BIOGRAPHICAL MEMOIRS

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Biogr. Mems Fell. R. Soc. 2002 **48**, 233-249, published 1 December 2002

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Biogr. Mems Fell. R. Soc. Lond. **48**, 233–249 (2002)

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Elected F.R.S. 1966

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FAMILY BACKGROUND AND EARLY LIFE

Ralph Ambrose Kekwick was born on 11 November 1908 at Leytonstone, Essex. Records of the Kekwick family go back to 1750, when they were living near Warrington in the parish of Daresbury. They were then Quakers and were involved in the local dye industry. In about 1800 they started to move south, and Ralph's grandfather, John Kekwick (1815–82), lived first in Abingdon and then, after the death of his first wife, moved to Bromley-by-Bow, where he worked as a corn factor. A second marriage outside the sect made him unacceptable to the Society of Friends and thus broke the family association with the Quakers. John Kekwick had two daughters and six sons by his second wife; of these, Ralph's father, Oliver A. Kekwick (1865–1939), was the youngest but one. He eventually became a managing clerk in a firm of ships' chandlers in Albert Docks, London. Ralph's maternal great-grandfather, James Price (1820–1900) had an administrative post at the Guildhall, London, and was responsible for the organization of the Lord Mayor's procession and banquets at the Guildhall. His eldest son, James Price (1840–1911), Ralph's grandfather, followed his father into employment at the Guildhall. James Price had three daughters and a son; Ralph's mother, Mary E. Price (1868–1958) was his eldest child. At the age of 13 she became a pupil-teacher at Bromley St Leonard's Church school, Bromley-by-Bow, where she had been a scholar. She was compelled to give up teaching when she married in 1898, in accordance with the regulations then in force, but she was called back to teach in Leyton during World War I at a boys' elementary school and, although Essex reinstated their 'no married women' rule after the war, London County

Council had less strict regulations and she continued to teach until she reached retirement age. Ralph was the youngest of her three children; she had an elder boy, Leslie Oliver (1899–1975) and a girl, Phyllis Mary (1902–78); with her strong character and interest in education she was a considerable formative influence in Ralph's early life and had taught him to read before he started school.

Ralph attended infants' and elementary schools in Leytonstone and then in 1919 gained a scholarship to Leyton County High School for boys. He remembered two outstanding masters, W.F. Woolner-Bird, who taught mathematics, and W.E. (later Sir Emrys) Williams, who aroused his interest in English literature. Ralph enjoyed his schooldays and was keen on all forms of sport. His elder brother, Leslie, lived at home while studying for a degree in chemistry at University College London (UCL), and it was his accounts of the experiments that they were doing that excited Ralph and firmly set him on a course towards a career in science.

In 1925, aged 16, Ralph passed the School Certificate with a sufficient number of subjects and distinctions to make him immediately eligible for university entrance. His father was in poor health at the time and it was decided that Ralph should go up to university rather than stay on at school for two more years to take the Higher School Certificate.

University College London, 1925–31

Ralph Kekwick entered UCL in October 1925, a month before his 17th birthday, and obtained a first-class honours degree in chemistry in June 1928. F.G. Donnan, F.R.S., was head of the Chemistry Department at that time, J. Norman Collie, F.R.S., was Professor of Organic Chemistry and W.E. Garner (F.R.S. 1937) was Professor of Physical Chemistry. Ralph found that it was physical chemistry that most caught his imagination. Two other people who joined the honours chemistry course in 1926 were Frank (later Sir Frank) Young (F.R.S. 1949) and C.M. Blow. Ralph and Frank Young travelled abroad together in vacations and remained lifelong friends. They borrowed many of their textbooks from Lewis's library in Gower Street, near UCL, and in December 1927 encountered John Pryde's *Fundamentals of biochemistry*. Ralph read this during the Christmas vacation and immediately became enthralled with a subject that he had not previously known existed. UCL did not run an undergraduate course in biochemistry at that time but did have a small biochemistry department headed by Professor Jack (later Sir Jack) Drummond (F.R.S. 1944), who, in the spring term of 1928, gave a series of open lectures. Attendance at these lectures increased Ralph's interest in biochemistry and for advice on the prospects of a career in this discipline he visited Walter Morgan (F.R.S. 1949) who, in 1925, as a young PhD student, had joined the Biochemistry Department at the Lister Institute of Preventive Medicine, London; Walter's enthusiastic endorsement of the subject settled the matter for Ralph and he determined to pursue a career in the physico-chemical aspects of biochemistry.

R. Keith Cannan, who was a lecturer in Drummond's laboratory, was at that time the leading figure in the country in the areas of physical biochemistry that Ralph wished to enter, but although he was able to offer him a place as a research student he had no funds to support him. Ralph's parents could not afford to finance him in postgraduate work but Professor Donnan obtained a two-year research grant of £130 per annum for him from the Department of Scientific and Industrial Research. This entailed spending the first postgraduate year in the Chemistry Department before he could transfer to biochemistry. Professor Donnan suggested

that he should make some measurements of osmotic pressure on alginic acid, a polysaccharide from seaweed, but apparently gave him no help at all and from a scientific point of view Ralph rated the year (1928–29) as a complete disaster. If Professor Drummond had not kept an eye on him he might have given up during that year, although he had a busy social round as he not only played cricket in the college first eleven and in the college and university's rugby first fifteens but was also secretary of the college cricket and rugby clubs and a member of the Union Society Committee. In the autumn of 1929 he was eventually able to join Keith Cannan and started work, using the hydrogen electrode in a Clark cell, to study the hydrogen-ion dissociation curve of the crystalline albumin of the hen's egg, which was then a model protein. However, just as the work was getting going in the autumn of 1930, Cannan was appointed to the chair of biochemistry at New York University, USA. He advised Ralph to stay at UCL during 1930–31 and to apply for a grant that would enable him to go to New York in the following year. Ralph's stay at UCL was facilitated financially by the award of the Bayliss–Starling Memorial Scholarship and by demonstrating in biochemistry to medical and zoology students.

USA, 1931–33

In the summer of 1931 Ralph was one of a small but distinguished group to be awarded a Commonwealth Fund (Harkness) Fellowship, which enabled him to spend two years in the USA. When he joined Keith Cannan they resumed work on the dissociation curve of egg albumin. They were held up by the discovery that residual ammonium sulphate used in the crystallization of the protein interfered with the estimation of titratable basic amino acids but, in time, they overcame this problem by using sodium sulphate for crystallization. The resulting two papers published in the *Biochemical Journal* (1, 2)* are classics representing the best practices both in theory and method then acknowledged, and this training had without doubt a formative influence on Ralph's subsequent studies. The work drew attention to the availability of the side-chain titratable groups in proteins and was to become of importance later as the significance of concealed functional groups became clearer. While in the USA, Ralph also spent a few months with E. Newton Harvey, at Princeton University and at the Marine Biological Laboratory at Wood's Hole, Massachusetts, in attempts to determine the effect of anaerobic conditions on the permeability to water of eggs of the sea urchin, *Arbacia punctulata*.

Ralph found New York an exciting place to live at that time and enjoyed the many opportunities to listen to jazz and to go dancing; he also had vivid memories of Toscanini conducting the New York Philharmonic Orchestra at Carnegie Hall, and of Wagner operas enjoyed at the Metropolitan Opera House. While staying at International House, a postgraduate student residence in New York, Ralph met his future wife, Barbara Stone. She was a graduate in English from Wells College, New York, who was taking a master's degree course in librarianship at Columbia University. They were married in June 1933, and during the summer they spent at Wood's Hole they were joined by members of Barbara's family. During the previous summer, Ralph had visited the Pacific coast and many of the national parks with two other Commonwealth Fund Fellows: Douglas Hill, who was subsequently to become the Director of the Shirley Institute in Manchester, and Ronald Bradbury, who became the city architect of Liverpool. In September 1933 Ralph returned to England with his American wife.

* Numbers in this form refer to the bibliography at the end of the text.

University College London, 1933–37

Guy Marrian (F.R.S. 1944) had filled the lectureship at UCL vacated by Keith Cannan, but in 1933 he also left, to take up the chair in biochemistry in Toronto, Canada. The vacant position was therefore offered to Ralph when he returned from New York. He found himself with a rather heavy teaching load but tried to maintain his research interests with the intention of adapting the hydrogen-ion titration work to a study of horse serum albumin. In the 1930s T. Svedberg, a physicist/engineer in Uppsala, Sweden, had gained worldwide attention and admiration for his development of the ultracentrifuge, but at that time his knowledge of proteins was somewhat fragmentary. Ralph had been critical of a comment of Svedberg's in a paper published in *Nature* suggesting that preparations of whey proteins from cow's milk might be artefacts, and he made a casual remark to a visitor that Svedberg needed a good protein chemist, unaware that the visitor was from the Rockefeller Foundation, which was extensively funding Svedberg's work. This chance remark led to Ralph's being offered a Rockefeller Fellowship to work in Svedberg's laboratory. Thus in March 1935 Ralph left for Sweden, not returning to UCL until January 1936. The Physical Chemical Institute in Uppsala was a very stimulating and exciting place to work in the mid-1930s. The oil turbine centrifuge had recently reached a peak of development with the application of the Lamm scale optical system for following and delineating sedimenting boundaries of dissolved macromolecules. The small group of visitors to the laboratory formed what was effectively an international club, with members from the USA, The Netherlands, Canada, France and South Africa. One, Jack Williams, from the University of Wisconsin at Madison, USA, became a lifelong friend, as did a colleague of Svedberg's, Kai O. Pedersen, with whom Ralph worked closely while in Uppsala. They investigated a respiratory enzyme that was then of major importance, the 'old yellow enzyme' described by O.H. Warburg, For.Mem.R.S., and W. Christian. The protein was characterized by the full procedure that was then becoming standardized at Uppsala: determination of molecular mass by sedimentation velocity and diffusion and by sedimentation equilibrium, supplemented by measurements of the electrophoretic mobility by the moving-boundary method developed by A.W.K. Tiselius (For.Mem.R.S. 1957). Both the diffusion and the sedimentation equilibrium data were subjected to the tests then available for dispersity, and the enzyme preparation was accordingly judged to be monodisperse. This experience, with its demonstration of the power of advanced technology, had a profound and lasting influence on Ralph.

The Lister Institute, 1937–41

A grant of £3400 to the Lister Institute of Preventive Medicine, London, in 1935 from the Rockefeller Foundation to meet the cost of acquiring from Uppsala an oil turbine and equilibrium ultracentrifuge largely shaped the course of Ralph Kekwick's subsequent career. The centrifuge was constructed in Svedberg's workshops and shipped to London. The Lister Institute met the cost of erecting a special building to house the equipment, the construction being supervised by Arthur S. McFarlane, who in 1935 had joined the Lister Institute. Forty-foot concrete piles were necessary in the foundations to support the structure because the site, which was adjacent to the tidal River Thames, was below water level at high tide. The six-inch steel housing of the rotor of the oil turbine centrifuge was mounted on a 40-ton concrete plinth,

which was insulated from the rest of the building to prevent vibrations from being transmitted to the optical system. Provision was also made for Tiselius's classical electrophoresis apparatus to be constructed in the Uppsala workshop and housed in the new Biophysics Building. When Ralph returned to UCL in 1936 he was well placed to assist McFarlane with the installation of the Svedberg machine, and he spent at least one day a week helping to set up the equipment. In 1937 a grant from the Medical Research Council (MRC) enabled Ralph to become an attached worker in the Lister Institute's laboratories in Chelsea. He was simultaneously offered a post by Jack Williams in Madison, Wisconsin, USA, who had also acquired an oil turbine ultracentrifuge, but Ralph chose to remain in the UK and the Lister Institute was to be his scientific address for the remainder of his working life.

In 1938 Svedberg attended the ceremonial opening of the new Lister Institute Biophysics Building and in the same year the first paper from the laboratory was published by McFarlane and Kekwick on 'The physical properties of bushy stunt virus protein'. This paper is of interest even today because it reports not only sedimentation equilibrium velocity studies but also a direct determination of molecular mass by sedimentation equilibrium, a method that was particularly appropriate because the large size of the virus particle prevented the determination of the diffusion constant by the methods then available. A steady flow of papers based on ultracentrifugal measurements followed in the subsequent years and, although many are now only of historical interest, they confirm the dominance of Ralph's department in this field in those years. His attention also became directed towards the use of the Tiselius electrophoresis apparatus for analysing protein preparations. In 1939 his paper on human serum (3) was among the first to give accurate proportions of the four main components, in large part owing to the painstaking use of the Lamm scale method of analysing photographic records, which he had adapted for use with the electrophoresis apparatus. This work remained a standard by which later preparations of pathological sera were judged. Among these, his own work on multiple myelomatosis stands out for the successful separation of the individual components and particularly for their subsequent characterization by ultracentrifuge measurements. The value of the sodium sulphate method used for the preparation of the γ-globulin fractions led to the paper describing the procedure becoming Ralph's most cited publication (4). Similarly, three papers with Basil R. Record, who had joined the laboratory in 1938, followed the changes in horse serum that occurred during the course of immunization with diptheria toxoid; they showed that antitoxin appeared initially in the γ-globulin component and later in the β-globulin, with which the bulk of the antitoxin was eventually associated. These papers were of fundamental importance in identifying γ-globulin as the main antibody-containing component and in demonstrating the use, for preparative purposes, of the large-scale U-tube electrophoresis apparatus (5–7). In recognition of his published contributions to the physico-chemical characterization of proteins, Ralph was awarded a DSc degree by London University in 1941. The outbreak of World War II in time deflected Ralph from the smooth development of these essentially physico-chemical studies and showed his intrinsic versatility in a striking way. In the summer of 1939 he left for the USA with his wife and infant daughter, Elizabeth, who had been born in late 1938. The aim of this trip was partly to visit his wife's family and also to attend the Third Microbiological Congress in New York. The congress had barely started in September when war was declared. Because of the uncertainties in Europe, Ralph's wife and daughter stayed on with her family while he returned to the UK in the lively company of many microbiologists, including Sir John Ledingham, F.R.S., Allan Downie (F.R.S. 1955) and Alexander (later Sir Alexander) Fleming (F.R.S. 1943), on an American ship that

was brightly floodlit at night because the USA had not then entered the war. In the event, Ralph did not see his wife and daughter again until they were allowed to come back to England in 1944.

Carshalton, 1941–43

On his return to the Lister Institute, Ralph expected to be immediately assigned to work related to the war effort, but this did not materialize for some time and he continued the experiments with Basil Record on the properties of diphtheria antitoxic horse sera. In 1940, however, Dr (later Sir) Percival Hartley, F.R.S., who was then head of the MRC Biological Standards Division, brought to the attention of Kekwick and McFarlane certain practical problems connected with the preparation of serum and plasma for transfusion and, largely under their own initiative, they set about tackling these problems. After the Munich crisis in 1938, steps had been taken to establish a service to provide blood for transfusion in the event of air-raids, and blood transfusion depots had been set up ready with stocks of blood to treat casualties. The MRC convened a Blood Transfusion Committee to advise on the promotion and coordination of work in this rapidly expanding field, and in 1940 Dr A.N. (later Sir Alan) Drury, F.R.S., was appointed as the committee's chairman. At that time only blood from donors of group O, found in roughly 40% of the population, was used for transfusion of whole blood; the donations from other ABO groups were used to prepare serum, and later plasma, for transfusion. On storage, sterile human serum developed a haze—owing to the liberation of lipid from serum lipoprotein—that was visually indistinguishable from bacterial contamination (which rendered the serum unfit for transfusion). McFarlane found that by shaking serum with excess ether, freezing at −25 °C and then thawing, the unstable lipid was removed in the ether layer. The extracted serum freed from ether could then be sterilized by Seitz filtration and remained crystal clear indefinitely. Electrophoretic analysis showed a large decrease in the β-globulin component, and similar treatment of citrated plasma led to the separation of fibrinogen in addition to lipid, leaving a clear, filterable, stable product.

As a further consequence of Hartley's visit, McFarlane became interested in the design of a freeze-drying plant for the bulk processing of human plasma and serum under rigorously aseptic conditions because a need was foreseen for stocks of dried plasma or serum that could be reconstituted in emergency situations. Before the war, R.I.N. Greaves in Cambridge had designed a small unit for preserving animal antisera by freeze-drying in medical flats, and in wartime this equipment had been diverted for preserving human serum; however, its capacity was small. Inadequate space was available in the Lister Institute for developing a larger plant, and by this time bombing in central London had become more intense, so in 1941 Ralph and McFarlane moved to the unoccupied London County Council Serum Institute at Carshalton, Surrey, taking with them the Tiselius apparatus so that products could be examined for the effects of drying and reconstitution. The move to Carshalton coincided with Basil Record's departure to join the Army Operational Research Group to work on anti-aircraft radar. Ralph adapted very readily to the change from laboratory work to small-scale chemical engineering. Test runs on the freeze-drying plant lasted for 48 hours; because there was no automatic recording equipment, they took it in turns to sleep in the laboratory with an alarm clock to wake them hourly to take thermocouple readings and to check the vacuum. The ether extraction and filtration of plasma and serum was also moved to Carshalton, and in 1942 Margaret

Mackay, a physiology graduate from Australia who had been working with Greaves in the plasma drying unit in Cambridge, joined the team. She introduced strict controls to exclude contaminated material and to maintain aseptic conditions during processing. The bulk freeze-drier did not prove very successful because it was a complex and rather temperamental piece of apparatus. It was soon abandoned for its original purpose and adapted for the removal of ether from the treated serum. During 1942–43 the ether-freeze treatment of human serum and plasma was expanded; 1000 litres of serum and 2500 litres of plasma were processed and the product was successfully used clinically by Dr John Loutit (F.R.S. 1963) and Dr Janet Vaughan (F.R.S. 1979), who were directors of the South London and Slough Blood Transfusion Depots, respectively.

Return to the Lister Institute, Chelsea, 1943

Early in 1943 Sir Alan Drury succeeded Sir John Ledingham, F.R.S., as director of the Lister Institute, still continuing his administrative responsibilities for the four London Blood Transfusion Depots and his chairmanship of the MRC Blood Transfusion Research Committee. He recalled Ralph, and Margaret Mackay, to Chelsea to continue production there. McFarlane had been seconded to the National Institute for Medical Research to supervise the installation of new equipment, and in 1944 he took up an appointment at that institute. Ralph was appointed to the staff of the Lister Institute and took charge of the Biophysics Laboratory. In the same year the MRC established at the Lister Institute a 'Unit for Research into, and Filtration of, Blood Plasma and Serum for Transfusion' which was run jointly by Ralph and by Margaret Mackay. The filtration unit processed for some time the entire plasma output of the four London Blood Transfusion Depots. The ether extraction process was discontinued in favour of a treatment devised by Dr M. Maizels (F.R.S. 1961), Director of the Maidstone Blood Transfusion Depot, in which plasma was treated with kaolin; this treatment adsorbed most of the fibrinogen and some of the clotting factors, with the result that the product could be sterilized by Seitz filtration. At first large pools of treated plasma (about 500 donors) were dispensed into transfusion bottles and despatched for freeze-drying to a new plant in Cambridge, but a serious clinical problem arose owing to contamination of the large batches with the viruses of serum hepatitis, for which no tests were then available. One infected donation could contaminate an entire pool. To reduce this risk as far as possible, untreated plasma from 10 donor pools was dispensed aseptically for freeze-drying and the kaolin step was discontinued. During this period much of Ralph's time and thought was given to the design and construction of suitable equipment for performing these operations.

Human plasma fractionation, 1944

In 1944 a development occurred that was to influence the remainder of Ralph's research career. Confidential information became available through the MRC about the method for fractionation of human plasma proteins introduced in the USA by E.J. Cohn. This procedure, developed in the Physical Chemistry Department of Harvard Medical School, involved the separation of the plasma proteins by precipitation with ethanol at low temperature, first into groups and then by further fractionation to the isolation of selected proteins. The process had

been organized on an industrial scale and some products had become available to British surgeons, who were impressed by the value of fibrinogen, fibrin foam and thrombin in the treatment of cranial injuries and skin grafting. A request came from the armed forces to the Lister Institute for the provision of these proteins, the Institute being the only laboratory in the country with the ultracentrifuge and electrophoresis apparatus necessary for monitoring the protein fractionation procedures. Consideration was then given to setting up a plant for plasma fractionation with cold ethanol but the difficulty of obtaining the necessary equipment, especially a continuous-flow refrigerated centrifuge from the USA at that stage in the war, together with a bureaucratic decision that—because of the armed forces' requirements for ethanol—none would be available for protein fractionation, meant that Cohn's procedure could not be followed. Ralph began investigating the use of ether in place of ethanol as an agent for precipitating fibrinogen and prothrombin from plasma because the earlier work to remove precipitated lipid had shown that this solvent was well tolerated by proteins in solution. At this point Basil Record was recalled from the Army Operational Research Group to help with the fractionation development. In retrospect one wonders at what level the decision was made not to make ethanol available in view of the urgent medical need for the products and the fact that it takes two molecules of ethanol to make one of ether! The volatility of ether made it necessary to devise an aseptically closed operation system in place of open stainless steel vessels used by Cohn; his procedure relied on a final Seitz filtration to sterilize the products. Preliminary experiments showed that at 0 °C fibrinogen was readily precipitated by the addition of 11% ether (by volume) and a simple adjustment to the pH of the residual plasma precipitated a fraction rich in prothrombin, which was then converted to thrombin. The generation of fibrin foam involved aerating the fibrinogen solution by rapid stirring, quickly adding thrombin to the resultant foam and, as soon as clotting started, pouring the mixture into a tray for freezing and drying. This procedure required skills more akin to making a good omelette than those that Ralph possessed of thorough and painstaking attention to details, so this procedure was therefore generally left to Margaret Mackay, who had the necessary knack.

Initially the fractionation work had to be performed with the simplest of equipment, frequently constructed in the Institute's workshop. In a cold laboratory the precipitation vessels were immersed in freezing mixtures to control the temperature, and precipitates were recovered in a belt-driven centrifuge borrowed from Cambridge. Ralph's subsequent efforts to get a refrigerated centrifuge made in the UK were at first frustrated when the London factory in which it was being constructed was destroyed by a bomb during an air-raid and another source had to be found. Despite these constraints, in a surprisingly short time a method was devised for routine production. When the war ended, Drury, conscious of the need for continuity of the work to supply civilian needs, persuaded the MRC in 1947 to replace the 'Plasma Filtration Unit' with the MRC 'Blood Products Research Unit' with himself as honorary director, Margaret Mackay as scientist in charge and Ralph as consultant advisor. This project continually called for that combination of fundamental science and engineering skills that Ralph had already ably demonstrated in the Carshalton phase. As in the Cohn procedure, the approach lay in the selective use of five variables in an aqueous system: pH, solvent concentration, protein concentration, ionic strength and temperature. With the pilot-plant production in operation the ether system was extended to provide γ-globulin for measles prophylaxis, as this was some years before a measles vaccine became available. Later the same fraction was used for treatment of hypogammaglobulinaemia. Because of the limited solubility of ethyl ether in water, after precipitation of the globulins, albumin remained in solution and precipitation with

ethanol had to be used to recover the albumin fraction. Ten to twelve litres of plasma were fractionated weekly in the pilot plant, and the quantities of albumin recovered were sufficient only for its use in selected conditions. A detailed description of the fractionation system and equipment was given by Kekwick and Mackay in 1954 in a Special MRC Report (9). Basil Record left the group to take up a post at the Microbiological Research Establishment at Porton in 1948, and in the following year Leon Vallet joined the Blood Products Research Unit, initially with the remit of investigating the inactivation of viral contaminants of plasma or serum by ultraviolet light.

Postwar developments

With the ending of the war, the Biophysics Department at the Lister Institute grew rapidly and, although he continued to be involved with plasma fractionation, Ralph was able to return to some more fundamental studies when time and assistants permitted. Postwar relaxation of travel restrictions brought a stream of visitors to the Institute, including many who were interested in the new plasma fractionation process. The ether method required comparatively little equipment and was easy to adapt, although some, not surprisingly, found the thought of using such a volatile, inflammable, solvent rather daunting. At the first International Biochemical Congress in Cambridge in 1949, Cohn was an invited speaker and Ralph looked forward to meeting him. Cohn, a forceful, extrovert character, had seen plasma fractionation in the USA organized on a national scale, and he had become a powerful figure in blood transfusion. After the war, aided by a large team of scientists and engineers, he had developed his concept of component therapy in which blood, both the cellular components and plasma proteins, were separated and used selectively. Ralph's efforts were on a smaller scale and performed with much more limited facilities, although with successful results insofar as supplying safe serum and plasma fractions for clinical use was concerned. When they met, however, to Ralph's disappointment, Cohn showed little interest in the methods developed in the UK and there was no rapport between the two men.

By 1950 it became obvious that the expanding civilian demand for dried plasma and plasma protein fractions could not be met by the existing production arrangements, and consideration was given to the design of a new blood products laboratory to be built on a site leased from the Lister Institute on its estate outside London, in Elstree, Hertfordshire, where therapeutic sera and vaccines were produced. This expansion was a further drain on Ralph's time and involved him, together with Drury, William d'A. (later Sir William) Maycock and Margaret Mackay in prolonged discussions with architects and makers of equipment for a new plasma-bottle freeze-drying plant. Ralph, with Vallet, specified the design of large, mobile, stainless steel vessels with the provision of independent thermostatic control in a cold laboratory for the precipitation stages. For the new plasma-drying plant Ralph collaborated with a refrigeration engineer, E.C.G. Lanyon from J. and E. Hall Ltd, who had previously been in charge of the Army freeze-drying plant. Because there was a shortage of steel after the war, the intention was to base the design on the reuse of the chambers of an old wartime drying plant. The trials of a prototype had a chequered course, but the engineers eventually built eight production units. Vallet transferred to Elstree to supervise the installation of freeze-drying and fractionation equipment and the design and setting up of an ancillary laboratory for control and development work. In 1954 the Blood Products Laboratory (BPL) opened as a department of the

Lister Insititute under the supervision of Dr Maycock. In 1957 Margaret Mackay moved to Elstree as an MRC External Staff member to engage in research in close association with the production facilities. Ralph continued in his position as consultant to the new laboratory, but his visits became infrequent. Having seen his creation come of age he seemed content that it should be left to make its own way.

Blood clotting components

In 1952 Ralph was appointed Reader in Biophysics in the University of London. The availability of the plasma protein fractions from the routine production facilitated more detailed fundamental studies on selected proteins in the Biophysics Department at Chelsea.

In collaboration with Margaret Mackay, M. Nance and Basil Record, Ralph determined conditions under which virtually pure fibrinogen could be prepared on a laboratory scale, making use of the ultracentrifuge and electrophoresis apparatus to control each step (10). The final product was 97–98% clottable, which—allowing for the small loss of peptide on fibrin formation—was a very satisfactory approximation to complete purity. With Ernest Caspary, a thorough characterization of this material was made, yielding values for its sedimentation and diffusion constants and molecular weights (11) that are still accepted today. A noteworthy feature of this investigation was the subjection by Caspary of the diffusion measurements to the deepest form of analysis then possible.

In the 1950s haemophiliacs were in great need of preparations more potent than fresh blood or plasma to control haemorrhage. The anti-haemophilic factor, Factor VIII, was known to co-precipitate with fibrinogen, and Professor J.V. (later Sir John) Dacie (F.R.S. 1967) at Hammersmith Hospital, London, found that injections of fibrinogen preparations reduced the clotting time of haemophilic blood. Nevertheless the activity was too low to be of clinical value, and the activity varied considerably between batches. The arrival of a young medical doctor with boundless energy and infectious enthusiasm, Peter Wolf, enabled this problem to be tackled in Ralph's laboratory. Wolf devised an assay, based on rates of clotting, that he called the prothrombin conversion ratio, and from this he was able to estimate Factor VIII activity. Although it was not very accurate, the method enabled conditions for precipitation and purification to be monitored so that eventually a concentrate that contained about 85% of the plasma Factor VIII with a purification of 20–25-fold on a protein basis was prepared. Seitz filtration of these preparations led to a massive loss of activity and it was here that the aseptic fractionation system became indispensable. This material was the first clinically effective concentrate of human Factor VIII. It was immediately used on human subjects, and the encouraging results with six cases of haemophilia were reported in *The Lancet* in 1957 (12). In the same year Ralph received the Oliver Memorial Award for outstanding contributions to blood transfusion. The purification procedure for Factor VIII was inserted into the production sequence at the BPL, Elstree, and distribution was controlled by the MRC Haemophilia Committee. Reports of mild transient reactions in some recipients led Ralph to modify the purification conditions and, with P. Walton, to devise a more accurate test of activity. He obtained a concentrate with 50 times the potency of normal human plasma with a 90% recovery of activity, which, freeze-dried and stored at −25 °C, retained its activity for long periods and was free from side-effects. Production of Factor VIII by this procedure went on until the early 1970s, when it was discovered by Judith Poole in the USA that Factor VIII separated in

a small precipitate, the 'cryoprecipitate', formed when plasma was allowed to thaw slowly from the frozen state. The precipitate yielded Factor VIII both of higher purity and in larger batches than those previously obtained, and at the BPL this method then superseded the earlier purification procedure.

In time the necessity arose for the plant at Elstree to be enlarged to facilitate the fractionation of larger quantities of plasma, and the implied use of increased amounts of ether raised questions of health and safety and of fire hazards. Fractionation with ethanol was being used almost exclusively in other countries by then, but Ralph, who had been informed of these considerations, felt keenly about the proposal to switch to ethanol and made a strong case for continuing with ether as a more gentle precipitating agent of proven value for plasma proteins. An MRC trial was in progress in which patients with hypogammaglobulinaemia were given large weekly injections of immunoglobulin. A batch prepared by precipitation with ethanol was included in the trial for a comparative study; no differences in clinical response or adverse effects were observed and Ralph's objections could no longer be sustained. The new greatly enlarged plant at Elstree, using ethanol as the precipitant, came into service in September 1974.

Today, a volatile, inflammable and relatively expensive solvent that had to be used without the usual stabilizers against oxidation would be an unlikely choice as a reagent for the precipitation of proteins on a manufacturing scale. Put in its historical context, however, with the knowledge gained from the use of ether to remove unstable lipoproteins from plasma, and the lack of availability of ethanol, Ralph saw how ether could be used to provide the urgently needed fibrinogen, thrombin and fibrin foam and thereby gave the UK an independent supply of these protein products for the treatment of wounds. The extension of the method into a scheme of plasma fractionation to provide other proteins of clinical use followed, and for nearly 30 years, with substantial increases in scale, the plant supplied these proteins for clinical use in England and Wales. The products had an impressive record of safety and freedom from adverse reactions, a record to which Ralph's aseptic technique most certainly contributed. People may wonder how, with a large-scale use of ether, it was possible to avoid fires in the days when smoking was much more prevalent than it is today, and indeed Ralph himself smoked a pipe. In the days of the pilot plant at Chelsea a large ashtray was kept outside the cold room so that cigarettes could be extinguished and pipes parked. The only fire in the area happened when Ralph was away and was not caused by smoking. Ether was added to plasma from a large separating funnel and vibration from a stirrer motor had loosened the glass stopcock on the funnel with the result that leaking ether was ignited by a spark from the brushes on the motor. Fortunately the funnel was nearly empty at the time and the fire was quickly extinguished. It is possible that Ralph was not told about this, because he always maintained that there had never been a fire!

Collaborative studies at Chelsea

Throughout his career Ralph very readily collaborated with other biochemists and clinicians to provide valuable and critical ultracentrifuge and electrophoretic characterizations, although, in accordance with the customs of the times, the results frequently appeared simply as addenda to the main papers. One such collaboration was with members in the Biochemistry Department in his own institute who were then engaged in the research that was to culminate

in the complete identification of the chemical groupings responsible for the differing ABO specificities of human blood, work that will always be linked with the names of Walter Morgan and his colleagues. In 1950, in a short addendum to one of Morgan's papers, Ralph recorded the essential physico-chemical properties of a newly isolated blood-group-specific glycoprotein with strong group A activity (8). The substance was shown to migrate slowly but essentially as a single component in electrophoresis over a wide pH range, and sedimented in a rather similar fashion, giving a single but spreading boundary. An estimate of the molecular mass of about 280 000 Da was found. The results were interpreted as indicating a highly asymmetric molecule of moderate polydispersity, but essentially free from contaminants. Two further papers in 1952 reported essentially similar properties for the human H and Le^a blood-group active substances. This work gave the biochemists a convincing demonstration of the essential purity of the blood-group glycoproteins and the large size of their molecules, knowledge that was crucial for their continuing investigations on the structure of the determinants.

Another long-standing collaboration was with Professor Nicholas Martin, Professor of Chemical Pathology at St George's Hospital Medical School, London. The use of the electrophoresis and ultracentrifuge facilities was extended to Martin and he became a research associate at the Institute. Interested in the serum protein changes in liver disease, he followed the effects of the infusion of the initial 'ether–ethanol'-separated albumin preparations in patients. A great enthusiast who shared Ralph's enjoyment of a good story, his weekly visits were much appreciated and he kept the department in touch with clinical developments. Ralph also provided a service to many other London hospitals to distinguish between Waldenström macroglobulinaemia and myelomatosis. The first case of Waldenström macroglobulinaemia was described by Kai Pedersen in 1958, who found excessive amounts of 19 S γ-globulin in the patient's serum in contrast to serum from myelomatosis patients, which contains excessive amounts of 7 S γ-globulin. Ultracentrifugal analysis permitted a simple differential diagnosis of these conditions.

A study with J.M. Creeth and colleagues in 1963 on urine proteins originating from renal tubular dysfunction (13) was the last of Ralph's publications to include results obtained with the oil turbine machine. The use of the Svedberg ultracentrifuge instrument had revolutionized the physico-chemical characterization of macromolecules in the 1930s and 1940s but it had really become outdated in the 1950s, although the Lister model was kept in very good order and used regularly. Creeth joined the Biophysics Division in 1960, and with his arrival the department acquired a Spinco analytical ultracentrifuge, which was simpler to use and capable of much greater precision, having both schlieren and interference optics and accurate speed and temperature control. A combination of more modern methods was used to good effect in a collaborative investigation performed with Creeth and J.M. Jones on the serum protein α_2-macroglobulin (14). The function of this component was then little understood and its relation to the immunoglobulins was obscure. In the bulk fractionation procedure the α- and 19 S γ-globulins of normal human plasma separate out in a macroglobulin fraction; it was found that preparative ultracentrifugation, followed by gel-filtration and ion-exchange chromatography, allowed the two to be separated. Establishment of the molecular mass of α_2-macroglobulin and its subunit structure revealed that the molecule differed extensively from that of 19 S γ-globulin (IgM).

In 1968, after 30 years of service, the Svedberg analytical ultracentrifuge at the Lister Institute was dismantled and presented to the Science Museum in London, where it is on display. Only seven of these instruments had been constructed; three were operated in Uppsala

and one each was operated in London, Oxford, Madison and at the Dupont Laboratories in Pennsylvania. The former Lister model seems to be the only one to have survived. In the last third of the twentieth century the models that succeeded the Svedberg ultracentrifuge slowly fell out of use until today analytical ultracentrifugation is no longer part of mainstream physico-chemical investigations. Virtually everything that was dependent on the ultracentrifuge can now be done more simply, more quickly and on a vastly greater scale by other methods, but this does not detract from pioneers such as Ralph Kekwick, who helped to establish the benchmarks that enabled the new procedures to evolve.

In 1966 Ralph was given a personal Chair in the University of London and was elected a Fellow of The Royal Society.

Retirement

The ill-health of his wife, Barbara, forced Ralph at the age of 62 to take early retirement in 1971. A sufferer from asthma, she had become a permanent invalid as a result of a medical disaster while undergoing treatment in a London hospital. She was in need of 24-hour care and attempts to nurse her at home at the same time as maintaining his research interests became impossible. She died within 18 months of Ralph's retirement. In 1974 he married his former colleague Margaret Mackay. They had eight years together, sharing the pleasures of travel and theatre, and classical music which had always been an important part of Ralph's life. He played the piano and had a clear singing voice. Sadly, Margaret died suddenly in 1982 and Ralph lived alone for the remaining 17 years of his life. He continued to enjoy gardening, which had been one of his hobbies since the days as a child when he had helped his father growing vegetables on their allotment during World War I, and also bird-watching, for which the Essex reservoirs provided such wonderful locations. After retirement he did not take on any more scientific work but sustained a broad interest in science by joining the Royal Institution and attending the Friday evening discourses. Much of his spare time was also given to the Wanstead and Woodford Association for the Welfare of the Blind. He died at home in Woodford on 17 January 2000, aged 91.

The man and the scientist

Ralph Kekwick was a man of high integrity with strong loyalties to his family, his country, his college and to the institute in which he worked. He derived great pleasure from being elected a Fellow of UCL in 1971. Although reticent on matters of religious belief, he attended his local church and stood by Christian values. Never one to preach, he sought fair play and would not readily let churlish or uncharitable behaviour pass without comment. His reactions to situations were readily discernible in his facial expressions. He gave generously of his time to the problems of others and younger workers could always rely on him for friendly advice. Ralph treated everyone with equal respect and had good relations with ancillary workers in his department. Among them was Charlie Broder, who had come to the Lister Institute with little education or training but was from the beginning involved in the installation and running of the Svedberg oil turbine ultracentrifuge and eventually became head of the Lister Institute instrument workshop. Another stalwart of the department was his senior technician, Harry

Murray, a remarkable person who was not only competent to operate all the equipment of the Biophysics Laboratory and a superb photographer, but was also widely read in art and literature.

Ralph was normally willing to adopt new methods and new ideas when the advantages were obvious, but sometimes he displayed a conservative streak that made him seem to his younger colleagues to be resistant to change. Replacement of the method of applying wax by hand to seal the vacuum chambers in the freeze-drying plant by the use of self-sealing neoprene O-rings required considerable persuasion, as did the changeover to using modern electronic pH meters. This conservative streak also surfaced when consideration arose for changing from the ether process to the less dangerous ethanol fractionation procedure, although this resistance was understandable because it meant abandoning the procedure that he had devised under difficult circumstances with very limited facilities. However, these examples were rare and until he retired he continued to introduce new equipment and protein separation methods into the department.

Ralph was primarily an experimentalist and his great strengths lay in his depth of biomedical knowledge, his ability to view a project in the widest context and his deep fund of sound practical sense. He pioneered the use of the ultracentrifuge in England and kept up with the refinements of technique and equipment. His aim was to use the ultracentrifuge to study the physico-chemical properties of proteins rather than to contribute directly to advances in ultracentrifugal theory. The Uppsala school had presented a comprehensive account of the theory underlying every application then possible and he followed the procedures it had laid down. In later years he seemed content to leave the more advanced theoretical applications and analyses to his younger colleagues, but he retained a position of authority in the centrifuge field throughout his working life. His vital role in the development of plasma fractionation in the UK in the immediate postwar years largely dictated the course of his future research, and although the work on Factor VIII inevitably lacked the delicate precision and intellectual rigour of his earlier ultracentrifugal studies, he was content to know that he had been instrumental in the development of procedures that led to clinically important blood products. Apart from his wish to be a physical biochemist he had not planned his career, nor did he have any overriding ambition, and considered himself to be very fortunate to have been in the right place at the right time to take advantage of the opportunities as they arose.

ACKNOWLEDGEMENTS

We are grateful to Ralph Kekwick's nephew, Dr Roy Kekwick, for preparing the bibliography, and to his daughter, Mrs Elizabeth Till, Dr R. Kekwick and Professor Walter T.J. Morgan, F.R.S., for helpful comments on the manuscript. This memoir has drawn on autobiographical details recorded in a video interview conducted by the Biochemical Society in 1991.

The frontispiece photograph was taken *ca.* 1960, and is reproduced by permission of The Lister Institute of Preventive Medicine.

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