Abnormal Human Serum Globulins

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Although many diseases induce an elevation in serum proteins, only multiple myeloma and certain related but obscure disorders elicit the formation of pathological serum globulins. These diseases involve a tumor or hyperplasia of the marrow or other branch of the reticuloendothelial system. They are characterized by an increase in the number of plasma cells that are considered a site of antibody synthesis. In a previous study of the nature and biosynthesis of the abnormal proteins in multiple myeloma, we isolated globulins from the individual serums of many patients (*1*). The abnormal globulins have been characterized by their physicochemical constants (*2*) and by their content of *N*-terminal amino acids—that is, by the number and nature of the terminal residues having a free α-amino group (*3*). The biosynthesis of the abnormal globulins has been studied by use of isotopic amino acids (*4*).

It is well known from electrophoretic analysis of multiple myeloma serums that the increase in plasma protein is confined to a predominant component that is remarkably homogeneous when it is compared with normal human globulins (or with the globulin increment in infections or toxic conditions) (*5*). Yet, electrophoretically distinguishable globulins are elaborated by different patients. Most myeloma globulins are of the "gamma" type; that is, their mobility is within the wide range exhibited by normal gamma globulins, and they have a similar molecular weight and shape (*2*). However, the abnormal globulins are more homogeneous in electrophoresis and thus exhibit precisely defined mobilities. With different patients, the mobility of the "gamma" type abnormal globulins may vary from -0.7 to -1.7×10^{-5} $cm^2sec^{-1}v^{-1}$ at *p*H 8.6, compared with a mean of -1.3×10^{-5} $cm^2sec^{-1}v^{-1}$ for the normal gamma globulin. Some myeloma globulins (the "M" or "β" types) have a greater mobility at this *p*H; these are often molecularly heterogeneous and contain several components with unusually high sedimentation constants (*2*). Immunologically, the multiple myeloma globulins fall into two groups of proteins, the members of which are interrelated and cross-react with one another. The one group is closely related to normal gamma globulin, the other (the β type) is distantly related. However, each protein appears immunologically different, indicating individual specificity (*6*). *N*-terminal amino acid analysis has proved effective in the chemical characterization of these proteins and has provided evidence that structurally abnormal forms of serum globulins are synthesized in response to the disease (*3*).

The chemical characterization of pathological serum globulins is best illustrated by reference to the studies on cryoglobulins (that is, serum globulins that gel reversibly on cooling), for these proteins can readily be purified by virtue of their insolubility in the cold. Cryoglobulins occur rarely, even in multiple myeloma, but they have been reported most often in this disease (*7*). They are also occasionally found in an obscure syndrome called cryoglobulinemia. Some of the chief clinical symptoms of the latter disease result from the physical properties of the cryoglobulin—that is, temperature sensitivity of the extremities, high blood viscosity, purpura, and cardiac failure attributed to intravascular deposition of the viscous protein (*7, 8*). The relationship of cryoglobulins to normal plasma components has been difficult to define, because the abnormal proteins have seldom been isolated and described. To be sure, a "cold-insoluble" globulin has been obtained in small amount from normal, pooled human plasma, but its origin has been attributed to contamination with pathological serums (*9*). In the cases we have studied, up to 80 percent of the total plasma protein and up to 98 percent of the globulin are of a kind not detectable in normal human serum.

Because there is no uniformity in the sedimentation constant, isoelectric point, solubility, or other physical properties of the few cryoglobulins thus far characterized, we undertook studies to clarify the relationships of these proteins to one another and to normal globulins. Eight highly purified cryoglobulins have been investigated by physicochemical and *N*-terminal amino acid analysis (*10*). Several were too insoluble at 1°C to permit electrophoretic analysis at *p*H 8.6. However, all were homogeneous at whatever *p*H was suitable for study (*p*H 4.7, 6.7, or 8.6). Although the crystallization of normal serum globulins has never been observed, two of the cryoglobulins crystallized spontaneously in the cold. A summary of the physical and chemical properties of the cryoglobulins and of normal human gamma globulin is given in Table 1.

All of the cryoglobulins differed from the normal protein in two or more of the following properties: crystal form, solubility, isoelectric point, electrophoretic mobility, sedimentation constant, ultracentrifugal or electrophoretic homogeneity, and *N*-terminal amino acid residues. The same properties served to differentiate all the cryoglobulins from one another, with the exception of the first two listed, which appeared similar in all respects tested. These results confirm the physicochemical variability of the cryoglobulins produced by different patients; they establish for the first time that the *N*-terminal residues of these unusual plasma proteins are not identical and, thus, that the molecules are structurally different.

Quantitative end-group analysis by the fluorodinitrobenzene method of Sanger (*11, 12*) has permitted the classification of cryoglobulins into chemically different types. Three of the proteins, which will be referred to as *N*-aspartyl globulins, contained approximately 2 moles of *N*-terminal aspartic acid and were devoid of *N*-terminal glutamic acid or exhibited only traces of this end-group. Thus, in accord with their physical homogeneity they possessed essentially only one type of amino end-group. These proteins had a similar mobility at *p*H 8.6 and a similar S_{20}. The two noncrystalline globulins were tested immunologically and were found to be related to, but not identical with, normal gamma globulin. A second group of three proteins, to be designated as *N*-glutamyl globulins, have approximately 3 moles of *N*-terminal glutamic acid per 160,000 g and little or no *N*-terminal aspartic acid. The *N*-glutamyl globulins differ from the *N*-aspartyl globulins in electrophoretic properties and in ultracentrifugal homogeneity and can also be distinguished from one another by their distribution of sedimenting components. Two cryoglobulins had both aspartic and glutamic acids as *N*-terminal groups but in twice the normal amount. Thus, in the *N*-terminal locus all the cryoglobulins were structurally different from the gamma globulin obtained from pooled normal serum.

Physicochemical and *N*-terminal amino acid analyses have also been com-

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Table 1. Physical properties and *N*-terminal groups of human plasma cryoglobulins.

Protein	Crystal form	*pI*	Mobility at 1°C		S_{20}, *w*	*N*-terminal groups	
			*p*H 8.6	*p*H 4.7		Aspartic acid	Glutamic acid
						(moles/160,000 g)	
			10^{-5} $cm^2sec^{-1}v^{-1}$		S		
Normal γ_2		7.2	− 1.3	3.0	6.6	1.12	1.75
Th		7.5	− 1.1	3.0	6.6	1.80	0.16
Ag		> 6.4	− 1.1		6.6	2.04	Absent
Mi	Needles		− 1.3		6.75	2.06	0.16
R	Hexagons		Insoluble	3.0	7.6,* 11	2.00	2.64
Gu			Insoluble	3.3	6.0	2.29	2.21
Se			− 0.8		6.4	Absent	2.65
WK		5.5			7, 18,* 28	0.12	3.14
I			Insoluble	3.3	7.6,* 11	0.15	2.77

* Mobilities and sedimentation constants were determined in 0.1 ionic strength Veronal buffer *p*H 8.6 or in 0.1 ionic strength acetate buffer *p*H 4.7. All the cryoglobulins migrated with a single sharp boundary upon electrophoresis. S_{20}, *w* is extrapolated to infinite dilution except for ultracentrifugally heterogeneous globulins, in which case the major component is indicated by an asterisk. *S* signifies Svedberg units, and *pI* signifies isoelectric point.

pleted on eight myeloma globulins that were not cryoglobulins. In seven cases the distribution of *N*-terminal aspartic and glutamic acids was unlike that found in normal human gamma globulin or in any of its commonly available subfractions. Both *N*-aspartyl and N-glutamyl globulins occurred in this group of proteins. It is of great interest that two of the myeloma globulins proved to be practically devoid of *N*-terminal aspartic and glutamic acids and contained amino end-groups not found in normal globulin fractions (*13*). Both proteins had a molecular weight of 160,000 but migrated with a mobility of only -0.7×10^{-5} $cm^2sec^{-1}v^{-1}$ at *p*H 8.6. One contained 2 moles of *N*-terminal leucine per 160,000 g; the other had about 1 mole of *N*-terminal leucine and about 1 mole of an unidentified *N*-terminal amino acid (or peptide). The unidentified substance behaves like phenylalanine on some paper chromatograms, but it moves faster than any known amino acid in the tertiary amyl alcohol system. Alanine has been identified as one of the *N*-terminal groups in another protein.

We have suggested previously that normal human gamma globulin consists of at least two components, one of which possesses only *N*-terminal aspartic acid, and the other only *N*-terminal glutamic acid (*3*). At first glance, this hypothesis is given some substance by the finding that *N*-aspartyl and *N*-glutamyl globulins predominate in the serum of some patients. It might seem tempting to suggest a correlate of this hypothesis, namely, that patients with multiple myeloma or cryoglobulinemia synthesize one or the other of these or still other normal components excessively and preferentially. However, the present observations demonstrate a far wider variety of amino end-groups and of physical properties than can reasonably be accounted for by such an interpretation. Indeed, we conclude that all the cryoglobulins that we have studied are unnatural proteins in that they are formed only in disease. Alternatively, it may be argued that all these proteins do, in fact, occur physiologically but only in trace amounts.

In this interpretation one is led to the unlikely conclusion that at least seven different types of cryoglobulins occur in normal serum. The *N*-leucyl globulins likewise appear to be abnormal proteins. It is more difficult to decide whether all myeloma globulins are truly abnormal and individually specific proteins, or whether, rather, each results from the profuse and selective synthesis of but one of the many normal globulin components. It is conceivable that an abnormal cell can produce abnormal proteins, but in extensive studies with Steven Schwartz we found no correlation between the morphology of the myeloma plasma cell and the nature of the pathological serum globulin or the proteinuria.

Quantitatively, as well as qualitatively, the change in protein synthesis induced by multiple myeloma is profound. Besides the copious synthesis of abnormal plasma globulins, the profuse excretion of unique urinary proteins (Bence-Jones proteins) is a common characteristic. Neither aberration is invariably present, nor are the two mutually exclusive, yet one or both are always found. Some patients produce twice as much abnormal globulin as a healthy person does total serum proteins, and others excrete as much as one-third of their daily nitrogen intake by way of a protein found only in this disease. In this striking parasitism of nitrogen metabolism may lie a clue both to the mechanism of normal growth and protein synthesis and to the nature of its derangement in disease.

Like the myeloma globulins, the Bence-Jones proteins are of different types (*12*). The urinary proteins have a molecular weight that is one-fourth of the weight of the serum globulins, and they are identified by unusual heat coagulation properties. They are more often excreted if the patient fails to make an abnormal globulin, but for tracer studies we have chosen several subjects in whom the two anomalies in protein synthesis occurred together.

The isotopic studies have as yet failed to reveal any precursor relationships between the abnormal globulins and the Bence-Jones proteins. Contrary to one prevalent view, the results have demonstrated that the urinary proteins are not formed by renal cleavage of the unnatural globulins (*4*). Rather, the Bence-Jones proteins are rapidly synthesized *de novo* from the free amino acids of the body pool and are excreted rapidly. In recent experiments, C^{14} glutamic acid was injected into a patient who had indwelling venous and urethral catheters so that blood and urine specimens could be taken frequently. Radioactive Bence-Jones protein appeared in the bladder within 35 min after injection of the labeled amino acid. The maximum specific activity of urinary protein was attained within 6 hr, and the radioactivity thereafter fell off at a logarithmic rate with a half-time of decline of about 8 hr. Similar results were obtained with another patient who was given C^{14} lysine. Fractionation and isotopic analysis of the blood proteins of these subjects is in progress.

From the physicochemical, chemical, and isotopic studies, the following picture is emerging. Patients with multiple myeloma have a perverted mechanism of protein synthesis. They may make abnormal globulins that are individually specific, whether by the test of immunological or chemical analysis. They may excrete Bence-Jones proteins that likewise appear to be individually characteristic. They may exhibit both aberrations in protein synthesis. No function is known either for the abnormal serum globulins or for the urinary proteins. The abnormal globulins accumulate as if they were made by a defective assembly line that had lost the ability to produce the diverse spectrum of normal globulins. The Bence-Jones proteins are essentially incomplete proteins, both in physicochemical and antigenic features. They are excreted as waste products, perhaps as the unused or unfinished precursors of normal globulins, or perhaps as abortive products of the deranged mechanism of serum protein synthesis. Although normal tissue proteins are not converted directly into Bence-Jones protein, the parasitism of nitrogen metabolism results in a wasting of the patient. The rapidity of synthesis and excretion of the urinary proteins testifies that they

are made directly from metabolites diverted from normal pathways. Further study of the nature and biosynthesis of proteins in multiple myeloma should aid in the elucidation of the mechanism for normal serum protein synthesis.

References and Notes

1. This work was aided by grants from the National Cancer Institute of the National Institutes of Health (C-1331-C3), the American Cancer Society, and the Lasdon Foundation. The *N*-terminal amino acid analyses were initiated in the Department of Biochemistry, University of Cambridge, with the aid and encouragement of Fred Sanger. Aiko Miyake assisted in some of the analyses.
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10. I am indebted to the following persons for specimens of purified cryoglobulins and for information on their properties: David Barr and Ella Russ, Cornell University Medical College, New York (proteins R and I), and A. S. McFarlane, National Institute for Medical Research, Mill Hill, London (protein WK). Serums containing cryoglobulins were kindly supplied by Charles B. Huggins, University of Chicago, Chicago, Ill.; Steven A. Schwartz, Hektoen Institute for Medical Research, Cook County Hospital, Chicago, Ill.; Elliott F. Osserman, College of Physicians and Surgeons, Columbia University, New York; F. W. Gunz, Pathology Department, Christchurch Hospital, Christchurch, New Zealand; and Jan Waldenström, University of Lund, Lund, Sweden.
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13. Preliminary analyses indicate that *N*-terminal glutamic acid, aspartic acid, and serine are also present in human serum globulin, fraction III. However, valine appears to be the major *N*-terminal group of the β_1 iron-binding globulin.

Arthur Berridale Keith, Student of Mankind

To live a full and useful life is one of man's most cherished desires. To make major contributions to the study of mankind is given only to the elect. To be humble when showered with honors demonstrates the inner spirit.

In these rare gifts and achievements Sir Arthur Berridale Keith was well endowed. His 88 years were indeed full. Born on Quarry Farm near Aberdeen, his pride in Scotland never left him. His delightful Scotch burr inspired all those who heard his voice. His charm of manner was beguiling.

As a medical student at Marischal College in Aberdeen, human anatomy became his absorbing interest. For dissection, he won a prize. This turned out to be Tylor's *Anthropology,* a book destined to influence his life, for now he commenced the study of mankind—a study that was to nominate him, five decades later, as the greatest living anthropologist.

His first job was as assistant for 2 months in the Murthly asylum near Perth with the special assignment of studying insanity, entertaining the deranged patients, and dissecting the scores of brains in the post-mortem room.

His next job was as assistant to a general practitioner in England. Then came the first real break. Keith was offered and accepted the position of medical officer to a British gold company in Siam, where he remained for 2 years. His first scientific publication resulted from the dissection of 32 Primates. Home in London, his main goal was "to pursue my studies into the origin and antiquity of mankind."

For 5 years he worked in the London Hospital, then became conservator of the Museum of the Royal College of Surgeons of England from 1908 to 1914. The next quarter-century was employed in research on the endocrine glands but more particularly on the physical characters of ancient man.

Keith's contributions to knowledge were recognized publicly in 1921 when King George V bestowed the title of Sir Arthur, the name by which he was known to his countless admirers and student friends. Honors and distinctions rained freely upon Sir Arthur.

Honorary degrees accoladed his writings and his lectures. Election as rector of Aberdeen University in 1930 was a crowning climax to his career. Three years later Sir Arthur became master of Buckston Browne Farm near Downe in Kent, a research center administered by the Royal College of Surgeons; he held this position until his death on 7 January 1954.

The last time I saw him was in 1950. On this cold British summer's day we sat together in his study beside a glowing fire. Sir Arthur listened to the results obtained by the Peabody Museum-Harvard expedition to the Near East in search of new data on ancient and modern man. His questions were clear, his comments encouraging. For 30 years he had guided my researches in this area.

My last impression was of a tall, spare figure enveloped in an old Scotch tweed cape leaning on a long stick as the rain beat down on his tasseled tam-o-shanter with the silver buckle. A radiant smile came over his thin, ascetic face. The rain and mist soon hid him from view.

To attempt to sum up a great man's life in a few words is a hopeless task; to reveal qualities of the inner man is even more difficult. Sir Arthur was soft-spoken, often with hesitant speech as his brain outran his words, kindly to those who sought guidance, swift and sharp to his critics, and above all generous with his time to those who needed encouragement; he well remembered his early days.

His many students, and in these I include all those who learned from his great knowledge, are forever linked together in a common bond by gratitude to their teacher, Sir Arthur Keith. Mankind is the richer for his living, the poorer for his passing.

HENRY FIELD

Coconut Grove, Florida

To do hard things without show of effort, that is the triumph of strength and skill.—A. J. Rowland.