, and he again responded to increased prednisone therapy.

Another two patients (P. V. and C. A.) were undergoing chronic, poorly controlled rejections when studied 188,

301, and 395 days after transplantation. Both of these patients had serum creatinine levels in the 4–5 mg/100 ml range, and had biopsy evidence of obliterative arterial disease and glomerular basement membrane thickening typical of chronic rejection. Two studies with C4, two with C3, and one simultaneous study were performed in the allograft group.

One patient with chronic glomerulonephritis and nephrotic syndrome was included in the present series. At the time of study this patient was in remission with normal renal function, no proteinuria, and was receiving azathioprine and prednisone therapy.

Three patients with hereditary angioedema (HAE) were studied simultaneously with radiolabeled C4 and C3 during asymptomatic periods; a fourth patient with HAE (J. C.) had mild abdominal pains, but no angioedema. In all four patients, the diagnosis had previously been established by demonstrating the functional absence of the inhibitor of C1. One of the patients (J. C.) had the genetic variant form of disease in which the inhibitor is present as a protein but does not function. With one exception (W. S.) patients with hypercatabolism were studied at the same time as one or two control subjects.

Preparation and labeling of C4 and C3

C4 and C3 were purified from the serum of healthy donors at the laboratories of the Scripps Clinic and Research Foundation by previously published techniques (6, 7). The purified preparations were trace labeled with ^{125}I and ^{131}I to a specific activity of 10–30 $\mu\text{c}/\text{mg}$ using the Chloramine-T method (2, 8). Immediately after labeling, human serum albumin was added to the preparations at a final concentration of 0.2%. A single preparation of labeled C4 was treated with C1-esterase (9) so as to achieve full conversion to the hemolytically inactive form (C4i).

Purification procedures were carried out entirely with autoclaved buffers containing 10^{-3} M chloramphenicol. After each step, the activity-containing fractions were passed through a bacterial filter before they were concentrated and submitted to the next step. The labeled preparations which had been mixed with the human serum albumin carrier were passed twice through bacterial filters into sterile, rubber-stoppered, sealed vials. Aliquots of the contents of these vials were analyzed for bacterial contamination and injected into three rabbits for pyrogen testing according to the requirements of the USP (10). Batches that, after 3 days of bacteriological testing showed evidence of bacterial growth, or those that gave rise to more than 0.5°C temperature increase in the rabbits over the test period of 3 hr were rejected for clinical use.

Measurement of C4 and C3

C4 as β 1E globulin and C3 as β 1C globulin were measured immunochemically by the radial immunodiffusion assay (11) as previously described (12, 13) in daily serum samples and in the radiolabeled preparations. The experimental error of this method has been shown to be $\pm 10\%$ (12).

The activities of each of the preparations of purified labeled C4 and C3 used in these studies were measured in stoichiometric hemolytic assays (14, 15).

To estimate any losses in activity which might have occurred during the purification and labeling procedures, the number of effective molecules of C4 and C3 observed in the hemolytic assays were expressed as a function of the weight of C4 and C3 measured in the radial immunodiffusion assay. The numbers of effective molecules of C4 or C3 per

microgram of purified preparation were compared with those obtained for C4 or C3 contained in normal human serum assayed under identical conditions. For C4, the purified labeled preparations contained from 25 to 100% of the activity of the same amount of C4 protein contained in normal human serum; for C3, the range was from 16 to 39%.

Administration of labeled components

All subjects received 30 drops of Lugol's solution per day orally to block thyroidal uptake of radioiodine. In addition, liberal NaCl intake was encouraged where feasible to promote optimal renal clearance of iodide (16). Syringes containing 10–15 μc of ^{131}I - and (or) ^{125}I -labeled proteins were weighed before and after intravenous injection of the labeled components: venous blood was drawn at 10 min, 1, 2, 4, 8, and 18 hr, and daily thereafter for approximately 1 wk. Samples were collected as serum and plasma ethylenediaminetetraacetate (EDTA) and stored at -70°C . 2-ml aliquots of plasma were counted and the amount of protein-bound radioactivity therein was determined as follows: an equal volume of 20% trichloroacetic acid (TCA) was added and 2 ml of supernatant fluid counted; twice this value was subtracted from the plasma count. Radioactivity was counted in a 3 inch NaI scintillation crystal by a Baird Atomic model 530 spectrometer. Determination of ^{131}I activity in the presence of ^{125}I was calculated by subtraction of the portion of the ^{125}I spectrum which overlaps the low energy ^{131}I peak. The count rate for ^{125}I did not exceed 5×10^5 cpm.

Urines were collected on a 24 hr basis, aliquoted, and frozen until pipetted. TCA precipitation of urine samples revealed no significant protein-bound radioactivity. Stools were homogenized and aliquots counted in five studies; collections were then discontinued because stool radioactivity was found to be insignificant.

Analysis of data

An estimate of plasma volume from dilution of the injected protein-bound radioactivity in the 10-min plasma sample always coincided with the volume predicted on the basis of body weight and hematocrit in instances where the *in vivo* behavior of the complement protein was that of a two compartment (intravascular/extravascular) system. The measured plasma volumes and hematocrit values were used to calculate the blood volumes (normal = 5.1–8.7% of body weight). Preparations which contained material which formed a third, rapidly removed compartment yielded falsely high plasma volumes as expected. The protein-bound counts observed in the 10-min plasma sample were given the value of 100% and the percentage of these counts observed in subsequent plasma samples were plotted as a function of time on semilogarithmic paper. The curve was then resolved by inspection into exponentials with numerical slope and intercept values. Analysis of catabolic rates and rate constants for distribution into extravascular spaces were performed according to the Matthews model (17). Certain of the preparations contained some material which was rapidly removed from the plasma space of control subjects so as to give a third exponent. Such a three exponential curve was normalized to two exponentials by subtracting from the total injected that proportion of denatured material indicated by the intercept of the third exponent (18), and reconstructing the curve. The catabolic rate, k_m , derived from the plasma curve was expressed as per cent of the plasma pool per hour.

By TCA precipitation there was 5–10% and occasionally as much as 25% free iodide in the material injected. Since the

amount of free iodide injected was known, it was subtracted from the radioactivity appearing in urine. Since the half-life of excretion of free iodide in man is considerably less than 24 hr (19, 20), two-thirds of the injected free iodide was arbitrarily subtracted from the first urine collection (usually about 20 hr), and one-third from the second 24 hr collection. When the amount of catabolically released iodide excreted in the urine was expressed as a percentage of the protein-bound iodide in the plasma space at the midpoint of the period of collection, a direct measure of the catabolic rate of the protein was obtained (k_u) (21, 22). Cumulative excretion of iodide was subtracted from the injected dose and the resulting whole body curve plotted on the same axes as the plasma curve. Parallelism of the whole body curve and the plasma slope indicates the rapid excretion of catabolically released iodide upon complete degradation of the protein (23).

In a steady state, synthesis and catabolism are equal; therefore, a synthetic rate can be calculated from the catabolic rate and the plasma pool of the protein and expressed as milligrams per kilogram per hour (17). Although stable serum levels of a protein may reflect a steady-state situation, compartmental shifts may occur without regard to changes in synthesis and catabolism (18); therefore, the calculations of synthetic rates and rate constants from which extravascular/intravascular distributions are derived are based on a steady-state assumption which could not be directly confirmed.

RESULTS

Control group

Purified and radioiodinated human C4 and C3 circulate in the plasma of normal individuals and immunologically stable patients for several days after intravenous injection. The half-life for both C4 and C3 in plasma ranges from 46 to 70 hr. A representative study for C4 is shown in Fig. 1; similar curves were found for C3. There is a rapid decline in plasma protein-bound activity during the 1st day as intravascular to extravascular distribution occurs; thereafter, plasma protein-bound radioactivity assumes a straight line exponential function which represents the net effects of catabolism and extravascular to intravascular redistribution. When the injectate does not contain significant amounts of denatured material, the plasma curve can be resolved into two exponentials (Fig. 1) with intercept and slope values which are used to calculate the catabolic rate as per cent of the plasma pool per hour (k_m). Since such a two compartment curve was obtained for both C4 and C3 in most of the studies, the metabolism of these complement components may be assessed by the two compartment model defined by Matthews (17) and previously applied to a number of other plasma proteins.

Total radioactivity remaining in the body, calculated by cumulative subtraction of the excreted radioiodide from the total injected, yielded an exponential curve generally parallel to the terminal slope of the plasma radioactivity curve (Fig. 1). In some instances, however, some deviation in the whole body slope occurred

for both C4 and C3. Such a divergence was always in the upward direction; that is, less radioactivity was recovered in the urine than expected on the basis of the plasma curve. At most, the discrepancy amounted to 10% of the injected dose. Since this phenomenon occurred in individuals who did not have impaired renal function, and who also had no reason to be trapping released iodide, it must be concluded that, after alteration in the plasma space, some of the C4 and C3 molecules are sequestered and more slowly degraded thereafter. Since stool radioactivity never amounted to more than 1% of the total iodide excreted in the five studies where stools were also measured, any divergence between the whole body curve and the plasma curve cannot be attributed to this alternate route of excretion. Sweat radioactivity was not measured (20).

When the radioactivity in urine as determined on a 24 hr basis was divided by the midpoint value for total plasma radioactivity during the period of urine collection, a measure of catabolism was obtained (k_u). When

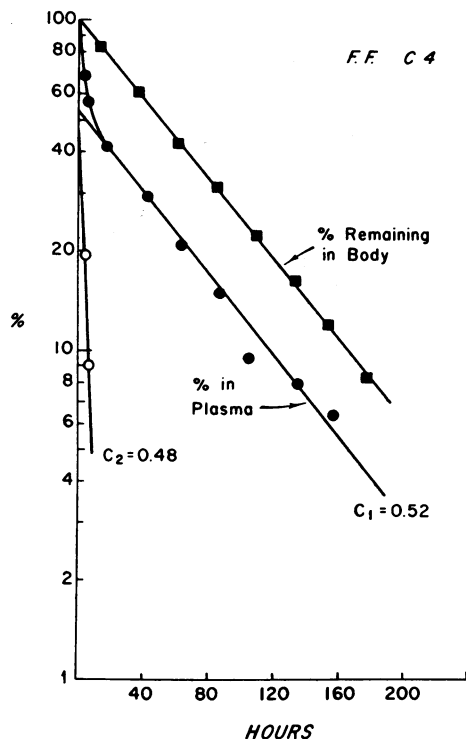


FIGURE 1 Metabolism of ^{125}I -C4 in a control patient with a stable renal allograft. The closed circles (\bullet) are the plasma protein-bound radioactivity values expressed as a per cent of the injected dose. The open circles (\circ) mark the second exponential of the plasma curve and are derived by subtraction of the extrapolated first exponential from the total plasma values. The intercepts of the two exponentials add up to 100%. The squares mark the amounts of ^{125}I -C4 remaining in the body and are derived by cumulative subtraction of urinary radioactivity from the amount injected.

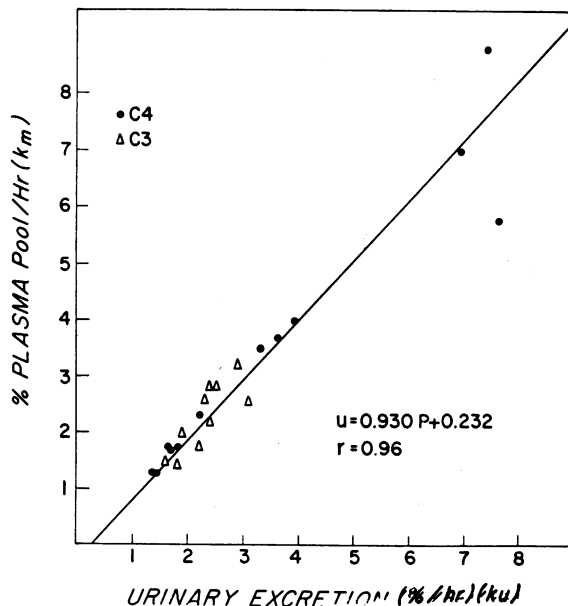


FIGURE 2 Catabolic rates for C4 and C3: correlation of results calculated from multiexponential analysis of plasma curves (k_m) with those obtained from the urinary excretion of radioiodide (k_u). Corrections for injected free iodide and denatured protein were applied as described in the text. All studies in which accurate urine collections were available are included, and each point represents a single study. The r value for C4 alone is 0.96 ($n=12$) and for C3 is 0.82 ($n=10$).

the mean catabolic rate value for k_u for each patient was compared with the k_m calculated from the two exponent plasma curve, an excellent correlation was obtained as shown in Fig. 2. Measurements of catabolic rates of C4 and C3 by both methods were in close agreement in all subjects studied, including those with greatly increased catabolic rates. For each 24 hr period the k_u value calculated on the basis of plasma radioactivity was fairly constant, whereas relating urinary excretion to extravascular or whole body radioactivity did not produce a constant catabolic rate. This fact is consistent with the assumption that catabolism of these plasma proteins occurs in or near the plasma space (22).

Since urinary excretion of radioiodide is retarded in the presence of severe renal failure, an accurate determination of the k_u was not possible in one of the stable renal allograft recipients (R. L. No. 2). The use of the k_m derived from the plasma protein-bound radioactivity curve alone is valid in all instances, however. In Table I are shown the catabolic rates (k_m), synthetic rates, and extravascular/intravascular ratios for the control subjects. Since the rates and compartments of the patients with stable renal allografts, the anephric patients, or the normal volunteers were similar, these three groups were combined. In the control group the range (mean ± 2 sd)

TABLE I
Metabolism of Complement Components

		Age	Sex	Catabolic rate	Serum level	Synthetic rate	Total body pool	Extravascular/ intravascular ratio
		yr		% plasma pool/hr	mg/ml	mg/kg per hr	mg/kg	
<i>C4 (β1E-globulin)</i>								
Controls								
Allograft	F. F.	36	M	2.6	0.70	0.99	70	0.83
Allograft	R. L. No. 1	33	M	2.3	0.85	1.34	107	0.83
Allograft	R. L. No. 2	33	M	2.2	0.52	1.01	60	0.30
Anephric	A. T.	38	M	2.0	0.67	1.10	89	0.62
Allograft	R. G.	36	M	1.3	0.45	0.46	45	0.27
Allograft	W. R.	21	M	1.7	0.51	1.05	70	0.26
Normal	S. R.	29	M	1.3	0.51	0.35	35	0.31
Normal	S. Rd.	33	M	1.7	0.42	0.41	29	0.21
Allograft	J. L.*	47	M	1.4				0.60
Allograft	A. S.*	35	M	1.2				0.28
Allograft	C. K.*	39	M	1.6				1.22
Normal range, ±2 SD				0.9-2.7	0.21-0.64			
Allograft rejection								
	W. S.	18	M	3.5	0.50	0.91	65	1.54
	M. J.	36	F	1.7	0.43	0.52	38	0.22
	P. V.*	22	M	3.1				2.00
Hereditary angioedema								
	N. L.	53	M	7.0	0.04	0.10	4	1.90
	M. D.	35	F	5.8	0.10	0.23	12	2.06
	J. C.	35	M	8.8	<0.03	<0.11	<1	—
	J. L.	21	M	3.7	0.14	0.30	15	0.86
Chronic glomerulonephritis								
	R. I.	52	F	4.0	0.35	0.43	34	2.21
<i>C3 (β1C-globulin)</i>								
Controls								
Allograft	R. G.	36	M	1.5	0.94	1.12	98	0.31
Allograft	W. R.	21	M	2.0	1.42	2.74	166	0.21
Normal	S. R.	29	M	1.5	1.17	0.93	77	0.24
Normal	S. Rd.	33	M	1.8	1.17	1.22	86	0.28
Anephric	M. S.*	36	M	1.4				1.78
Allograft	M. S.*	36	M	1.3				1.42
Allograft	A. S.*	35	M	1.2				1.15
Allograft	O. K.*	33	M	1.3				1.71
Allograft	J. L. No. 1*	47	M	1.4				1.06
Allograft	J. L. No. 2*	47	M	1.0				0.48
Allograft	C. K.*	39	M	1.5				0.15
Normal range, ±2 SD				0.9-2.0	0.94-1.82			
Allograft rejection								
	M. J.	36	F	2.2	1.30	2.04	134	0.60
	C. A.*	8	F	2.5				1.29
	P. V.*	22	M	2.1				0.36
Hereditary angioedema								
	N. L.	53	M	2.8	1.37	1.57	111	0.99
	M. D.	35	F	2.8	0.98	1.12	72	0.79
	J. C.	35	M	3.2	1.33	1.78	142	1.56
	J. L.	21	M	2.6	0.91	1.37	82	0.55
Chronic glomerulonephritis								
	R. I.	52	F	2.6	1.44	1.14	85	0.85

* At the time of the early studies in 1963, immunochemical and functional assays for C4 and C3 were not available; hence, accurate estimates of pool sizes and synthetic rates are not possible for these patients.

for the catabolic rate (k_m) of C4 was 0.9–2.7% plasma pool/hr, whereas that of C3 was 0.9–2.0% plasma pool/hr (Fig. 3). Since the individuals in the control group had stable serum levels of C4 and C3 as measured by the radial immunodiffusion assay, the mean values observed during the study were employed to determine synthetic rates and pool sizes. Synthetic rates in the control group varied from 0.35 to 1.34 mg/kg per hr for C4 and from 0.93 to 2.74 mg/kg per hr for C3. The ratios of extravascular to intravascular pool sizes varied from 0.21 to 1.22 for C4 and from 0.15 to 1.78 for C3. Another way of expressing this partition is to say that 45–83% of total body C4 and 36–87% of C3 is intravascular. It is noteworthy that in patient A. T. who had a draining thoracic duct fistula, the ratio of protein-bound radioactivity of lymph/plasma reached a value, 0.75, corresponding to, but slightly higher than the ratio of immunochemically determined C4, 0.68 (Fig. 4).

Effects of denaturation. Two of nine preparations of C4 and one of six preparations of C3 contained denatured material as defined by a rapid clearance of a portion of the radioactivity from the plasma space, forming an early compartment as a third exponential. This amounted to 20–50% of the protein-bound radioactivity injected. Three-compartmental analyses of such studies yielded k_m values which were higher than expected from

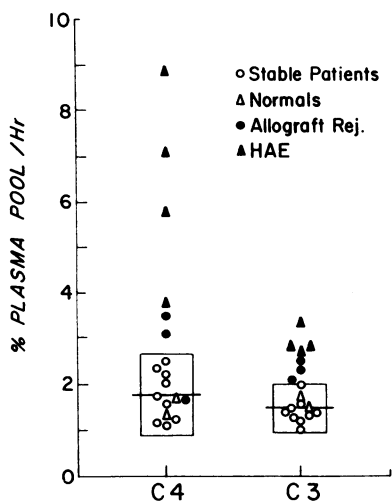


FIGURE 3 Catabolic rates for C4 and C3 as derived from analysis of plasma curves (k_m). The indicated mean and 2 sd range for the controls includes stable patients with renal allografts, anephric patients, and normal subjects. Marked hypercatabolism of C4 and moderate hypercatabolism of C3 is present in each of four patients with hereditary angioedema (HAE), studied simultaneously with radiolabeled C4 and C3. Five allograft rejection periods were studied in four patients (Table I). There is increased catabolism of C3 in all three instances of allograft rejection, and increased catabolism of C4 in two of three allograft rejection periods.

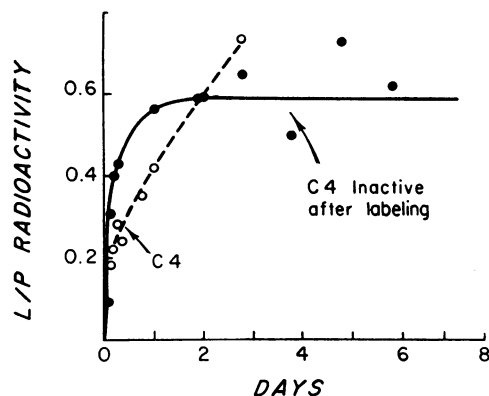


FIGURE 4 Appearance of protein-bound radioactivity in thoracic duct lymph after intravenous injection of radiolabeled C4 (open circles, patient A. T.) and of nonspecifically inactivated C4 in a second patient (closed circles, E. C.). The lymph values are expressed as a ratio of the corresponding plasma protein-bound values. Thoracic duct drainage in A. T. was terminated on the third day. The inactivated preparation was cleared more rapidly from the plasma space and appeared more promptly in the lymph space than the functionally active and nondenatured C4 preparation. Both materials reached a concentration in lymph which was less than that in plasma, corresponding to the ratio of lymph to plasma C4 measured immunochemically.

results with other preparations in control subjects and did not correlate with the k_m values derived from urinary excretion. Therefore, normalization of a three compartment curve to a two compartment curve was performed as described in methods for all recipients of a preparation which formed three compartments in the control subject. The amount of radioactivity excreted during the first 2 days after injection was never enough to account for all of the rapidly removed component. Apparently, much of the denatured material was not immediately catabolized with the result that the over-all catabolic rate derived from urinary excretion provided an excellent correlation, as shown in Fig. 2, with the catabolic rate derived from the normalized plasma curve.

Effects of nonspecific inactivation. The few preparations which demonstrated the presence of denatured material as defined by *in vivo* behavior did not differ on immunoelectrophoretic analysis or in terms of function from those preparations which formed a two compartment system. Losses of functional activity during preparation and labeling did not correlate with the presence of denatured material. Seven of nine C4 preparations and five of six C3 preparations produced two-compartment curves in control individuals; therefore, some loss of functional activity is consistent with normal intravascular behavior.

Specific inactivation of C4 by C1 esterase. A C4 preparation was treated with C1 esterase to produce C4i. As shown in Fig. 5, C4i disappears rapidly from the

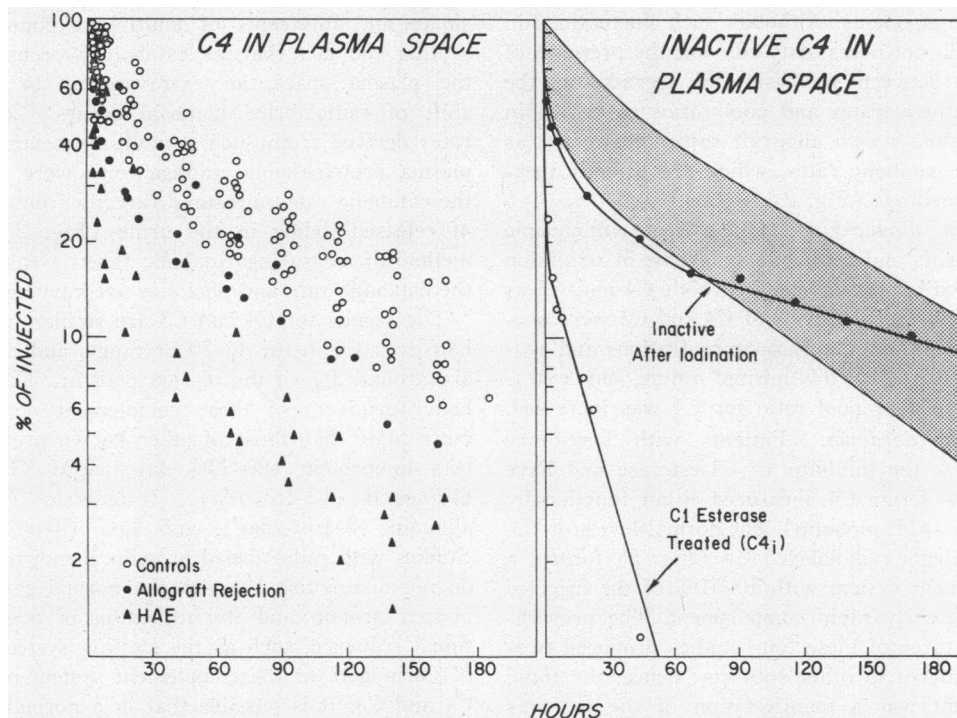


FIGURE 5 In vivo behavior of radiolabeled and functionally active C4, nonspecifically inactivated C4, and C1-esterase treated C4 in the plasma space. On the left are shown individual plasma protein-bound radioactivity values for the C4 control group (open circles). Two of three patients with evidence for allograft rejection (closed circles) show more rapid elimination of C4 from the plasma space (W. S., P. V.). The third patient (M. J.) had a normal catabolic rate for C4. Four patients with hereditary angioedema (HAE) show very rapid elimination of C4, although their 10-min samples indicate a normal initial intravascular distribution. On the right, the range of values for the controls is shown as the shaded area. A C4 preparation which had lost all functional activity after iodination displays a grossly normal plasma curve, except for an early exponential decline (third compartment) which represents the presence of denatured material. In contrast, in vitro treatment with C1-esterase of a functionally active (60%) C4 preparation results in an extremely rapid elimination in vivo of C4i. A catabolic rate calculation from such a plasma curve is not possible, since 75% of the injected protein is eliminated within 10 min.

circulation, with less than 5% of the injected protein-bound radioactivity present in the plasma space 24 hr after injection. The native C4 preparation from which the C4i was prepared had 60% functional activity and gave a two compartment exponential curve in the same patient which fell within the normal range indicated in Fig. 5. Quite clearly then, activation of the complement system should result in rapid elimination of labeled C4 molecules, including those with impaired function, since the C1-esterase-treated C4 did not have 100% functional activity. Fig. 5 also shows the in vivo behavior of a C4 preparation which had been completely inactivated during labeling. This material produced a three compartment curve with 64% of the injected dose in the early rapid component. The disappearance of the remaining material falls within the normal range. Fig. 4 shows that the functionally inactive C4 appeared in the thoracic

duct lymph of this patient very promptly during the rapid disappearance phase of the plasma curve.

Experimental group

Renal allograft rejection. Fig. 5 shows the plasma curves for radiolabeled C4 in three patients undergoing rejection as compared with those for 11 control subjects. C4 was removed rapidly from the plasma space in two (W. S. and P. V.) of these three patients. In the third rejecting patient (M. J.), simultaneously administered radiolabeled C3 disappeared at an accelerated rate, but C4 did not. The k_m values for C4 and C3 in these patients are shown in Fig. 3 and Table I. The instability of C4 and C3 concentrations in the sera of patients during renal allograft rejection has been previously well documented (24, 25), and suggests that an asynchronous state exists between synthesis and utiliza-

tion of these components. Although such fluctuations in plasma levels do not necessarily rule out the presence of a steady state between synthesis and degradation, the values for synthetic rates and pool ratios as shown in Table I for patients with allograft rejection are not as reliable as the catabolic rates, which are directly measured by two methods (Fig. 2).

Chronic glomerulonephritis. One patient with chronic glomerulonephritis and nephrotic syndrome in remission (R. I.) revealed hypercatabolism of both C4 and C3 as shown in Table I. Serum levels of C4 and C3 were normal and stable. Although serum concentrations and synthetic rates fell within the normal range, the extravascular/intravascular pool ratio for C4 was increased.

Hereditary angioedema. Patients with hereditary angioedema lack the inhibitor of C1-esterase and have consistently low serum C4, measured either functionally or as a protein (β 1E globulin), but normal levels of C3. In all four patients radiolabeled C4 (Fig. 5) formed a three compartment system with 10–61% of the injected material in the early rapid compartment. The preparations used for three of these four studies produced two-compartment curves in other subjects; hence, the rapid early component was a manifestation of the patient's disease, and not a result of denatured protein. The catabolic rates (k_m) in the patients with HAE ranged from 3.7 to 8.8% of the plasma pool/hr for C4 (Fig. 3, Table I). These elevated catabolic rates were confirmed by urinary excretion rates (k_u) as shown in Fig. 2. As shown in Table I, the serum concentrations of C4 were extremely low, whereas the synthetic rates were lower than those of the control group. The calculated extravascular/intravascular pool ratios for C4 were elevated in the two patients with very high catabolic rates.

In all four HAE patients the C3 plasma curves formed a two compartment system with moderately increased catabolic rates (k_m) of 2.6–3.2% of the plasma pool/hr (Fig. 3, Table I). The serum C3 levels were within the normal range as were the synthetic rates and the extravascular/intravascular pool ratios (Table I).

DISCUSSION

The *in vivo* behavior of purified and radioiodinated C4 (Fig. 1) and C3 after intravenous injection is similar to that of a number of other plasma proteins (26). After rapid mixing within the plasma space, there is a decline in plasma protein-bound radioactivity as the material diffuses into extravascular spaces, followed in 1–2 days by a phase of exponential decline as equilibrium is reached between catabolism and intravascular/extravascular redistribution. The half-life calculated from the terminal slope is often used as a rough measure of the turnover of a plasma protein, but it is only partially related to the catabolic rate. The latter is a function of the

slopes and intercepts of multiple exponentials, representing the fact that, as catabolism occurs in or near the plasma space, an extravascular to intravascular shift of radiolabeled material occurs (26). Catabolic rates derived from such a multiexponential analysis of plasma protein-bound radioactivity were confirmed by the catabolic rates measured directly from the excretion of released iodide in the urine (Fig. 2). The latter method of measuring catabolic rates is valid even when the catabolic rate and pool size are varying (22).

The results for C4 and C3 are similar in that plasma half-lives are in the 46–70 hr range, and catabolic rates are around 2% of the plasma pool/hr. Thus, the metabolic turnovers of these complement components are more rapid than those of other known proteins, such as beta lipoprotein (20–22%/day), IgM (14–25%/day), fibrinogen (12–16%/day), transferrin (9–17%/day), albumin (8–16%/day), and IgG (4–6%/day) (26). Studies with radiolabeled proteins in normal individuals do not distinguish between the normal catabolism of a plasma protein and its utilization in a specific functional sequence, such as the clotting system in the case of fibrinogen, or the complement system in the case of C4 and C3. It is possible that in a normal individual a small amount of complement fixation may be occurring at all times. Nevertheless, hypercatabolism above such a baseline resulting from a specific and vigorous stimulus for complement activation, as in HAE, is readily apparent (Fig. 3).

The significance of a modest elevation in catabolic rate, as in renal allograft rejection (Fig. 3), is of course dependent upon the normal limits defined by the control group. Patients with renal allografts without evidence for clinical rejection activity at the time of study were ideal controls since both groups were receiving the antipurine drug, azathioprine, as well as prednisone, which is known to increase protein catabolism. There were mild elevations in BUN and serum creatinine in all rejecting patients and in one nonrejecting patient. The anephric patients were included to rule out any contribution of renal tissue to complement catabolism. Since the simultaneous study of C4 and C3 in two normal subjects produced results similar to stable renal allografts and the anephric patients, it was concluded that none of the following have important effects on the metabolism of C4 and C3: mild uremia, absence of all renal tissue, antipurine drugs, or steroid hormones.

The catabolic rate for C3 obtained in the control group was 0.9–2.0% of the plasma pool/hr (Table I). In the report of Alper and Rosen (1) in which C3 was purified in a similar fashion, but labeled by the iodine monochloride technique, the mean ± 2 SD range for catabolic rate was 1.3–3.4%/hr. In their studies which were also analyzed by the Matthews method, the number of

exponential components of the curves obtained was not stated. The other published study of C3 metabolism by Petz et al. (2) employed material prepared and labeled by the same laboratory that provided C3 and C4 for the present study. These workers found a range of 1.6–2.7%/hr for the C3 catabolic rate, and took note of the error introduced by the presence of denatured material which is cleared rapidly from the plasma space in the 1st 15 min, as evidenced by falsely high plasma volumes; data from such studies were excluded from their control group. Only one of the C3 preparations used in the present study yielded abnormal values for blood volume, and it contained material which produced a third exponential in control subjects (W. R., S. R., and S. Rd.). It is possible that inclusion of denatured material which forms a third early exponential is responsible for the higher normal range observed by Alper and Rosen (1). The range of 0.9–2.7%/hr for the C4 catabolic rate (Table I) is remarkably similar to that of C3.

Current techniques for preparing and radioiodinating C4 and C3 usually result in a nonspecific loss of functional activity as assessed in stoichiometric assays. It is further apparent that denaturation, as defined by *in vivo* behavior, and loss of functional activity during preparation are not equivalent abnormalities. A considerable loss of functional activity can be present in a preparation which behaves normally after injection. Furthermore, such a nonspecifically inactivated molecule can undergo specific alteration by C1-esterase yielding a molecule (C4_i) which experiences a rapid and distinctly different *in vivo* clearance (Fig. 5). Of course, some loss of the active site would preclude full participation in formation of C3-convertase, but such a secondary step should not affect the catabolism of C4 itself. Similar studies on the susceptibility of C3 inactivated during preparation and labeling to the action of C3-convertase have not as yet been performed, but it is assumed that alteration of the active site occurs without significantly affecting the molecule as a substrate. *In vitro* preparations of C3_i are also rapidly catabolized (1, 2). Our conclusions regarding the effects of purification and radiolabeling of C4 and C3 can be summarized as follows: (a) denatured protein, as defined by its rapid disappearance from the plasma space, either within the first few minutes after injection or in an exponential fashion during the first several hours, must be identified and appropriate corrections made in the catabolic rate calculations; (b) some nonspecific functional inactivation of protein, as defined by loss of hemolytic activity, does not affect *in vivo* behavior of C4 with regard to compartmental distribution and need not alter susceptibility to the C1 enzyme.

Synthetic rate calculations based upon a steady-state assumption are made from the values for catabolic

rates, serum concentrations, and plasma volumes. Alper and Rosen (1) reported a mean ± 2 SD range for the synthetic rate of C3 as 0.87–1.89 mg/kg per hr, while Petz et al. (2) found a range of 0.90–1.42 mg/kg per hr. In the present study the synthetic rates for C3 were calculated in only four control subjects and varied from 0.93 to 2.74 mg/kg per hr. For C4 the synthetic rate varied from 0.35 to 1.34 mg/kg per hr (Table I).

Estimations of the extravascular/intravascular distribution of proteins from any of the methods of analysis, including the Matthews model employed here, are less reliable than the catabolic and synthetic rates and are invalid in the presence of a non-steady-state situation. In the present method of analysis, the ratio of extravascular to intravascular protein mass is based upon the rate constants for transfer from plasma to lymph space, and vice versa. The extravascular/intravascular ratio for C3 in the control group varied from 0.15 to 1.78 (36–87% intravascular). Alper and Rosen (1) found a range of 0.57–1.25 (44–63% intravascular, while Petz et al. (2) had a range of 0.16–0.85 (54–86% intravascular). In the present study, similar extravascular/intravascular ratios were found for C4 varying from 0.21 to 1.22 (45–83% intravascular). Direct sampling of the extracellular fluid, possible in one patient receiving radiolabeled C4 (Fig. 4), confirmed the distribution into the extravascular space predicted from the compartmental model which assumes that after equilibrium the specific activity in lymph will be slightly greater than that in plasma (23, 26).

Renal allograft recipients with clinically recognizable rejection reactions have unstable serum levels of C4 and C3 (25). Modest elevations in catabolic rates of these components were observed during such periods (Fig. 3, Table I). The elevations and depressions of C4 and C3 present during rejection periods suggest that an asynchrony exists between synthesis and utilization, although extravascular/intravascular pool shifts cannot be excluded. Short-term, hour-to-hour estimates of catabolic and synthetic rates are not possible when radiolabeled proteins are employed for metabolic studies; therefore, conclusions regarding variable rates of complement consumption and synthesis during allograft rejection are inferential. The presence of deposited fragments of C3 and C1 in renal allografts which have undergone rejection episodes (27–29), and the present findings of hypercatabolism of C4 and C3 during such periods point to a temporal relationship of allograft rejection and complement activation. Synthesis may not always compensate for increased utilization, since prolonged and poorly controlled rejections generally result in persistent complement depressions (12, 30).

The single case of chronic glomerulonephritis (R. I., Table I) in this series illustrates hypercatabolism of C4

and C3 in the presence of stable and normal serum concentrations. As noted, the rejecting patients (W. S., M. J., Table I) had hypercatabolism with normal serum levels. Examples of normal serum C3 levels in hypercatabolic patients with acquired hemolytic anemia were reported in the study of Petz et al (2) and the same authors have recently reported that hypercatabolism of C3 can occur in lupus erythematosus in the presence of normal serum C3 levels (31).

In hereditary angioedema, persistent reductions in the serum levels of C4 measured either functionally or as a protein have been attributed to hypercatabolism of this component resulting from its destruction by the uninhibited enzyme, C1 esterase (13). Direct confirmation of this postulated hypercatabolism is now available (Fig. 3, Table I). Both the common type of HAE and the "genetic variant" form, in which nonfunctional inhibitor protein is circulating, exhibit accelerated catabolism of C4. In addition, previous observations (13) of a high correlation between C4 protein levels and C4 activities in sera from patients with HAE suggested that the product of the action of C1 on C4 (C4i) was rapidly cleared from the plasma space. The single study with C4i produced in vitro by treating C4 with C1-esterase provides direct confirmation of this postulated rapid clearance (Fig. 4).

Two other abnormalities of C4 metabolism in HAE are deserving of mention. In all four patients, plasma curves contained a rapidly cleared third exponential, even though control studies with the same preparations in normal individuals yielded two exponential curves. This third exponential indicates the existence of an additional "space" other than the plasma or usual "extravascular space"; this "space" serves as a sink for the injected C4. Since all four patients were asymptomatic during the time of the studies, the "third space" may correspond to areas of subclinical angioedema, possibly the gut, which use up C4 at a rapid rate. In addition to the abnormal "third space," the calculated extravascular/intravascular ratios for two of the four HAE patients vastly exceeded those of the control group. Neither of these patients was clinically edematous. This expanded extravascular compartment may also represent areas of subclinical angioedema in which the rate of C4 utilization is not accelerated.

The moderately elevated catabolic rates for C3 in HAE were not anticipated. Measurements of serum C3 levels both by protein (32) and by function (13) had indicated normal levels of this component in HAE, and it had been postulated that the reaction sequence triggered by fluid phase activation of C1 in HAE did not progress to involve the C3 step. One of the two patients reported by Alper and Rosen (1) had an increased catabolic rate for C3, and all four of the patients in the

present study did so. It is clear, therefore, that the reaction sequence in HAE does involve C3, presumably as a result of the generation of C3-convertase by the action of C1 on C4 and C2, in a fashion entirely analogous to the in vitro experiments of Müller-Eberhard, Polley, and Calcott (4).

The relationship between the catabolism and the synthesis of C4 is problematical. In none of the patients with hypercatabolism was a compensatory increase in synthesis observed. In fact, an inverse relationship between catabolism and synthesis was noted, particularly in the patients with HAE. Although the calculated synthetic rates may be artifactually depressed by non-steady-state dynamics and by the grossly abnormal extravascular/intravascular ratios, the HAE patients would still have reduced synthetic rates even if the "true" rates were twice those calculated.

Another explanation for the apparent inverse correlation between catabolism and synthesis would be a negative feedback control of synthesis, in which the presence of the inactive product, C4i, actually retards the production of the native molecule. We know of no biologic precedents for such a negative feedback, in which an inactive metabolite inhibits synthesis of the active material, but studies of such rapidly metabolized proteins as complement components have been limited in number to date. If this negative feedback principle is postulated for C3 synthesis as well, it would have the virtue of explaining the somewhat anomalous findings of Alper and Rosen in progressive glomerulonephritis of childhood (1). In this disease, they found normal to moderately elevated catabolic rates for C3 in the presence of striking reductions of serum C3 levels; the calculated C3 synthetic rates were therefore low. It is possible that inactive C3 generated during the pathogenesis of progressive glomerulonephritis may be responsible for these observed low synthetic rates.

ACKNOWLEDGMENTS

The technical assistance of Mary Graves, Jean Paradysz, and Barbara Moyer is gratefully acknowledged.

This work was supported in part by U. S. Public Health Service Grants HE-11306, AI-07722, AM-05577, 5T1-AI-301, and 5MO1-FR-000-31.

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