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An appreciation of Elvin A. Kabat (1914–2000): Scientist, educator and a founder of modern carbohydrate biology

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Elvin Abraham Kabat, a world-renowned immunologist who specialized in the immunochemistry of carbohydrates, died on June 16th 2000 at the age of 85. In a remarkable career, extending from the 1930s to the 1990s, Elvin Kabat made many important contributions to science, including the first demonstration that antibodies are gamma globulins, the delineation of the size of antibody combining sites, the demonstration of complimentarity-determining regions in antibodies, and the elucidation of the structural basis for blood group specificities, to name just a few. He was also an outstanding teacher who served as an inspiring mentor to a succession of graduate students and post-doctoral fellows, many of whom went on to make significant careers in immunology and carbohydrate biology.

Elvin Kabat began his scientific career with a B. S. from City College, New York in 1932, a Master's degree in 1934 and, in 1937, a Ph.D. degree from Columbia University, New York. He joined the Columbia University faculty in 1941 and eventually held Professorships in Microbiology and in Human Genetics and Development there. In later years he also held joint appointments at the National Institutes of Health. His Ph.D. work at Columbia was carried out with Michael Heidelberger who is widely considered to be the father of modern immunology. Heidelberger's work, including Kabat's contributions, firmly established immunology as a quantitative science through the development of the quantitative immunoprecipitation methods. In 1937–1938 Kabat spent time at the Karolinska Institute in Uppsala, Sweden working with Arne Tiselius. Using antibody preparations purified at Columbia, Tiselius and Kabat demonstrated for the first time by electrophoretic techniques that antibodies are gamma-globulins. During the war years Kabat carried out war-related work at Columbia University on pneumococcal vaccines and the potent poison, ricin. This work remained classified for many years after the war, as did his report (with Ted Rosenbury) on the dangers of germ warfare.

At the end of the war, Kabat took up two important new lines of investigation, one on the immunochemistry of carbohydrate antigens and the other animal models of allergic encephalomyelitis. He continued the carbohydrate studies for the remainder of his career, but the latter work was brought to a sudden and unfortunate end when his National Institute of Health (N.I.H.) grant support was terminated during the turmoil of the McCarthy years. This action was apparently the

Elvin A. Kabat, Ph.D., a key figure in the development of glycobiology.

result of being denounced to the F.B.I. as a communist by a fellow scientist. Although cleared by the President's Loyalty Review Board, this was not sufficient to protect his N.I.H. grant. In this respect, he joined the ranks of many other famous U.S. scientists, including Linus Pauling, who also lost their grant support during that time. With the support of the Office of Naval Research and later the National Science Foundation, Kabat turned his attention to the study of the immune responses in man to polysaccharide antigens – a field in which he was to make many important contributions. Prime among these were experiments that resulted in the concept of the antibody combining site and the delineation of its physical properties. Using anti-dextran antibodies isolated from immunized volunteers (including himself) Kabat and co-workers showed that anti-dextran combining sites have a finite size, encompassing up to seven sugar residues. These experiments were made possible by the isolation, by Allene Jeanes in Prioria, Illinois and Bill Whelan and Jim Turvey in Bangor, Wales, of a series of α1,6-linked isomaltose oligosaccharides. Using these oligosaccharides as inhibitors of dextran-anti-dextran immunoprecipitation reactions, Kabat showed that the inhibitory power of these oligosaccharides, on a mole basis, increased with increasing chain length but that the increment in inhibitory power, per added sugar, diminished until an upper limit was reached at about six or seven sugar units. These studies were extended in subsequent years with a series of detailed experiments on dextrans and other polysaccharides with human and animal antibodies, and later with human and mouse monoclonal antibodies. One outcome of these experiments was the concept of 'cavity' and 'groove' type antibody combining sites, representing reactivities with terminal and internal sugar sequences, respectively. These ideas were later confirmed by X-ray crystallographic studies by other investigators.

As a compliment to his studies on anti-carbohydrate antibodies, Kabat was interested in the specificities of plant and animal lectins, which also recognize sugar units. Other investigators were already engaged in studies on the specificities of lectins. Kabat was able to use his extensive collection of polysaccharides and oligosaccharides to analyze their reactivities in greater detail. These studies often led to new insights into their specificities and opened the way for their use as specific reagents for immunochemical and immunohistological studies. Among the lectins studied were those from *Helix pomatia*, *Dolichos biflorus*, *Griffonia simplicifolia* and *Axinella* sponge and the chicken hepatic lectin (the last with Gilbert Ashwell at the N.I.H.).

Beginning in 1967, when investigators began to report on the amino acid sequences of Bence Jones proteins (immunoglobulin light chains) and later of antibody heavy and light chains, Kabat turned his attention to the analysis of the significance of these sequences for antibody structure and antibody-antigen interactions. Working originally by visual inspection, and later by computer analysis (with Tai Te Wu) he recognized many patterns in immunoglobulin sequences, for example the presence of conserved glycine residues at certain positions and of framework residues and sequences not involved in antigen recognition. The greatest achievement of these studies, however, was the demonstration of the hypervariable, complimentarity-determining regions (CDRs) in both the heavy and light chains of immunoglobulins. To analyze these sequences Wu and Kabat developed a search parameter, termed 'variability', which became a powerful tool in studying other polymorphic proteins, such as MHC molecules, as well as immunoglobulins. Later work by other investigators, mainly by X-ray crystallography, confirmed that CDRs correspond to the amino acid sequences comprising the antigen-binding regions of antibodies. As a part of these studies, Kabat also put forward the 'minigene' hypothesis that anticipated later findings on the assembly of immunoglobulin genes by recombination mechanisms.

It is interesting to recall the background to Kabat's entry into the field of the blood group carbohydrates, where his contributions have also been spectacular. While working with Heidelberger on quantitative immunochemistry of bacterial polysaccharides, Kabat read a paper published in 1936, in the *Journal of Experimental Medicine* by Karl Landsteiner and Merril Chase, describing the presence of blood group A substance in commercial pepsin. Kabat was stimulated by this report, knowing how little antigenic material can be obtained from red cells. He suggested to Heidelberger that they might "do some quantitative precipitin tests" using this soluble material and human anti-A sera. His mentor's response was that this was a good problem for him to pursue as an independent investigator. Thus, Kabat's interest in the blood group antigens remained latent, only to be rekindled by a proposal by Ernest Witebsky and colleagues, during World War II, that soluble A and B substances from hog and horse stomachs might be added to group O blood to neutralize the anti-A and anti-B, to make it a better universal blood. Some of the preparations were not meeting specifications in that they induced anaphylactic shock in guinea pigs, and Witebsky suggested to Kabat to look into the question. Kabat applied for a contract from the Office of Scientific Research and Development, which he received very late in the war, in 1945, shortly before V-E day! Thus, Kabat's seminal work on the structures of the blood group antigens began in 1945.

By the time Kabat's studies on the blood group antigens were launched, there had been some important developments. Walter Morgan and colleagues in the UK had shown that large amounts of blood group substances occur in human ovarian cyst fluids, and they had developed methods for their isolation. Witebsky and colleagues had succeeded in producing high titer anti-A and anti-B sera by immunizing volunteers with hog A and horse B substances. Thus, the scene was set for quantitative immunoprecipitation assays of the blood group antigens. These assays, coupled with hemagglutination assays, served initially to monitor the purification and characterization of the blood group substances, and their partially hydrolyzed forms. Later on, they were important in assigning the blood

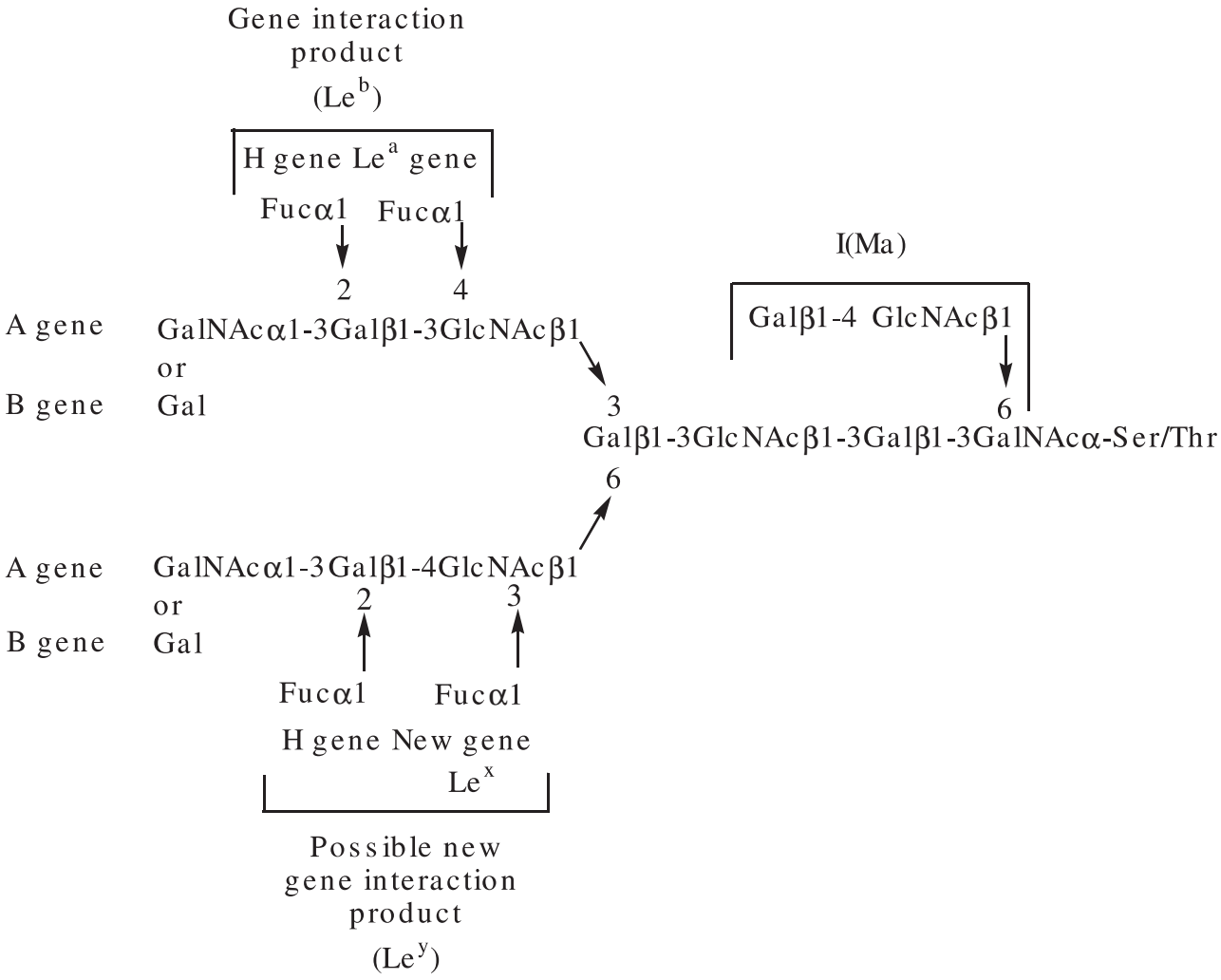

Figure 1. Composite structure for blood group-active oligosaccharides on ovarian cyst mucins proposed by Kabat and colleagues. The 'new gene' and the 'new gene interaction' products are now termed Le^x and Le^y antigens, respectively. The unsubstituted branch in the core region was later shown to be the determinant recognized by anti-I (Ma). More recent work has shown that blood group epitopes may also occur on this branch and that on erythrocytes, the branched backbone sequences have exclusively the Galβ1-4GlcNAc (poly-*N*-acetyllactosamine) sequence on which are expressed the i antigen determinants (when unbranched) and various I determinants (when branched) as reviewed in refs 1 and 2. Modified from ref. [10].

group determinants expressed on the substances by identifying and determining the sugar sequences of oligosaccharide fragments derived from them that specifically inhibited the binding of the blood group-antibodies. In the following two decades, the biochemical basis of the major blood group specificities was elucidated by Kabat's group in intense competition with Walter Morgan and Winifred Watkins at the Lister Institute in London.

In the first phase of this pioneering immunochemical work with blood group-active polysaccharides (mucins) of human and animal origins, it emerged that there were some similarities in these highly complex substances, and also differences between those of differing A, B and O(H) types. By mild acid hydrolysis, immunoreactivities were revealed with horse antibodies raised to pneumococcus type XIV polysaccharide. This indicated the presence of common sequences (lactosamine backbones) among their oligosaccharide chains. The other major conclusion was that the 'structural groupings' associated with each of the three blood group types and the type XIV cross reactivity were distinct. In the extremely labor-intensive groundbreaking studies that followed, various conditions of partial acid hydrolysis and alkaline degradation were applied to blood group A, B, H, Lewis^a (Le^a) and Le^b antigen-positive substances, to isolate and elucidate the sequences of the immunoreactive oligosaccharides. By 1966, the chemical structures of these major blood group antigens had been determined, and a composite structure for the carbohydrate chains on the epithelial mucins

that bear the various blood group determinants had been proposed. As depicted in Fig 1, the genes (coding for glycosyltransferases) involved in the biosynthesis of the major blood group antigens were anticipated.

The significance of this work extends beyond our current understanding of the biochemical basis of the major blood group antigens. This work opened the way to the elucidation of several blood group-related carbohydrate antigens, later to be referred to as carbohydrate differentiation antigens, whose expressions change sequentially from the earliest stages of embryogenesis right through differentiation events in adulthood, and also in oncogenesis [1]. Among these are the I and i antigens expressed on specific parts of the backbones of this family of oligosaccharides, and the Le^x and Le^y antigens. The latter sequences were designated 'new gene' products (Fig 1) when first discovererd on the epithelial mucins. The foundations had been laid for understanding the roles of members of this family of carbohydrate antigens as ligands of carbohydrate-binding receptors. Notable examples are the roles of the Le^x- and Le^a-related oligosaccharides as recognition elements for the selectins [2].

Kabat described Michael Heidelberger as a Leonardo da Vinci-type Renaissance man in a splendid portrayal of his mentor, who had discovered Kabat's own talents almost by chance [3]. Kabat himself was perhaps more of an Evangelist, a teacher with a deep commitment to science, and whose text book entitled *Experimental Immunochemistry*, jointly written with Manfred Meyer, served as the Bible of precise laboratory techniques for immunologists for many years.

In Kabat's laboratory, strict rituals for ensuring the highest standards in experimental practice were an obligatory start point, irrespective of seniority and previous experience. Thus, as recalled in the Boston Globe (June 20, 2000), to those who worked in his laboratory, Kabat was known as an exacting and highly principled boss. In a commemorative issue of *Molecular Immunology* honoring Kabat's 70th birthday, two former students, Nobel Laureate Baruj Benacerraf, and Stuart Schlossman wrote: "To be 'Kabatized' and survive meant you could do well anywhere". Members of the Kabat clan of former associates have a lasting kinship, unified, not only through having had the unforgettable experience of working with him, but also by the memory of a demanding mentor who could nevertheless unwind, and share his enormous talent as a humorous raconteur of personal and scientific stories (some of the reminiscences have been published [4–9]).

Kabat was a member of the National Academy of Sciences, and received numerous other honors and awards for his scientific contributions, among them the Golden Hope Chest Award of the National Multiple Sclerosis Society (1962), the Karl Landsteiner Memorial Award of the American Association of Blood Banks (1966), the Fogarty Scholarship of the National Institutes of Health (1974–1975), the Fifth International Convocation of Immunology Award (1976), the Louisa Gross Horwitz Prize, shared with Michael Heidelberger and Henry Kunkel (1977), the Philip Levine Award of the American Society of Clinical Pathologists (1982), and in 1991, the National Medal of Science (the highest award given by the United States for scientific achievement). The latter award, coming as it did from the U.S. government, was a personal vindication long overdue.

Elvin Kabat is survived by his wife Sally, his three sons, Jon Kabat-Sinn, Geoffrey and David, and six grandchildren to whom we submit this appreciation of our mentor on behalf of the Glyco community.

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