the composition of nonhelical regions but also from differences in the relative amount of helical structure and in the lengths of the helical segments. Variation in composition might, therefore, reflect significant differences in structure.

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Beta-1C-Globulin: Metabolism in Glomerulonephritis

Abstract. *The metabolism of β_{1C}-globulin labeled with iodine-131 was studied in six normal individuals and in three individuals with glomerulonephritis who exhibited markedly reduced serum concentrations of this protein. Fractional catabolic rates were similar in both groups, and therefore the low concentration of serum β_{1C}-globulin in glomerulonephritis appears to be chiefly secondary to decreased synthesis.*

It has been known for decades (*1*) that total serum complement activity is markedly lowered for several weeks after the onset of acute glomerulonephritis. In addition, some children without a history of acute glomerulonephritis, but with intermittent nephrotic syndrome and characteristic pathological changes of the glomeruli (progressive glomerulonephritis), have persistently low levels of complement activity for a year or more (*2*). In both acute and progressive glomerulonephritis, concentration of the third component of complement, β_{1C}-globulin (C′3), has been shown to be markedly decreased in serum (*2, 3*).

Several independent observations have indicated that the lowered activity of complement in these disorders is the result of continuing complement fixation. Immunofluorescent studies have shown β_{1C}-globulin, as well as γ-globulin and fibrinogen, on the glomeruli of patients with glomerulonephritis (*4*). Some features of acute and progressive glomerulonephritis can be produced in rats by the injection of rabbit antiserum to rat kidney (*5*). In this experimental disease, activity of serum complement is lowered and β_{1C}-globulin is fixed to glomeruli (*5*).

We have attempted to obtain more direct evidence for the basis of persistently lowered activity of complement in the serums of individuals with glomerulonephritis by the use of I^{131}-labeled β_{1C}-globulin. $Beta_{1C}$-globulin was prepared by the original method of Müller-Eberhard, Nilsson, and Aronsson (*6*), and it was labeled with radioactive iodine by the technique of MacFarlane (*7*). A mean of one atom of iodine (or less) per molecule was incorporated. Over 80 percent of the radioactivity was attributable to β_{1C}-globulin as measured by precipitation of counts in antibody excess by an antiserum specific for this protein.

The labeled β_{1C}-globulin was filtered on a Millipore filter and examined by immunoelectrophoresis before and after incubation with hydrazine and $EAC'_{1,4,2}$ (sheep red blood cells sensitized with rabbit antibody to sheep red cells that have complement components 1, 4, and 2 on them), and, after addition to fresh serum, by radioimmunoelectrophoresis, with the use of antiserums to whole human serum and to β_{1C}-globulin. With both antiserums, the labeled protein gave an arc identical in appearance with that of native β_{1C}-globulin in fresh serum. The same material converted to an arc of more rapid mobility (β_{1A}- or β_{1G}-globulin) when it was incubated with hydrazine or $EAC'_{1,4,2}$ in the same manner as β_{1C}-globulin in whole serum. Millipore-filtered, labeled, and dialyzed β_{1C}-globulin increased the hemolytic activity of serum from patient V.O. (with progressive glomerulonephritis) to the same extent that the unlabeled protein did. These findings confirm the observation of Müller-Eberhard and coworkers that labeling with radioactive iodine has no effect on the complement activity of C′3 (*8*).

Concentration of serum β_{1C}-globulin was estimated by an immunochemical method (*9*). Six normal subjects and three individuals with glomerulonephritis were studied (*10*). After intravenous injection of from 2 to 6 μc of I^{131}-labeled β_{1C}-globulin, radioactivity was assayed in plasma, urine, and, in some cases, stool. In no case was significant protein-bound radioactivity found in the urine, nor was there significant excretion of radioactivity in the stool. Curves of protein-bound radioactivity in plasma and urinary excretion in normal individuals (Fig. 1) and in individuals with glomerulonephritis (Fig. 2) showed no striking differences. Since the concentrations of β_{1C}-globulin in serums of individuals with glomerulonephritis were between 4 and 20 percent of normal (5 to 30 mg as opposed to a normal of 150 ± 10 mg per 100 ml), the low levels resulted primarily from depressed synthesis of β_{1C}-globulin.

Catabolic and synthetic rates were calculated from curves of protein-bound radioactivity in plasma by the Matthews method (*11*) and from plasma concentrations of β_{1C}-globulin. Plasma volumes were calculated from the amount of radioactivity in plasma samples obtained 10 minutes after injection and the amount of radioactivity in the labeled protein that was injected. In several instances, plasma volumes were also determined by I^{125}-labeled human serum albumin, and the figures

Table 1. Summary of the metabolism of I^{131}-labeled β_{1C}-globulin in six normal subjects and three individuals with glomerulonephritis.

Subject	Age (yr)	Sex	Status*	β_{1C}-globulin in serum (mg/100 ml)	Fractional catabolic rate (% plasma pool/hr)	Rate of synthesis of β_{1C}-globulin (mg/kg per hr)	Extravascular/plasma pool ratio	Plasma volume† (ml/kg)
T.G.	26	M	N	150	2.0	1.2	1.1	39
D.Z.	26	M	N	165	2.8	1.7	0.4	37
F.A.	27	M	N	150	3.0	1.9	0.8	43
R.L.	26	M	N	162	2.3	1.5	0.6	40
L.R.	26	M	N	150	2.5	1.3	0.7	33
J.P.	26	F	N	150	1.7	1.1	0.8	42
Mean (± 2 SD)				154 ± 14	2.40 ± 0.98	1.5 ± 0.6	0.71 ± 0.48	
J.F.	11	F	AGN	6	3.6	0.1	1.1	38
V.O.	13	F	PGN	7	2.4	0.1	0.8	50
J.L.	12	F	PGN	30	1.8	0.3	1.1	55

* N, normal; AGN, acute glomerulonephritis; and PGN, progressive glomerulonephritis. † Patients V.O. and J.L. had hematocrits of 38 and 34 percent.

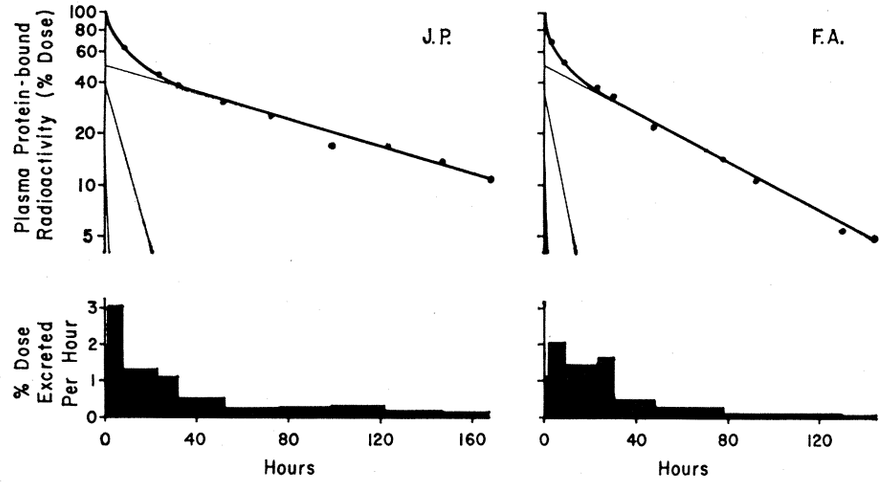

Fig. 1. (Upper portion) Protein-bound radioactivity in plasma as percentage of the initial (10-minute) value plotted on a semilogarithmic scale versus time for two normal individuals. Plasma curves have been resolved into three exponentials, which are also shown in upper portion of figure. (Lower portion) Percentage of the dose excreted in urine per hour in these same studies.

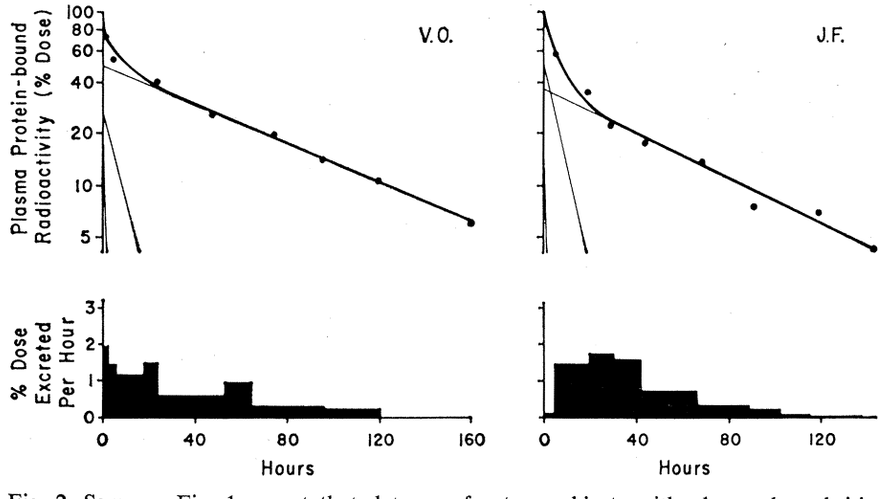

Fig. 2. Same as Fig. 1 except that data are for two subjects with glomerulonephritis. It is evident that these curves do not differ greatly from those in the normal individuals.

agreed within 5 percent. If a significant amount of labeled protein had been removed before 10 minutes, plasma volumes calculated from dilution of I^{131}-labeled β_{1C}-globulin would have been too high. These data are presented in Table 1 and show the markedly lowered synthesis of β_{1C}-globulin in individuals with glomerulonephritis.

Prolonged decrease of hemolytic complement in serum of individuals with glomerulonephritis is apparently due chiefly to the selective lowering of the concentration of serum β_{1C}-globulin, since highly purified β_{1C}-globulin (*12*) alone restores the hemolytic activity of complement in serums from these individuals (*9, 13*). Deposition of β_{1C}-globulin on glomeruli may well be an initial and transient event in acute glomerulonephritis. This protein is detectable on glomeruli even after complement activity returns to normal in both natural (*14*) and experimental (*5*) glomerulonephritis.

Our failure to find strikingly increased disappearance of labeled β_{1C}-globulin from the plasma of individuals with glomerulonephritis cannot be attributed to contaminating protein (less than 20 percent of the total radioactivity as labeled β_{1F}- and β_{1H}-globulin) in the β_{1C}-globulin preparation. Even if one assumes a very long half-life (for example, 20 days) for this contaminant, made very unlikely by the actual plasma curves, catabolic rates would be increased only two and a half or three times. If synthesis were normal, the catabolic rates would have to be up to 25 times the normal to explain the very low concentrations of serum β_{1C}-globulin that we observed in some of the patients. In addition, as seen from the plasma and urinary excretion curves, no rapidly catabolized component is evident.

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Satellite Deoxyribonucleic Acid from Bacillus cereus Strain T

Abstract. *DNA isolated from exponentially growing cultures of* Bacillus cereus *T has a single component (density 1.696 g cm^{-3}) in a cesium chloride density gradient whereas DNA isolated from spores shortly after the initiation of germination has two components: a major one (density 1.696 g cm^{-3}) and a satellite (density, 1.725 g cm^{-3}). The DNA of both components is double-stranded. By the first cell division there is no satellite DNA.*

The germination and outgrowth of bacterial spores has been proposed as a useful model system for the study of cellular differentiation (*1*). Evidence has been obtained for changes in the pattern of RNA synthesis (*2*), in classes of ribosomes (*3*), in metabolic activity (*4*), and in structures (*5*). We have examined some of the physiochemical properties of the DNA isolated from spores and outgrowing cells of *Bacillus cereus* T, and have obtained evidence for two DNA components, one of which differs quantitatively and qualitatively from the single component found in logarithmically growing cells of *B. cereus*.

Spores were prepared from cells of *B. cereus* grown in "G" medium (*6*). Germination after heat activation (65°C, 2 to 4 hours) was initiated by the addition of spores to "G" medium containing $10^{-3}M$ L-alanine and $10^{-3}M$ adenosine. Germination was stopped at intervals after initiation by pouring portions of the culture over an equal volume of ice maintained at −20°C. The cells were washed once in SCET buffer [1*M* NaCl, 1 percent cetyltrimethylammonium bromide (weight/volume), $10^{-3}M$ EDTA, and $10^{-3}M$ tris, *p*H 8.0], suspended in the same buffer, and incubated at 37°C overnight with 1 mg of Pronase (Calbiochem) per milliliter. Treatment with Pronase greatly increased the viscosity of the germinated spore suspension, and the spores appeared empty under the phase-contrast microscope. Dormant spores were not appreciably affected. The partially lysed cells were then heated for 4 hours at 65°C to complete lysis and to allow autodigestion of Pronase. Cellular debris was then separated by centrifugation and the supernatant was poured into approximately 4 volumes of 95-percent ethanol. The fibrous precipitate that rose to the top of the ethanol was collected and dissolved in saline-citrate (0.15*M* NaCl + 0.015*M* $Na_3C_6H_5O_7$, *p*H 7.0) according to the procedure of Marmur (*7*). RNA was digested by incubation at 37°C for 30 minutes with 1.0 unit of T_1 ribonuclease and 100 μg of pancreatic ribonuclease per milliliter. Sodium perchlorate was added to a final concentration of 1.0*M* and the incubation mixture was repeatedly deproteinized by a mixture of chloroform and isoamyl alcohol (*7*). The DNA was finally precipitated with ethanol, and the precipitate was dissolved in saline-citrate solution. Such solutions were stored at 4°C over chloroform until used.

Fig. 1 (above right). Buoyant densities in CsCl of DNA extracted from cells in the mid-log phase of growth (*A*) and spores 1 minute after initiation of germination (*B*). *Escherichia coli* DNA (1.71 g cm^{-3}) was used as a reference density marker.

Fig. 2 (bottom right). Buoyant densities in neutral and alkaline CsCl of one and two component DNA from *Bacillus cereus* T.: DNA at neutral *p*H from cells in the mid-log phase of growth (*A*), DNA at *p*H 12 from cells in mid-log phase of growth (*B*), DNA at neutral *p*H from cells shortly after germination (*C*), and DNA at *p*H 12 from cells shortly after germination (*D*).

Analytical methods were essentially those of Meselson, Stahl, and Vinograd (*8*). For analysis in cesium chloride solutions at neutral *p*H, mixtures were prepared by the procedure of Schildkraut, Marmur, and Doty (*9*). When alkaline CsCl solutions were used as solvent, solid CsCl was added to the DNA solution, the density was adjusted