Obituary

Winifred May Watkins (1924–2003)

by Robin D. Marshall

Blood transfusion is, in general, a safe procedure, in large part as a result of many years of extraordinary research by Winifred Watkins and her colleagues at the Lister Institute of Preventative Medicine, which led to the elucidation of the overall relationships in the 'ABO Lewis' blood group antigens.

Winifred described her hypothesis about the relationships, after considerable reflection, in 1958. Her written message at that time was clear, unequivocal and set down in simple terms, for she wrote with an economy of words allied with a powerful use of language. The idea was novel, the experimental procedures used to test the hypothesis were innovative, and the results were radical. Her achievements in that borderline area that straddles the fields of medicine and biological sciences are unique. They will remain as a monument and a constant reminder of her, as will her other internationally acclaimed excellent advances in medical science. Winifred Watkins was born in Shepherd's Bush, London, on 6 August 1924, the second daughter of a process engraver whose hobby was painting. Her early education was at the local St Stephen's Parish Elementary (now V.A. Primary) School in Shepherd's Bush. It appears to have been a contented and stimulating time for her, and, in 1935, she was awarded a London County Council Scholarship, at Godolphin and Latymer Girl's School in Hammersmith. It was a school with fine traditions of scholarship, including the serious teaching of chemistry, physics and biology, combined with other advanced approaches to education.

It must have suited Winifred to be in a community in which co-operative activity based on self-discipline was paramount, and where there was a minimum of rules and a maximum of personal responsibility. Winifred attended high school during times of great change, necessitating the ability to learn to adapt to new situations, joining the school in the same year as a new Head (Joyce Bishop) who ushered in change. The pupils were encouraged to debate news of both national and international events that were destined to profoundly affect the lives of everyone worldwide, as well as reflecting on wider responsibilities to the community. Winifred remained in contact with the school throughout her life.

There was talk, in the latter 1930s, of evacuating all children of school age from London to the country in the event of war. On 1 September 1939, a few days before the school was due to reassemble for the start of the year in which Winifred was due to sit her London School Certificate examinations, the Head called together all staff and students, and addressed them briefly about the likelihood of war. The whole school then set off on foot for Ravenscourt Park railway station where they boarded trains to take them away from possible bombing of London, not knowing their destination. On arrival at Windsor, it was discovered that there was no long-term accommodation for the 400 girls and staff from Godolphin and Latymer School. Three weeks later, they were transported by train to Newbury where the local schools provided generous teaching facilities and where lodgings were found. The boys' school at Newbury had teaching laboratories and Winifred was given permission to join the boys' laboratory classes for science lessons.

After a year as an evacuee, Winifred returned to live at home in London, a city that would soon come under The Blitz. She attended school in company with a relatively small number of other students who had also returned home and they were taught by a depleted staff.

It appears to have been a time

of goodwill at the school, and Winifred gained her Higher School Certificate in the summer of 1942. Although she had originally wished to study medicine, she was precluded from doing so because wartime regulations prevented entry to university, and so she accepted a laboratory post at the Lister Institute, which was classed as her (obligatory) 'war service'. The Institute, at the time, was drab, the building having been superficially damaged during earlier air raids, with some laboratories being deprived of natural light, and having few, if any, windows glazed and intact. Winifred always recalled those early years, working in spartan laboratory surrounding, as being happy ones. She attributed this, in part, to the ethos generated by the intellectually stimulating group of scientists in the Biochemical Department, which alone remained in Chelsea throughout the war. It was at this time that Winifred enrolled for evening classes at Chelsea Polytechnic (now College) in order to study chemistry, while being fully employed at the Lister Institute during the day. She was awarded a BSc Honours degree in Chemistry by London University in 1947.

Those early days at the Lister Institute were recalled by Winifred as being interesting for a number of reasons. Walter Morgan had introduced research on blood group antigens at the Institute in 1940. Within months of Winifred joining, she had produced sufficient practical data to merit publication in a reputable journal, yet questions were asked as to whether an 'unqualified', 20-year-old, female technician should appear as an author of a serious scientific treatise. It says so much about Winifred that she stuck to her guns, and I would imagine that she persuaded others through reasoned argument, a charming manner and a smiling face. In later life, she explained with amusement her having to get written permission from the Director of the Institute, in order to be an author on a paper, published in 1944, describing her own results. Convention at the time was that authors should have formal qualifications, which Winifred did not have until 1947. Not for the first (nor the last) time, Winifred changed established patterns of thinking by asking searching questions. She would, on occasion, ask what seemed at first to be a naïve question, and later reality would dawn that there was no simple answer.

There was another interesting aspect of those days; with her keen sense of humour, Winifred sometimes alluded to the need to be on good terms with all of one's colleagues, especially in 1944. As she would explain, one never knew who would be joining you under the protective shield of a laboratory bench that one would dive under at the time that the noise from the motor of the latest overhead flying bomb ceased.

Winifred relinquished her post at the Lister Institute in 1947, after graduating, and became a postgraduate student at St Bartholomew's Hospital Medical School, London, under the supervision of the immunochemist, Arthur Wormall. She studied the action of nitrogen mustards (cytotoxic agents used at the time to treat myeloid leukaemia) on the changed immunological properties of body proteins after reaction with these agents. Her innovative work as a postgraduate student led to several publications, and upon being awarded a PhD in biochemistry by the University of London in 1950, she returned to work at the relatively new, small Blood Group Research Unit, initiated by Walter Morgan, at the Lister Institute.

She was asked to investigate the relationship between the antigen on O blood cells, previously thought to be the product of the O gene in the ABO system, and the A and B antigens. The work led to the realization that the O antigen was the precursor substance of the A and B antigens, and that the O gene was in no way analogous to the A and B genes. It was an independent genetic system. It was proposed that the precursor molecule be called H substance, and the finding was a major breakthrough in understanding the ABO Lewis system, although the suggestion led to some curious reactions. Winifred was both surprised and amused by the view expressed in a letter sent by a distinguished scientist that the idea served solely

to "stultify" thinking. Not for the first time, Winifred had to persevere and, with good humour, wait for reality to dawn. Watkins had used the techniques of periodate oxidation, sequential degradation and exoglycosidases, isolation of chemically derived fragments and binding studies with both lectins and antibodies in the quest for her radical proposal in 1958, concerning the biosynthesis of the blood group substances, that explained the serological, genetic, antigenic and chemical basis. The techniques used and the deductions made from the results were new, and they were to have major implications for the growth of the whole field of glycoconjugate research.

In order to proceed further with her studies, she spent from 1960 to 1961 as a Henry Wellcome Travel Fellow in William [Zev] Hassid's department in the University of California at Berkeley. There she acquired practical knowledge of the awakening field of the glycosyltransferase enzymes. The next few years were spent with her colleagues on testing her hypothesis, with the realization that the gene products that specified the ABO Lewis antigens were specific glycosyltransferases. These latter enzymes were the primary gene products and the blood group antigens were secondary products.

The crux to the understanding lay in the specificities of the particular glycosyltransferases. Both her suggesting and experimental proving of the relationship between the components of the ABO Lewis blood group system in antigenic, chemical, serological and biosynthetic terms were recognized internationally as outstanding. The Oliver Memorial Fund made its Annual Award to Winifred in 1965 in recognition of her outstanding contributions in the field of blood transfusion. Two years later, in 1967, she received (jointly) the Karl Landsteiner Award of the American Association of Blood Banks, followed by the Paul Ehrlich-Ludwig Darmstadter Medal and Prize (also jointly) from the Paul Ehrlich Foundation, Frankfurt-am-Main, Germany in 1969. It was in 1969 that she was elected to Fellowship of the Royal Society of London and, in 1970, the University of London awarded her the William Julius Mickle Fellowship.

Watkins and her colleagues then demonstrated the presence of specific glycosyltransferases in post-mortem samples from baboon and human stomachs and submaxillary glands, tissues rich in blood-group activity, and another striking finding emerged. The A and B transferases are expressed in both secretors and non-secretors, and this confirmed that the absence of A and B activity in non-secretors results from the absence of the H precursor material. Those results began a number of efforts that led to both confirmation and extension of Watkins' original hypothesis. In addition, the ABO gene locus was identified, confirming Bernstein's 1924 (the year of Winifred's birth) suggestion that inheritance of the ABO group involved three alleles at the ABO locus. The A and B transferases were shown to exhibit, severally, weak, overlapping enzymic specificity, to be able to use the 'wrong' sugar and to catalyse synthesis of the 'wrong' immunodeterminant. Moreover, antibodies prepared against the two enzymes cross-react, indicating at least some homology between the two. Those results have great significance in some areas of forensic science.

Winifred continued with her research until shortly before her death, after a stroke, on 3 October 2003. She shed new light on how immunologically active structures appear in the course of normal development, and on how normally unexposed epitopes re-appear when there is a lack of expression of glycosyltransferases in malignant cells. The principles are, in general, similar to those that are applicable to the expression of blood group antigens, and her work will stimulate research in many areas of glycoconjugates.

She also had long collaborative efforts with colleagues in Kenya and Thailand in studying drug action in malaria, and a number of publications resulted from the work. Winifred Watkins was always extraordinarily energetic and also of good humour.

Winifred not only developed an important area of biomedical science, but she also acted as a great stimulus to many others, both in her own laboratory and also throughout the world. She began work in Walter Morgan's department, and her efforts led to entirely new ideas and knowledge about complex carbohydrates in general, including blood-group active systems. In due time, she became both Head of the department and Professor of Biochemistry at the University of London.

As well as the awards mentioned above, shortly after her unique contributions were made public, she was presented with the Kenneth Goldsmith Award of the British Blood Transfusion Society in 1986,

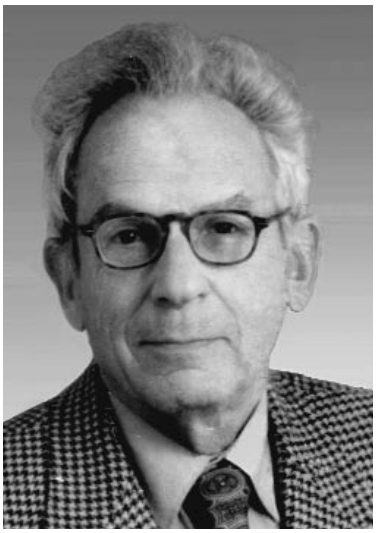
the Royal Medal of the Royal Society in 1988, the Franz Oehleckler Medal of the German Society of Transfusion Medicine and Immunohaematology in 1989, and the Phillip Levine Award of the American Society of Chemical Pathologists in 1990 (jointly). She was awarded a DSc degree by the University of London in 1963 and an Honorary DSc in chemistry by the University of Utrecht in 1990. She was elected to membership or fellowship of a number of prestigious bodies: The Royal Society of London (Fellow, 1969), The Royal College of Pathologists (Fellow, 1983), the International Society of Blood Transfusion (Honorary Member, 1984), the Polish Academy of Sciences, (foreign Member, 1988), The Royal College of Physicians (Fellow, 1990), the Japanese Biochemical Society (Honorary Member, 1990), the British Blood Transfusion Society (Honorary Member, 1996), The Royal Swedish Academy of Sciences (Member, 1988), the Academy of Medical Sciences (Fellow, 1998) and the Biochemical Society (Honorary Member, 2000).

In addition to great distinction in science, Winifred had an engaging personality and a smile of comprehension would sometimes appear at times when somebody was becoming a shade pompous or was bewailing shortcomings in his/her laboratory. Who ever had a perfect laboratory?

She would disagree and smile indulgently at suggestions that women should receive positive discrimination, but she had very strong views concerning the need for pupils, regardless of sex, from their earliest schooldays to be provided with the same opportunities in every subject, including science. She was never strident on the subject for, by nature, she would always rather explore an idea before making comment. That exploration may well have been in her mind as she served as a Governor at both Dulwich College and Alleyne's School from 1987 to 1995. An evening spent discussing the philosophy of the educational process with Winifred was wonderfully refreshing. She had many friends worldwide, and to be a guest worker in her laboratory, be it for a year or an afternoon, was to enjoy the work and to feel a sense of excitement.

Robin Marshall, now retired from Strathclyde University, was a long-term colleague of Winifred Watkins.

Elliott N. Shaw (1920–2003)



by Charles A. Kettner
(DuPont Pharmaceutical Company, USA; retired)
and Herbert Angliker
(Novartis Research Foundation, Basel, Switzerland)

Elliott N. Shaw died in his sleep at home in Basel, Switzerland on 25 November 2003. Elliott had just returned from a holiday to Italy with his wife Charis. They were married in 1955 in Milan, Italy, after meeting at the Rockefeller Institute. Elliott is also survived by his children, Jeffrey and Roxanne.

Reviewing Elliott's contributions to science is a very humbling experience. We, like many others, were very fortunate to have had him as a mentor and as a colleague. We know of no individual who has made a larger contribution to our understanding of proteolytic enzymes. First, in the 1960s, Elliott did pivotal work on the understanding of catalytic mechanism of proteases. He was able to identify the active-site histidine residue of serine proteases by selective alkylation with substrate-derived chloromethyl ketones; this was the first example of 'affinity labelling'.

Elliott is often referred to as 'The Father of Modern Affinity Labelling', but this title only partially reflects his contributions. Elliott was among the first to recognize the importance of proteases in biological control, and led the way in these studies. Elliott developed highly effective inhibitors that allowed him to identify unique roles for proteolytic enzymes. Perhaps just as important, he developed reagents that are used by almost everyone who studies proteolytic enzymes. To attest to Elliott Shaw's early

insights, inhibitors of four different proteolytic enzymes are presently being used a drugs, while inhibitors of a number of other proteases are in the drug-development process in the pharmaceutical industry.

Elliott was born in Youngstown, Ohio, on 6 April 1920. He received his BS degree from the Massachusetts Institute of Technology in 1941 and his PhD in organic chemistry in 1943. He was a Research Fellow in Medicinal Chemistry at the Squibb Institute for Medical Research, New Brunswick, New Jersey from 1943 to 1948. Elliott was a Research Fellow and an Associate in Biochemistry at Rockefeller University, New York, from 1948 to 1957. He joined the Tulane University School of Medicine, New Orleans, Louisiana, where he was an Associate Professor and Professor of Biochemistry between 1957 and 1965. Next, he moved to the Biology Department at Brookhaven National Laboratory, Upton, New York State (1966–1982). While at Brookhaven, Elliott was Chairman of the Biology Department (1974–1979) and was an Adjunct Professor in the Biochemistry Department at the State University of New York, Stony Brook, New York State, (1973–1982). He moved to the Friedrich Miescher Institute in Basel, where he remained until 1994, when he retired from active research.

As he was trained as a synthetic organic chemist, Elliott was among the first to work at the interface of chemistry and biology. Early understandings of the roles of individual amino acid residues in catalytic function arose from studies of serine proteases. Elliott synthesized tosylphenylalanine chloromethyl ketone (TPCK) and demonstrated that this reagent bound to chymotrypsin in a substrate-like manner, but, instead of being hydrolysed, it alkylated a single histidine residue in the active site of the enzyme[1]. Shaw coined the term 'affinity labelling' to describe this selective chemical modification. Later, the close proximity of this histidine to the active-site serine residue and to an aspartate residue was confirmed in an X-ray crystal structure of chymotrypsin. These observations led to the identification of the roles of the aspartate–histidine–serine catalytic triad in catalytic mechanism of serine proteases, which is described in all biochemistry textbooks.

Elliott applied a similar approach in developing an affinity label for trypsin, which hydrolyses peptide bonds after basic arginine or lysine residues. The resulting reagent, tosyl-lysine chloromethyl ketone (TLCK), like TPCK, selectively alkylates the active-site histidine residue[2]. Furthermore, TLCK does not inhibit chymotrypsin and, conversely, TPCK does not inhibit trypsin. Thus, two reagents were available which could distinguish between the activities of two enzymes based on their primary specificity. They have become commonly used tools for early characterization of proteases as trypsin-like or chymotrypsin-like.

Earlier in Elliott's career, he and Mares-Guia[3] observed that aromatic amidines and guanidines were much more effective trypsin inhibitors than the corresponding aliphatic compounds. This allowed them to identify a hydrophobic binding site between the catalytic site and the anionic binding site of the basic side chain. This interaction has yielded a prototype for many drug-development programmes that target physiologically important trypsin-like enzymes.

Before 1970, the primary focus of research in Elliott's lab was on mechanistic studies to identify catalytic residues by affinity labelling. The direction of his research shifted to the identification and study of physiological important proteases using affinity labelling. Focusing on trypsin-like enzymes, he recognized the need for more effective and selective affinity labels. This was achieved by preparing peptide chloromethyl ketones, taking advantage of the extended binding sites (reviewed by Kettner and Shaw[4]). One compound in this series, which was particularly useful, was D-Phe-Pro-Arg chloromethyl keytone (PPAC). PPAC is a selective inhibitor of the blood-coagulation enzyme thrombin. It was successfully used *in vivo* to demonstrate important roles for thrombin in arterial thrombosis and used in determining the crystal structure of thrombin[5]. The binding of PPAC was used as a model for developing a thrombin inhibitor in the pharmaceutical industry.

In the mid-1970s, Elliott also began an effort to identify important roles for thiol proteases. One of the first barriers to overcome was the fact that thiol proteases are similar to serine proteases, reacting with many of the same inhibitors and substrates. Elliott's lab prepared peptide diazomethyl ketones, which were the first selective affin-

ity labels for thiol proteases[6]. His laboratory, and others, developed synthetic methods for peptide fluoromethyl ketones to complement the diazomethyl ketones[7].

Like the serine proteases, it was important to have reagents that would distinguish between different enzymes. Selective inhibitors for cathepsins B, C, L, and S and the calpains were obtained by optimizing the affinity–labelling moiety and the amino acid sequence for individual proteases. Since most target thiol proteases are intracellular, compounds were designed which were cell-permeable. The result has been a rather extensive library of selective thiol-protease inhibitors, which has allowed the investigation of the roles of thiol proteases in macrophage protein turnover, in parasite cell cycles, in platelet activation and in the pathogenesis of glomerulonephritis.

Elliott can easily be held as model for a senior scientist. In the early 1970s, a number of laboratories were independently studying proteolytic enzymes. Elliott, along with E. Reich and D. Rifkin, brought these researchers together in a Cold Spring Harbor Symposium in 1974. This was a pivotal conference and its published proceedings[8] led to establishing the study of proteolytic enzymes as a field and led to many others joining this field.

Throughout his career, Elliott fostered quality research on proteolytic enzymes, often encouraging collaborations between different laboratories. Very fittingly, he was the chairman of the 1986 Gordon Research Conference on Proteolytic Enzymes and their Inhibitors. He was a member of the Editorial Board of the *Archives of Biochemistry and Biophysics* (1969–1982) and was Executive Editor (Protein Structure) from 1972 to 1976. He was also on the Editorial Advisor Board for *Biochemistry* from 1974 to 1979.

He was a member of the NIH Biochemistry Training Committee (1962–1966), the American Cancer Society Advisory Committee on Research on Therapy (1967–1971) and The American Chemical Society, Division of Biological Chemistry, Nominating Committee (1975). Elliott was also a member of the Society for Biological Chemistry, the New York Academy of Science and the Harvey Society.

After his retirement, Elliot's interest in research did not diminish. He continued to keep up with the latest literature. Besides his commitment to research, Elliott was interested in reading, history, politics, gardening and, most of all, classical music.

In conclusion, Elliott Shaw was known for his scientific integrity. He held others to high scientific standards and held himself to even higher standards. Borrowing from the old Jimmy Stewart movie *It's a Wonderful Life*, we can ask: "Where would science be if it hadn't been for Elliott Shaw?"

References

1. Schoellmann, G., and Shaw, E. (1962) Biochem. Biophys. Res. Commun. **7**, 36–40
2. Shaw, E., Mares-Guia, M. and Cohen, W. (1965) Biochemistry **4**, 2219–2224
3. Mares-Guia, M. and Shaw, E. (1965) J. Biol. Chem. **240**, 1579–1585
4. Kettner, C. and Shaw, E. (1981) Methods Enzymol. **80**, 826–842
5. Bode, W., Mayr, I., Baumann, U., Huber, R., Stone, S.R. and Hofsteenge, J. (1989) EMBO J. **8**, 3467–3475
6. Leary, R., Larsen, D., Watanabe, H. and Shaw, E. (1977) Biochemistry **16**, 5857–5861
7. Rauber, P., Angliker, H., Walker, B. and Shaw, E. (1986) Biochem. J. **239**, 633–640
8 Reich, E., Rifkin, D.B. and Shaw, E. (eds) (1975) Proteases and Biological Control, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Charles A. Kettner obtained his PhD from Texas A&M University in 1974. He joined Elliott Shaw's laboratory at Brookhaven National Laboratory and worked with Elliott until 1980, when he joined the DuPont Company. Throughout his career he has been a disciple of Elliott's, working to develop inhibitors of physiologically important proteolytic enzymes until his recent retirement at the level of Research Fellow.

email: sket122169@aol.com

Herbert Angliker obtained his PhD from the University of Basel, Switzerland, in 1979. Afterwards, he held post-doctoral positions at the Institute of Physical Chemistry of the University of Basel and at the Wesleyan University in Middletown, CT, USA. In 1984, he joined Elliott Shaw's group at the Friedrich Miescher Institute, Basel. After Elliott's retirement, Herbert changed to the service department of the institute, establishing the DNA sequencing service and is presently in the group of the DNA microarray service.

email: angliker@fmi.ch