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Research Article

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Metabolism of Properdin in Normal Subjects and Patients with Renal Disease

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ABSTRACT Properdin deposition has been recognized in glomeruli of patients with acute and chronic nephritis and lupus nephritis, and low serum properdin levels have been found in these disorders. These findings suggest that properdin may be involved in the production of glomerular damage and that low properdin levels may be due to hypercatabolism. The study was designed to examine the metabolism of properdin in normal subjects and to look for an abnormality in five patients with systemic lupus erythematosus with renal involvement and in six patients with membranoproliferative glomerulonephritis or dense deposit disease (MPGN). Highly purified human properdin was prepared by elution from zymosan, followed by DEAE-cellulose and carboxymethyl-Sephadex chromatography, and labeled with ^{125}I by the iodine monochloride method. Parameters of metabolism were determined by monitoring plasma and urinary radioactivity at frequent intervals after the intravenous injection of 1-2 μCi of labeled material. The fractional catabolic rate (FCR) of properdin in normal subjects was found to have a very narrow range of 0.78–1.08% of the plasma pool per hour (mean 0.95%). In systemic lupus erythematosus, the FCR was regularly elevated with a range of 1.21-2.30% (mean 1.70%). In MPGN, FCR was elevated in three patients (1.22, 1.94, and 2.08%) and within or below the normal range in three (0.76, 1.00, and 1.00%). Properdin levels were reduced in two patients who had the highest FCR's noted in the study. Properdin synthetic rates in normals varied from 4.1 to 14.3 μg/kg per h (mean 9.1) and was not found to be reduced in any patient. Properdin catabolism was found to be normal in a patient deficient in the C3b inactivator. These studies show that properdin is hypercatabolized in patients with renal disease and that decreased properdin levels when they occur in these patients can be entirely explained on the basis of this hypercatabolism.

INTRODUCTION

The properdin system or alternative pathway of complement activation was first described by Pillemer et al. in 1954 (1), and it has subsequently been shown that one of its components, properdin, is a 5.2S glycoprotein of mol wt 184,000 (2). Despite the demonstration almost 20 yr ago that properdin participates in a nonspecific natural defense mechanism, no clear understanding of the specific function of this molecule has been reached, and its precise physiological role remains to be discovered. There is some indirect evidence, however, that properdin may be involved in the pathogenesis of certain forms of renal disease: properdin levels have been found to be low in acute glomerulonephritis, membranoproliferative glomerulonephritis or dense deposit disease (MPGN)[1] (3), and in systemic lupus erythematosus (SLE) nephritis (4–6). Immunofluorescent studies have demonstrated properdin in glomeruli in acute and chronic nephritis (7) and in SLE nephritis (6). To ascertain the cause of low properdin levels and the significance of glomerular de-

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[1] *Abbreviations used in this paper:* FCR, fractional catabolic rate; KAF, conglutinogen-activating factor or C3b inactivator; MPGN, membranoproliferative glomerulonephritis considered to include dense deposit disease; SLE, systemic lupus erythematosus.

TABLE I
Summary of Clinical Data

Diagnosis	Subject	Sex	Age	Disease duration	Renal biopsy	Serum creatinine	Medications at time of study
			yr	*yr mo*		*mg/100 ml*	
SLE	M. S.	M	17	5 2	ND	0.75	P,Cl
	S. T.	F	16	6 6	FPGN	0.7	P
	B. V.	F	14	4	ND	0.7	P,Cl
	S. F.	F	15	8	DPGN	0.7	P,Cl
	M. Si.	F	9	0 1	DPGN	0.65	P,Cl
MPGN	V. W.	F	14	0 11	DDD	0.55	0
	D. M.	M	8	0 2	MPGN	0.65	P,Cl
	P. M.	M	19	6	MPGN	2.67	0
	S. Ca.	F	16	3 6	DDD	3.7	0
	H. G.	M	9	5	MPGN	1.0	0
	P. T.	M	12	6 3	DDD	0.75	0

Abbreviations used: Cl, chlorambucil; DDD, dense deposit disease; DPGN, diffuse proliferative glomerulonephritis; FPGN, focal proliferative glomerulonephritis; ND, not done; P, prednisone.

posits, a metabolic study with ^{125}I-labeled purified properdin was performed in 11 patients and in controls. The properdin level has also been found to be low in a patient with hereditary deficiency of the C3b inactivator (KAF) (8, 9). This patient has a reduced serum level of immunochemical and functional C3 due to consumption via the alternative pathway. Properdin levels about half normal have been found to rise toward normal after temporary correction of the metabolic defect by infusion of normal plasma (8) or purified C3b inactivator (10). To explain the changes in properdin levels, a properdin metabolic study was also performed in this patient.

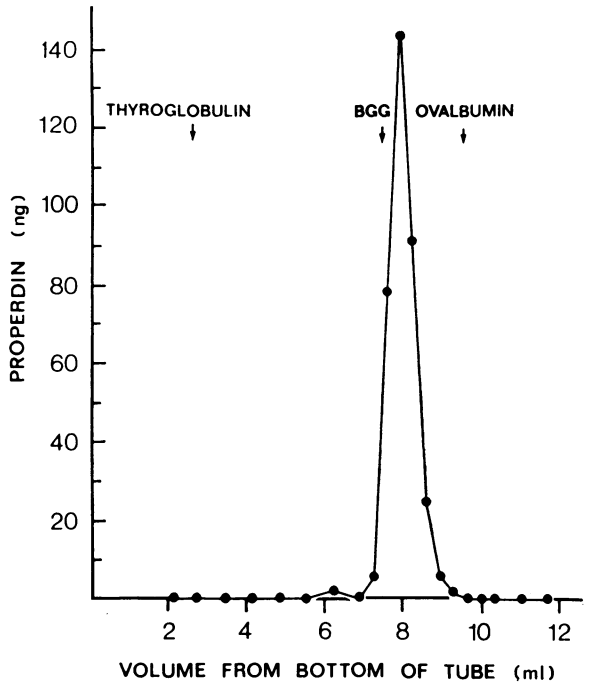

FIGURE 1 Study of purified properdin by sucrose density gradient ultracentrifugation. 0.5 ml of purified protein at a concentration of 25 μg/ml in phosphate buffered saline (PBS), fortified with 0.5% bovine serum albumin, was layered on a 12-ml 10–40% linear sucrose density gradient made in PBS and centrifuged at 41,000 rpm for 26 h at 2°C. Fractions were monitored for properdin by radioimmunoassay. Markers used were thyroglobulin (19.2S), bovine gamma globulin (BGG, 7.3S), and ovalbumin (3.5S).

METHODS

Subjects. Members of the hospital staff served as controls. Five patients with SLE nephritis and six with MPGN were studied, and a summary of their pertinent clinical data is given in Table I. Properdin metabolism was also studied in the patient with a hereditary KAF deficiency.

Preparation of ^{125}I-labeled properdin. Properdin was isolated as previously described (11) and found to be free of pyrogenic activity and hepatitis-associated antigen. It was labeled with ^{125}I by the iodine monochloride method (12) and sterilized by Millipore filtration (Millipore Corp., Bedford, Mass.) after extensive dialysis against phosphate-buffered saline to remove free label. 1% human serum albumin was added to prevent autoirradiation and nonspecific adsorption to glass, and the labeled properdin was stored at 4°C until used. Physicochemical and functional properties of the properdin preparation were determined as described (11) and by zymosan uptake of labeled properdin in the presence of normal serum (13).

Administration of ^{125}I-properdin. Each subject received 1–2 μCi of ^{125}I-properdin intravenously after blockade of thyroidal iodine uptake with potassium iodide. Exact amount of radioactivity administered was determined by measuring the weight of material injected and assaying a small sample of the dose for radioactivity.

Collection and analysis of samples. Blood was drawn into EDTA at 10 min after the injection and at frequent intervals thereafter for 5–6 days. Red cells and plasma

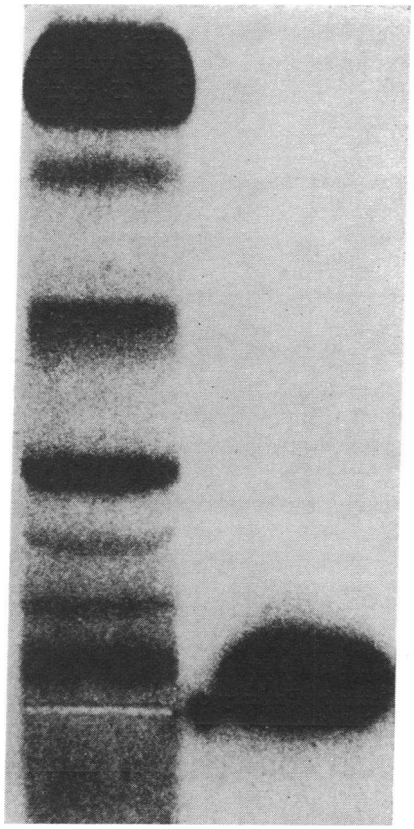

FIGURE 2 *Right:* Autoradiograph of agarose gel after electrophoresis of ^{125}I-properdin for 45 min at 50 v/cm. Buffer is 0.05 M veronal, pH 8.6. Anode to the top. *Left:* Protein stain of agarose gel after electrophoresis of normal human plasma under the same conditions.

were separated by centrifugation, and radioactivity of plasma was assayed in a Packard gamma scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Washed red cells in several patient and control studies were regularly found to be free of radioactivity, so this procedure was dropped from the protocol. Urine was collected throughout the studies, and total radioactivity determined for each sample. Comparison of whole plasma radioactivity and trichloroacetic acid (TCA)-precipitable radioactivity showed no significant difference in results even in patients with impaired renal function, so that only data of whole plasma radioactivity have been analyzed in detail. Similarly, TCA-precipitable radioactivity in urine was negligible even in the presence of gross unselective proteinuria.

Serum protein quantitation. Plasma samples obtained at the beginning, middle, and end of the study were assayed for properdin by electroimmunoassay (14) with a monospecific rabbit antiserum. Plasma levels of properdin factor B, C4, C3, and C5 were also measured by electroimmunoassay.

Calculation of parameters of metabolism. The Matthews method (15) was used to calculate (*a*) the catabolic rate of properdin as the fraction of the plasma pool catabolized per hour (FCR), (*b*) the synthetic rate as micrograms of properdin per kilogram per hour, and (*c*) the extravascular pool to plasma pool ratio. A second estimation of FCR (Campbell, Cuthbertson, Matthews, and McFarlane, 16) was obtained by plotting the ratio of urinary excretion of label to the mean plasma radioactivity during the time of production of each urine sample on the ordinate versus time on the abscissa. FCR and pool ratios were also determined by the method of Nosslin (17), who has shown that the plots of $1\text{-}P/E$ and of $\int_{t_0}^{t_i} E\,dt/E$ (ordinate) against $\int_{t_0}^{t_i} P\,dt/E$ (abscissa) are straight lines, the slopes of which are the FCR and extravascular to plasma pool ratio, respectively. (P is the plasma pool, and E is the extravascular pool at time t; E is given by $1\text{-}U\text{-}P$ where U is cumulative urinary radioactivity.)

RESULTS

The properdin preparation used in this study was in all respects identical physicochemically and functionally to that prepared by Pensky et al. (11). It contained no material which would enter an alkaline disc gel. Continuous sucrose density gradient ultracentrifugation of the purified properdin showed it to be homogeneous and free of high molecular weight aggregates (Fig. 1). Autoradiography of ^{125}I-labeled properdin after agarose gel electrophoresis in 0.05 M veronal buffer, pH 8.6, showed a single band of radioactivity at the origin (Fig. 2). When labeled properdin was incubated with zymosan in the presence of normal human serum at 37°C (13), up to 60% of the label was taken up by zymosan.

In all five patients with SLE, properdin disappeared more rapidly from the plasma pool than in the control subjects (Fig. 3). In three of the six patients with MPGN, properdin was also hypercatabolized (Fig. 4).

Results of immunochemical determinations of properdin, C3, C4, C5, and factor B are shown in Table II. C3 levels were reduced in five of the six patients

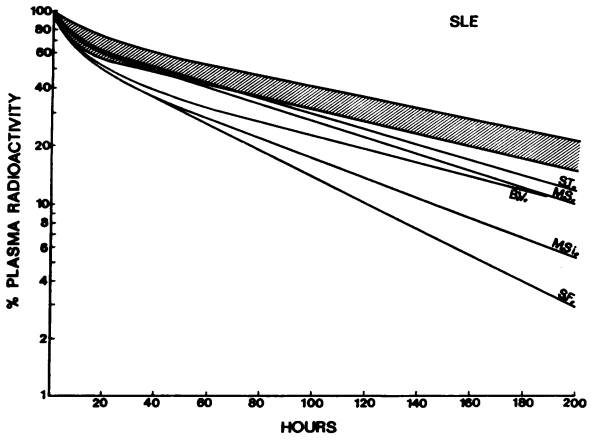

FIGURE 3 Disappearance curves of plasma radioactivity after intravenous injection of 1–2 μCi ^{125}I-properdin. Curves in nine normal subjects fell within the cross-hatched area. Curves obtained in five patients with systemic lupus erythematosus are shown as solid lines. The sample obtained at 10 minutes has been considered to have 100% of the initial plasma radioactivity level.

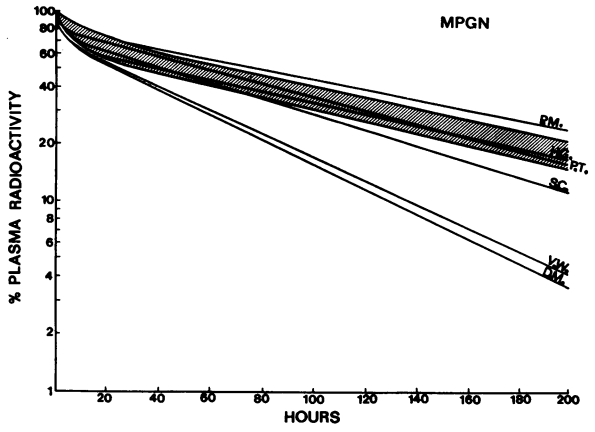

FIGURE 4 Disappearance curves of plasma radioactivity after intravenous injection of 1–2 μCi ^{125}I-properdin in six patients with MPGN. Cross-hatched area shows normal range.

with MPGN, a well-recognized feature of this disease. In SLE, however, despite other evidence of disease activity, C3 levels were normal in three of the five patients while C4 was regularly reduced. C3 levels were comparable in patients and controls.

A wide range in properdin levels was found in the normal subjects, values from 12 to 25 μg/ml being obtained (mean 17.2 μg/ml). A steady state was indicated in all subjects from serial properdin estimations, a necessary condition for the use of the Matthews method to calculate parameters of metabolism. Properdin levels were found to be low in two patients, S. F. with SLE and D. M. with MPGN.

Properdin synthetic rates were calculated from FCR (Matthews method), properdin level, and plasma volume; these results are shown in Table III with the several calculations of FCR and pool ratio. Synthetic rates in control subjects ranged from 4.1 to 14.3 μg/kg per h; values in the renal patients were always normal or elevated.

FCR calculated by Matthews method showed a very narrow range of 0.78–1.08%/h in control subjects. Values above this range were found in all SLE patients (viz., 2.3, 1.21, 1.21, 1.74, and 1.95%) and in three patients with MPGN (2.08, 1.94, and 1.22%).

TABLE II

*Immunochemical Determinations of Levels of Classical and Alternative Pathway Components**

Diagnosis	Subject	Properdin	Factor B	C3	C4	C5
		μg/ml	*mg/100 ml*	*mg/100 ml*	*% normal*	*% normal*
Controls	S. C.	16	29	136	84	115
	I. L.	21	36	112	112	145
	J. G.	18	22	128	94	145
	J. C.	24	29	100	66	108
	J. C.	25	30	109	72	100
	L. H.	15	29	100	88	70
	S. K.	12	39	128	72	118
	L. H.	12	28	100	100	80
	J. T.	12	33	194	98	145
	Mean±SD	17.2±5.1	30.6±4.9	123.0±29.9	87.3±15.3	114.0±27.9
SLE	M. S.	18	34	112	60	94
	S. T.	23	36	170	30	117
	B. V.	13	34	109	36	108
	S. F.	6	22	50	18	72
	M. Si.	26	18	26	16	140
	Mean±SD	17.2±8.0	28.8±8.2	93.4±56.8	32.0±17.7 ($P < 0.001$)	106.2±25.4
MPGN	V. W.	16	20	55	66	53
	D. M.	6	14	64	76	122
	P. M.	28	24	42	84	90
	S. Ca.	33	27	22	180	108
	H. G.	32	34	154	150	160
	P. T.	16	23	83	192	66
	Mean±SD	21.8±10.8	23.7±6.7 ($P < 0.05$)	70.0±46.0 ($P < 0.02$)	124.7±56.0	99.8±39.0
KAF deficiency	T. J.	8	1	22	100	40

* Where means are significantly different from controls (Student t test), P values are indicated in parentheses.

TABLE III

*Parameters of Properdin Metabolism**

Diagnosis	Subject	FCR			Extravascular to plasma pool ratio		Properdin synthetic rate
		Matthews	Campbell	Nosslin	Matthews	Nosslin	
			% plasma pool/h				*μg/kg/h*
Controls	S. C.	0.97	0.66	0.66	0.34	0.42	8.8
	I. L.	0.85	0.52	0.66	0.45	0.86	11.1
	J. G.	0.85	—	—	0.20	—	12.1
	J. C.	1.07	0.59	0.52	0.45	0.77	13.7
	J. C.	1.01	0.62	0.64	0.39	0.57	14.3
	L. H.	0.94	0.64	0.88	0.15	0.57	6.1
	S. K.	1.08	0.72	0.82	0.44	0.73	6.2
	L. H.	0.94	0.64	0.64	0.20	0.37	5.3
	J. T.	0.78	—	—	0.54	—	4.1
	Mean±SD	0.94±0.10	0.63±0.06	0.60±0.12	0.35±0.14	0.61±0.15	9.1±3.9
SLE	M. S.	1.21	0.89	1.10	0.28	0.53	9.5
	S. T.	1.21	0.70	0.72	0.30	0.65	13.3
	B. V.	1.74	0.90	0.95	0.64	1.04	9.1
	S. F.	2.30	0.86	1.25	0.32	1.83	7.1
	M. Si.	1.95	1.35	0.92	0.58	1.16	7.1
	Mean±SD	1.68±0.48 ($P < 0.001$)	0.94±0.24 ($P < 0.01$)	0.99±0.20 ($P < 0.01$)	0.42±0.17	1.04±0.51	9.2±2.5
MPGN	V. W.	1.94	1.29	1.36	0.40	1.09	12.7
	D. M.	2.08	1.88	1.07	0.43	1.22	4.6
	P. M.	0.76	—	—	0.24	—	16.1
	S. Ca.	1.22	0.91	0.92	0.33	0.69	14.3
	H. G.	1.00	0.43	0.58	0.50	0.64	18.6
	P. T.	1.00	0.71	0.67	0.27	0.38	7.7
	Mean±SD	1.33±0.55 ($P < 0.05$)	1.04±0.56	0.92±0.31	0.36±0.10	0.80±0.34	12.3±5.3
KAF deficiency	T. J.	0.95	0.74	0.74	0.24	0.34	2.9

* Calculations based on the methods of Matthews (15), Campbell et al. (16), and Nosslin (17) are indicated. Where means are significantly different from controls (Student t test), P values are indicated in parentheses.

Three patients with MPGN had a normal or low FCR (0.76, 1.00, 1.00%). The highest FCR observed (2.30 and 2.08%/h) occurred in the two patients (S. F. and D. M.) with low properdin levels; and since their synthetic rates of properdin were normal, the low properdin levels can be explained by hypercatabolism alone. There was good agreement between values for FCR based on the methods of Campbell and Nosslin; however, these methods gave lower values for FCR than that of Matthews. This was equally true of control and patient groups; and the seven of eight abnormal FCRs revealed by the Matthews analysis are confirmed by the alternative procedures, the exception being S. T., a patient with SLE who appears to have a normal FCR, as calculated according to Campbell and Nosslin. (Urine collections were incomplete for P. M., as well as for two control subjects, so that only Matthews analysis is possible in these cases.)

In the present study, properdin metabolism has been found to differ from that usually seen with other serum proteins. In general, the semilog plots of total body, plasma, and extravascular radioactivity become parallel as equilibration throughout all compartments is completed and final exponentials are adopted (18). The extravascular pool of radiolabeled properdin, however, reached an optimal value of between 20 and 50% of the injected dose, and then maintained this value throughout most of the duration of the study (see Fig. 5). This was seen in patients as well as controls and representative cases are shown in the figure.

The extravascular to plasma pool ratio as calculated by the Matthews method ranged from 0.15 to 0.54 in control subjects, and values in the patients were generally within this range (Table III). However, the Nosslin analysis, which incorporates urinary radio-

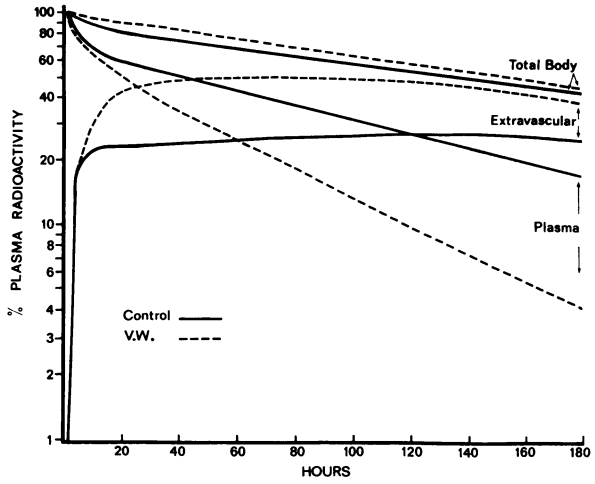

FIGURE 5 Total body radioactivity, plasma radioactivity, and extravascular radioactivity expressed as percent of plasma radioactivity 10 min after the injection of ^{125}I-properdin in a typical control subject and patient (V. W.). Ordinate: percent of initial radioactivity. Abscissa: time elapsed from injection.

activity data, indicated higher extravascular pools in all studies, the highest ratios occurring in patients with high FCR.

A properdin metabolic study was performed in the patient with KAF deficiency, and the plasma radioactivity decay curve and FCR were normal. This analysis indicated that the low properdin level in this patient is due to decreased synthesis (2.9 μg/kg per h).

DISCUSSION

The FCR of properdin in normal subjects was found to be only about 1% of the plasma pool/h, about half that found for C3 (19) and factor B (20). It is probable that properdin catabolism in normal subjects is largely a result of a general catabolic process, and it does not appear necessary to assume that activation or utilization is an important factor in properdin metabolism in health. It should be pointed out, however, that if activation or degradation products of properdin had the same plasma half-life as the parent molecule, the change in state during activation would not be reflected in plasma radioactivity levels (19).

Rapid properdin catabolism has been shown in this study to be a feature of two diseases in which localization of properdin in affected tissues has been demonstrated (6, 7). It seems likely, therefore, that as a result of the pathological process in these disorders, up to 1.3% of the plasma pool of properdin is catabolized hourly, in addition to 1%/h subject to a general catabolic process.

FCR's calculated by the Nosslin and Campbell analyses were generally 30–50% lower than those obtained by the Matthews method. It therefore may be that discrepancies between estimates of FCR could be explained by extravascular metabolism, for which the Matthews method does not allow. If the intercept value on the ordinate in the graph of $1\text{-}P/E$ against $\int_{t_0}^{t_i} P\,dt/E$ is less than one, extravascular metabolism is taking place (17). This value had a mean of 1.02 in controls, 0.96 in SLE, and 0.86 in MPGN, indicating extravascular metabolism in MPGN but probably not in SLE or controls. Since the discrepancy between FCR values was evident in both control and patient groups, extravascular metabolism does not appear to explain it fully.

The finding of normal properdin metabolism and reduced synthesis in the patient with KAF deficiency requires reconciliation with the observation that properdin levels rise briefly after plasma or KAF infusion in this patient (10). If properdin synthesis is depressed by an activation fragment of C3 or by other activated constituents of the alternative pathway, a rise in properdin levels would be expected to follow temporary correction of the metabolic defect.

Purified properdin such as that used in this study may be partially activated, since physiological concentrations will cause C3 cleavage on addition to serum (21), a property probably not shared by native properdin. Direct evidence for the existence of a zymogen precursor of activated properdin has been presented by Stitzel and Spitzer (22), who isolated an enzyme capable of converting inactive properdin to the activated state. Whether the properdin used in this study is heterogeneous with respect to activity, it is clearly physically homogeneous, as shown by Figs. 1 and 2.

Functional heterogeneity of purified properdin might explain the extravascular sequestration of up to half the injected material. It is possible that properdin in one functional state remains in equilibrium with the plasma pool and is subject to normal and pathological fates of native properdin; a second form may represent a molecule functionally altered so that it is sequestrated, perhaps in the reticuloendothelial system, and its catabolism is therefore delayed.

There was no correlation between properdin metabolism and renal pathology in this small group of patients, but the three MPGN patients with the longest disease duration had normal properdin FCR, suggesting that properdin hypercatabolism may be a feature predominantly of early disease.

This study has shown significant abnormality in properdin metabolism in SLE nephritis and MPGN and shows that hypercatabolism alone and not hyposynthesis explains earlier reports of low properdin levels. Renal hypercatabolism of properdin would be consistent with the demonstration of glomerular deposition. Studies in vitro have recently shown the formation of com-

plexes between C3 and properdin (23, 24) and since both C3 and properdin have been demonstrated in glomerular deposits in SLE (6), it is possible that these contain complexes of C3 and properdin formed in vivo. It is not clear whether, if this is the case, the glomerular material represents deposition of properdin-C3 complexes formed in the circulation sequestered in the glomeruli or whether one of these proteins is deposited primarily, with complex formation following. Nor is it possible to say whether this process is a cause or a consequence of glomerular damage. An altered state of the protein may at least partly explain unusual features of its metabolism in normal subjects.

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