BIOGRAPHICAL MEMOIRS

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Elected FRS 1974

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Kenneth Burton was an enthusiastic, lateral-thinking biochemist, with special skills in physical sciences, chemistry and mathematics who, although perhaps best known for his highly cited study of the conditions for the diphenylamine colour reaction for DNA, made outstanding contributions in several important fields. His calculations, which he refined over the years, of the thermodynamic parameters of many biological substances were of the greatest importance in the development of intermediary metabolism. Attracted to bacteriophage systems as a route to tackling the thorny problem of DNA replication, his early discovery that bacteriophage DNA replication precedes phage DNA synthesis set the stage for important developments in our understanding of viral systems and, ultimately, antiviral therapies. His pioneering studies of specific methods for the chemical degradation of DNA molecules were among the first steps towards the determination of nucleotide sequences in DNA at a time when direct chemical degradation seemed to be the only feasible approach.

Family background

Kenneth (Ken) Burton was born on 26 June 1926, the first child of Arthur Burton and Gladys (*née* Buxton). Ken's paternal grandfather, Joseph, had first worked as a miner (like his father before him), but became a signalman when the Great Central Railway (GCR) line was opened from Sheffield to Manchester; Arthur (born in 1894), his four brothers and his sister's husband also worked for the railway. His first job was in the booking office of the small Silkstone Common GCR station. In 1915 Arthur joined the Wiltshire Regiment (Royal Army Service Corps), in which he later became a sergeant. He served in France on the Somme and in the second battle of the Marne and twice received minor wounds, but spent most of the war training recruits in Warminster and, on discharge from the Army, rejoined the GCR.

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Childhood and early education

In 1921 Arthur married Gladys Buxton, the daughter of a Sheffield cutler, and Ken was born in the Jessop Hospital, Sheffield, in 1926. Twin brothers, born in 1930, died shortly after birth and Ken was brought up as an only child. The family moved house several times as Arthur took different railway posts; Ken recalled that they lived comfortably, but not affluently. In 1930 Arthur became Station Master at Beighton, Derbyshire, where they lived on the station, now demolished, and appropriately (given his maternal grandfather's occupation) the 7.30 'Master Cutler' express from Sheffield to London was Ken's alarm clock. Ken's mother died in 1932 when he was five years old, and Ken had a difficult few years. His grandmother, and then a cousin, came to Beighton to look after him and his father, and Ken also spent long summer holidays in his grandmother's home in Immingham, where she had gone to live after the death of her husband. Her house had no electricity or gas lighting upstairs and he remembered having to carry lit candles upstairs to bed. In 1935, Arthur married Ivy Beaumont, whom he had engaged as a housekeeper. Ivy, who died in 1976, did much to make Ken's childhood happy, although Ken felt that he did not always live up to her high standards of dress and behaviour. Ken was pleased that his stepmother lived long enough to attend his admission as a Fellow of the Royal Society. Arthur retired in 1954 aged 60 years and later moved to Harlaxton, Grantham, where he died in 1970.

Ken attended Beighton Infants' School (1931–33) and Beighton Primary School (1933–37). From 1937 to 1939 Arthur moved to suburban stations in Nottingham and the family lived in Sherwood. Ken completed his primary schooling in 1937 at Claremont Primary School, Nottingham, where he recalled a valuable citizenship course dealing with the outlines of insurances, mortgages and the structure of local government and, more significantly, where a very good science teacher sparked an interest in physics. His secondary schooling, which began later in 1937 at High Pavement School, Nottingham, did not start auspiciously. On the basis of the 11-plus examination Ken was placed in the top Form 1A, which was devoted to Greek and Latin, but he developed no fondness for Classics. He remembered some badly taught chemistry, quite good mathematics and a little biology through occasional country walks, but there was no physics. Form 1B (presumably thought to be of lower standard) studied physical sciences and German, but no Greek or biology. Pupils in Form 1C were not deemed to be Oxbridge material and studied biology and German and no classics, while Form 1D pupils were given art, woodwork and Spanish. Ken wanted to study science. His request to change streams was refused and, even at that age, he was infuriated by the bad system.

In 1939 Ken was evacuated to Mansfield, Nottinghamshire. Fortunately, the problem of his secondary education was resolved at the end of that year when Arthur was appointed Superintendent of the large railway marshalling yard that served the 45 collieries within 10 miles of Wath-on-Dearne, South Yorkshire. Wath was frequently foggy, and Ken remembered there being always a vile smell because the detached, sooty railway house (incongruously called Swiss Cottage) in which the family lived lay between the brewery and the bone-rendering soap works. However, from 1939 to 1943 Ken was able to attend Wath-on-Dearne Grammar School.

At Wath, Ken enjoyed O-level science taught by a Miss Knowles, a lively teacher who also gave the class an introduction to plant and animal physiology. His interest in science was stimulated further by popular books given to him by a friend of his father, a colliery chemist who had been blinded in a laboratory accident. In the sixth form, Ken studied physics, pure

mathematics, applied mathematics and chemistry. The chemistry teacher, Mr Williams, had studied agricultural chemistry at Bangor and gave many biological examples. (A classmate in that small form was Brian Finean, who became Reader in Membrane Biology at Birmingham University.) At this time, Ken developed an interest in electronics under the guidance of an uncle who worked as a crane driver at Immingham docks but who built and sold radio sets in his spare time. He gave Ken old radio magazines and components, which Ken supplemented with added items from jumble sales and then spent much time building simple electronic circuits with thermionic valves.

Much to the disappointment of his father (who had played soccer and cricket for his regiment at Warminster), Ken was not good at sport. But he enjoyed the outdoor exercise gained walking to and from school and swimming in the public baths near his home at Wath.

University education

Apart from an English master, none of the schoolteachers at Wath had Oxbridge connections or useful advice, but Ken decided to aim for physics and chose Cambridge because of its strength in physical sciences. He chose King's College, partly from seeing pictures of its chapel in railway carriages, but mainly from entry scholarship information in *The Times*. Wartime changes in the timing of entrance examinations meant that Ken was able to take the scholarship examination after his Higher School Certificate Examination. He was delighted to be awarded a Scholarship by King's, although the State Scholarship that he had already obtained (along with a West Riding County Major Scholarship) on the strength of the Higher School Certificate Examination already provided the essential funds to go to university.

For the first two years, Ken read for Part I of the Natural Sciences Tripos, which he graduated with Class I, taking physics, mathematics and chemistry along with the (then compulsory) electronics and just one year of optional biochemistry. The elementary chemistry laboratory had no electric points or electric lights, and Ken remembered a large class doing ether distillations under lit gas mantles. Ken was excited by the optional course in biochemistry and became very interested in the subject. The course was taught well, mainly by Ernest Baldwin, who was then writing his book *Dynamic aspects of biochemistry*, and, unlike the other subjects that Ken studied, the undergraduate course touched on the frontiers of current science. Despite his lack of biological background, Ken applied for and obtained deferment from call-up to the armed services to do Part II biochemistry, in which he graduated with Class IIi honours in 1945, winning college prizes in 1944 and 1945. By then the war had ended and he was told that he was 'deemed to have served' his war service.

On graduation, Ken did not explore the possibility of work in industry, because he had been awarded a Science Research Council scholarship to study for a PhD and thought that he could always go into industry afterwards. He was allotted a supervisor and a project—the study of a higher fatty acid dehydrogenase of mammalian liver. However, although this led to his first publication, neither his supervisor nor the project was of his own choosing and his inexperience, poor supervision and lack of equipment led to slow progress. He changed both supervisor and project and, under the supervision of Malcolm Dixon FRS, turned to a study of D-amino acid oxidase that was significantly more fruitful. Dixon's suggestion was that Ken should first study the biosynthesis of flavin adenine dinucleotide (FAD) and then use the D-amino acid oxidase apoenzyme to measure FAD concentration. However, he was

side-tracked and his thesis—'The dissociation of flavoprotein enzymes'—became a study of the influence of substrates, competitive inhibitors and flavins on the stability of D-amino oxidase and a preliminary study of other enzymes that cleaved FAD to flavin mononucleotide (FMN) (1)*. The fluorescence of FAD enabled him to show that quinine inhibited the enzyme by binding to FAD. Adenosine monophosphate or benzoate stabilized the thermolabile apoenzyme, and they each inhibited by competition with FAD and amino acids, respectively. In retrospect, Ken suspected that this was the first evidence for independent binding of two reactants to an enzyme. In his research, Ken benefited enormously from the help and advice of Gregorio Weber. He was grateful to Malcolm Dixon for teaching him to look for a simple way of getting the result and not to rush into a more immediately obvious approach, which might have needed more labour—a philosophy that Ken followed all his life and was careful to pass on to his own research students.

For much of his PhD research, Ken shared a large laboratory with Peter Mitchell (FRS 1974; awarded the Nobel Prize in Chemistry in 1978 for his work on chemiosmosis) and Herbert ('Freddie') Gutfreund (FRS 1981), Joan Keilin and T.-C. Tsao. Other PhD students in the department at the same time included Ita Askonas (FRS 1973), Rod Porter (FRS 1964), Sam Perry (FRS 1974), Frederick Whatley (FRS 1975) and Bill Elliott (later Professor of Biochemistry at Adelaide). Sam Perry and Rod Porter organized a weekly preparation of ATP from rabbit muscle, and anybody wanting some of this had to join in. Each batch, still impure, was analysed by myosin ATPase, myokinase and paper chromatography. The laboratory was a good environment in which to encounter different interests and contemporary techniques, such as Warburg manometry, colorimetry, protein fractionation by crude batchwise methods and paper chromatography. The coloured flavin nucleotides were ideal for chromatography and, in the department, Frederick Sanger (FRS 1954) was also using this technique to separate dinitrophenyl amino acids derived from insulin.

A missed opportunity occurred when Bob Comline in the Physiology Department found that the fluorescent material left at the origin in paper or alumina column chromatography during the preparation of FAD from yeast would activate acetylation. Microbiological assay showed the material to contain pantothenic acid, and Ken found it to contain 1 mole of adenine and 2 moles of ribose per mole of pantothenic acid. Unfortunately, he had no evidence that these residues were all in the same molecule. Shortly afterwards, Fritz Lipmann (ForMemRS 1962), in Boston, announced the discovery of coenzyme A (CoA), for which he was awarded the Nobel Prize in Physiology or Medicine (jointly with H. A. (later Sir Hans) Krebs FRS) in 1953. Furthermore, in those early days when Ken had CoA preparations, he predicted that CoA would give the key to the tricarboxylic acid cycle by entering the cycle as acetyl CoA (instead of pyruvate, which was thought to be involved). He was, however, 'talked out of doing any experiments by someone older, active and more experienced than he was.'

Marriage and family

Ken married Hilda Marsden, the third child of Albert and Mary Hannah Marsden, on 3 September 1955 at Heeley Church, Sheffield. Hilda's parents were both from farming families in the Mayfields Valley near Fulwood, Sheffield. Albert was the caretaker of a school with

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

a large garden, which he tended, together with a team of under-gardeners, and grew vegetables for school dinners. After graduating with a BSc in Horticulture from Wye College, London University, Hilda was a lecturer at Oaklands Agricultural College, Hertfordshire, and a biology teacher at Croft School House, Shillingstone, in Dorset. She later became a technician in Krebs's Medical Research Council (MRC) unit in Sheffield, but Ken and Hilda did not get to know each other until Hilda moved to Oxford with the MRC unit. After the birth of two children, Hilda returned to teaching.

Their son, Andrew James, was born in 1957 at home in Kidlington. He is a General Practitioner in Buckinghamshire. He and his wife, Alison (*née* Pierce), had four sons, one of whom sadly died as a teenager. Alison's brother is a biochemist in the Pasteur Institute in Lille, and his wife is Professor of Biochemistry at Lille University. Ken and Hilda's daughter, Angela Mary, was born in 1958, also at Kidlington. She completed a Diploma in Higher Education at the York campus of the College of St John, York and Ripon, and trained as a nurse and midwife in York. She currently works as a Senior Occupational Health Nurse Advisor. She is married to Stuart McKay. They live in Brighton and have three children.

University of Sheffield, 1949–52

On completing his PhD in 1949, Ken applied for an advertised Assistant Lectureship in Krebs's Department of Biochemistry at Sheffield University. Ken liked the vigorous approach of the Department, although the laboratory was very crowded (Ken had approximately six feet of bench space, and nobody, not even Krebs, had a private office). There were only two other university teaching staff (John Bacon and Monica Johnson) beside Krebs and Ken, but there was a large MRC Unit for Cell Metabolism, which included Bob Davies (FRS 1966), David Hughes, Reg Hems and Leonard Eggleston. Hans (later Sir Hans) Kornberg (FRS 1965) was a PhD student with Bob Davies. Teaching was to about 60 medical students and two science students who were studying BSc physiology, of which biochemistry formed half of the third-year course. One of the students was Walter Bartley, who had come to Sheffield as a member of a group of conscientious objectors acting as human guinea-pigs in research on nutrition and scabies and working in the laboratory. He was later in Krebs's MRC unit at Sheffield and Oxford and eventually returned to Sheffield as Professor of Biochemistry and Pro-Vice-Chancellor. Ken's teaching duties were relatively light. Krebs gave most of the lectures to the medical students, and Ken's duties mainly involved teaching practical courses.

Ken's PhD research on D-amino acid oxidase led him into the study of the *Neurospora crassa* L-amino acid oxidase that had recently been discovered by Bender and Krebs, and independently by Horowitz and Thayer. He tried unsuccessfully to concentrate the activity by adsorption on alumina or calcium phosphate (which could be centrifuged successfully at 2000 r.p.m., the highest speed available to him for litre volumes). So, because the activity was rugged and resistant to copper ions, he tried adding cupric sulphate plus enough alkali to make cupric hydroxide. The enzyme was adsorbed on the precipitate, and Ken showed that it had a firmly bound FAD prosthetic group (2).

Krebs showed Ken a draft paper that he had written involving thermodynamic data that Ken thought could be improved on by a better value for the oxidation–reduction potential of nicotinamide adenine dinucleotide (NAD^+). To obtain this, he studied the equilibrium

between propan-2-ol and acetone, catalysed by the enzyme alcohol dehydrogenase, and used the excellent thermodynamic data available for these compounds. Tom Wilson, an American visitor, collaborated to study the same reaction further, together with other dehydrogenase equilibria. Using these data, Ken and Krebs were able to reassess the free energy of adenosine triphosphate (ATP) and free-energy changes of glycolysis and the tricarboxylic acid cycle and the hydrolysis of acetyl CoA.

Because he thought them to be sterile, Ken never entered arguments over the term 'phosphate bond energy' but always used the phrase 'free energy of hydrolysis'. He appreciated that thermodynamic work had been useful in framing biochemical concepts but doubted whether it had really influenced the design of biochemical experiments; he considered the most valuable use of thermodynamic data to be the imaginative development by Krebs & Veech (1969) that gave evidence for different concentrations of metabolites such as ADP in mitochondria and the cytosol. Ken was one of the first to realize the importance of the chelation of adenosine phosphates by Ca^{2+} and Mg^{2+} ions and was the first to report trying to measure their formation constants, a problem to which he returned later at Oxford.

While at Sheffield, Ken attended the Sorby Lectures on the subject of bacteriophages, presented by the distinguished French microbiologist André Lwoff (ForMemRS 1958) and became interested in the possibility of using bacteriophage systems for the study of DNA replication. He was promoted to the post of lecturer in 1952, but was granted leave from the department to allow him to gain experience with Earl Evans's phage group in the Department of Biochemistry of the University of Chicago, which was concerned with the biochemistry, but not the genetics, of the T-phages of *Escherichia coli*.

University of Chicago, 1952–54

Ken went to Chicago expecting to be a research associate on a research grant, but the university insisted on seeing his BA certificate from Cambridge, which he did not have with him, and Earl Evans's secretary unilaterally changed his appointment to 'instructor' (for which, presumably, a degree certificate was not considered necessary!). However, he was required to do virtually no teaching and became a researcher in the group, which included Frank Putnam and Lloyd Kozloff. At that time, all that was known was that phages grew, but nobody knew how; Ken decided to investigate the effect of preventing protein synthesis on the growth of the phages. Using amino acid auxotrophs of the *E. coli* host, he discovered (3) the need for phage-directed protein synthesis immediately after infection of the host cells by the phage particles preceding the synthesis of phage DNA (identified by its 5-hydroxymethylcytosine content). This discovery proved to be of great significance in virology. Tomizawa & Sunakawa (1956), in A. D. (Al) Hershey's laboratory, discovered this effect independently by inhibiting protein synthesis with chloramphenicol, an approach that Ken deliberately avoided because of confusion at the time over whether or not chloramphenicol inhibited the incorporation of amino acids into cell-wall material.

This study required the measurement of DNA levels in cultures. Ken tried a variety of available methods and finally settled on the Dische diphenylamine reaction, which involved heating the DNA at 100 °C with diphenylamine in a mixture of sulphuric and acetic acids. One day he added the reagents with the intention of heating them the next day and, next morning, was surprised to find that, after standing overnight at room temperature, the samples had

developed a blue colour that was more intense than that obtained after heating at high temperature. The improved reaction was not only more sensitive but also less prone to interference by other substances, and Ken adopted these conditions as routine.

In Chicago, Ken frequently met Konrad Bloch (ForMemRS 1985), both in the laboratory and socially, and there was scientific and social exchange with members of the biophysics group, who were using the amino acid auxotrophs that Ken used in his research. The head of that group was Leo Szilard, who had worked with Einstein in Berlin and, working in England in the 1930s, had patented the concepts of critical mass and a nuclear chain reaction. (He had assigned the patents to the British Admiralty—after unsuccessfully offering them to the War Office—in the hope that the knowledge could be kept secret.) After emigrating to the USA in 1938, Szilard persuaded Einstein to write to President Roosevelt to point out the possibilities of nuclear energy and an atomic bomb. Later, a group of scientists joined him in another letter to President Truman in a vain attempt to prevent the bomb from being used against Japan. In 1946 Szilard turned to biology (Weart & Szilard 1978). Ken came to know him well and enjoyed the company of that 'quixotic, brilliant Hungarian scientist'. He clearly felt himself privileged to share Szilard's perceptive thinking and was pleased that he could help him with biochemical matters with which he was not familiar.

Ken gained much from the time spent in the USA, particularly from his association with the band of 50 or so workers on phages who met on several occasions, including at the 1953 Cold Spring Harbor meeting. There, he first met such people as Gunther Stent (whose laboratory in Berkeley he visited in the summer of 1953), James Watson (ForMemRS 1981), Francis Crick (FRS 1959), Maurice Wilkins (FRS 1959), Bill Hayes (FRS 1964) and Renato Dulbecco (ForMemRS 1974).

MRC Cell Metabolism Research Unit, Department of Biochemistry, University Of Oxford, 1954–66

In 1954 Krebs moved to Oxford, where he had been appointed Whitley Professor of Biochemistry; Ken accepted Krebs's offer of a position in the MRC unit, which moved with him. Ken was particularly pleased to be encouraged to continue his bacteriophage work, but Krebs gave him a completely free hand with regard to his research topic and Ken embarked on what was to be the most productive period of his research career.

It was a blow to find that the 'improved' overnight diphenylamine–DNA reaction that Ken had developed in Chicago gave no colour in Oxford. In a flash of inspiration, he recalled a story about the discovery of tryptophan by Sir Frederick Gowland Hopkins PRS (the legendary former Professor of Biochemistry at Cambridge) that he had first heard told as a student in the Part I course and also from Hopkins himself at the lunch during the Part II practical examinations. The tale started with a temperamental colour reaction for protein that Hopkins and Cole had shown depended on the acetic acid used in the reagent containing an aldehyde impurity that was produced by storage of the acid in sunlight. Ken realized that the acetic acid that he had used in Chicago had been stored in sunlight, whereas the acid used in Oxford had not. He tested the effect of several aldehydes on the development of the colour reaction and settled on the addition of traces of acetaldehyde to the diphenylamine reagent to solve the problem. The paper describing the 'Burton' diphenylamine–DNA colour reaction (4) was widely adopted as the preferred method for assaying DNA, and the paper later became a

Citation Classic. Many years later, Ken gave an account of the background story to this discovery in a letter to *Current Contents*.

From time to time in Oxford, Ken continued to take an interest in biochemical thermodynamics. In 1957 he provided a comprehensive compilation of free-energy data of biological interest as an appendix to a large and authoritative summary of intermediary metabolism by Krebs and Kornberg (5). In Cambridge, Ken had seen Peter Mitchell measuring metals in bacterial cell walls. He knew that free Mg^{2+} could be measured spectrophotometrically by using 8-hydroxyquinoline and realized that the formation constants of the ADP and ATP complexes could be measured. Although he was working at the limits of the method, the instruments and the ATP or ADP purities then available, Ken obtained what he believed to be the first well-founded estimate of the strengths of the complexes and confirmed suspicions that ATP had a much greater affinity than ADP for both metals. Again, although Krebs and Ken had previously reported values for the free energy of hydrolysis of ATP to ADP, it was clear that combining data for glutamine synthase and glutaminase would facilitate a more accurate calculation, and Ken was able to take advantage of a sabbatical visit to the MRC unit by Hannes Benzinger (who had worked with Krebs in Germany on the urea cycle) and Charlotte Kitzinger from the US Naval Medical Research Institute in Bethesda, Maryland. They had brought with them a large and sensitive calorimeter, and with the help of Reg Hems they were able to use it in an elegant experiment to study the glutaminase equilibria (7). The combined data gave what Ken believed to be the first really reliable figures for ATP hydrolysis (6).

In exploring the optimal conditions for his improved diphenylamine reaction, Ken came to an unexpected conclusion. It was known that pyrimidine deoxynucleotides were stable to the acid conditions of the Dische reaction, which thus detected only the deoxyribose associated with purine nucleoside residues (which account for one-half of the base complement of double-stranded DNA molecules). Ken had measured the fraction of the DNA-phosphorus released as inorganic phosphate and had found that, as would be expected from a DNA molecule of random nucleotide sequence, it accounted for approximately 25% of the total phosphorus. At that time, Erwin Chargaff and his co-workers (Tamm *et al*. 1953) had published experiments suggesting that hydrolysis with strong acid might prove useful as a method for degrading DNA specifically for the determination of nucleotide sequence arrangement. Recognizing that the reaction conditions that he was studying were significantly milder than those used by Chargaff, Ken concluded that his reaction 'may prove to be a useful tool [for the determination of nucleotide sequences in DNA] when the other products of the reaction and the action of the acid alone have been studied' (4).

In 1956 I arrived in Oxford from New Zealand to study for the DPhil in biochemistry and, at Krebs's suggestion, approached Ken to see whether he had a problem that would suit me. I had a background in chemistry as well as biochemistry and had read of the work by Chargaff's group; I hoped that I might be able to work on the possibility of using specific chemical degradation methods to determine nucleotide sequences. I was completely unaware of Ken's work and was delighted to find that not only did he have a novel approach to the problem, but he was also anxious to find a chemically orientated research student who was interested in exploring the diphenylamine-induced degradation of DNA further to see whether the reaction had any practical application for sequencing DNA. We showed that the degradation reaction occurred just as effectively—but without significant colour production—when DNA was incubated overnight at 30 °C with 2% diphenylamine under the less acid conditions of 66% formic acid and that the products of the degradation were the expected free purines plus pyrimidine

deoxynucleoside and oligonucleotide 3′,5′-diphosphates. We devised methods of separating the pyrimidine nucleotide products and were able to demonstrate the usefulness of the reagent for the quantitative study of the distribution of pyrimidine nucleotides in DNA (8), and Ken made a comparative study of the distribution of the shorter pyrimidine sequences in a range of DNAs of differing overall base compositions (9).

It must be remembered that this work was done in the days when no deoxyriboendonuclease of defined specificity was known and there was no polyacrylamide gel electrophoresis. At that time, studies of DNA chemistry in Britain were dominated by Cambridge, and the importance of Ken's Oxford-based pioneering work on DNA structure has tended to be forgotten in the excitement generated as later developments in nucleic acid sequencing unfolded elsewhere.

Meanwhile, other aspects of bacteriophage biochemistry were not forgotten. With his first DPhil student, Marianne (Mary) Kelemen and an American visitor, Liselotte Fessler (*née* Hecht), Ken explored phage growth in which nucleic acid synthesis was limited by inhibitors or by purine starvation. They found that, although the extent of phage DNA synthesis is controlled by the supply of preformed purines, the enzymes required for the synthesis of phage DNA were made whether exogenous purines were supplied or not. Furthermore, although there was no net synthesis of DNA, phage DNA could be synthesized in the absence of added purines by using purines derived from degraded bacterial DNA and the conversion of bacterial cytosine residues to 5-hydroxymethylcytosine (10). With Al Levin, an American visitor, it was shown that infection of *E. coli* with T2r+ or phage ghosts prevented the synthesis of bacterial enzymes that were required for the production of nucleic acid precursors. Alan Stone, a DPhil student, examined the increased deoxyribonuclease activity in *E. coli* after infection by T2 and T5 bacteriophages and in a separate study found that Ca^{2+} or Sr^{2+} ions acted synergistically on the rates and extent of hydrolysis of DNA by a streptococcal deoxyribonuclease, suggesting the presence of two metal-binding sites in the enzyme.

The mild reaction conditions of the diphenylamine–formic acid reagent were exploited in several important studies. Shapiro and Chargaff had used 0.1 M sulphuric acid to degrade DNA from a thymine-requiring mutant of *E. coli* cells grown in the presence of 5-bromouracil in place of thymine and had concluded that incorporation of the mutagenic thymine analogue had resulted in gross alterations in nucleotide sequences in the substituted DNA (Shapiro & Chargaff 1960). Using the diphenylamine–formic acid reagent, Ken found no difference between wild-type and bromouracil-substituted DNA (11), a conclusion also eventually reached by the Chargaff group on re-examination of the problem (Rudner *et al*. 1962). With postdoctoral student Mary Lunt, Ken took advantage of the fact that glycosidic bonds were stable to the diphenylamine–formic acid reagent and examined the distribution of glucosylated pyrimidine nucleotides in phage T2 DNA; they found that the glucose was attached predominantly to the 5′ residues of consecutive hydroxymethyl-deoxycytidine nucleotides. Similarly, Jens-Christian Siebke, a Norwegian visitor, found no pattern in the glucosylation of purine–pyrimidine dinucleotides produced by deoxyribonuclease.

I returned to Oxford in 1962–63 and again collaborated with Ken in a study of sequences of consecutive deoxycytidylic acid residues in DNAs of different base compositions. DPhil student Basil Smith and Ken investigated the size of DNA present in *E. coli* undergoing thymineless death and, using viscosity and sedimentation measurements, did not detect any unusual fragmentation. Mervyn Smith, another DPhil student from the Otago Department of Biochemistry (who returned to New Zealand to join the staff of that department a few years before I took up the chair in 1968), examined replicating DNA in T5-infected *E. coli* and

found a precursor to phage DNA that was of higher molecular mass than the DNA found in the phage particles. Unfortunately the DNA was too large to study further with the methods that they had available at the time.

Ken extended his work on possible methods for the base-specific degradation of DNA with a study of the effects of osmium tetroxide, a reagent known to attack double bonds. With DPhil student Bill Riley he found that, in dilute aqueous ammonia, thymidine residues were attacked about 45-fold more rapidly than deoxycytidine (12). They used this differential susceptibility to degrade mixed polypyrimidine nucleotides containing a single thymidylic acid residue preferentially and obtain information on the consecutive sequences of deoxycytidine nucleotides within the mixed oligonucleotides. Other postdoctoral students and scientists who visited Ken's laboratory in Oxford for shorter periods included Eugene Kennedy and Howard Fisher from the USA, Val Tanyashin from Russia, and Peter Bergquist from Auckland, New Zealand.

In 1964–65 Ken took sabbatical leave to be a Visiting Lecturer in the Harvard Medical School, where he worked with Professor Paul C. Zamecnik in the John Collins Warren Laboratories of the Huntington Memorial Hospital, Massachusetts General Hospital, Boston. With N. F. Varney and Zamecnik, he looked at the action of osmium tetroxide on tRNA and found a correlation between sensitivity of various amino acceptor activities and the genetic code. Ken also studied the osmium tetroxide reaction in greater detail and found that OsO_3NH was formed during the reaction, that a second molecule of ammonia seemed to be involved, that secondary structure could prevent osmium attack and that 4-thiouridine was oxidized very rapidly by the osmium reagent (13).

In Oxford, Ken assisted with the departmental teaching through short practical courses on nucleic acids and bacteriophages. From 1961 to 1964 he also taught in the first-year course for the physiology preliminary examination, taken by medical students and all science students reading biochemistry or physiology, with the brief to replace one of the two terms devoted to elementary organic chemistry with a new course on physical chemistry for biologists. Sir Hans Krebs retired in 1967 and moved his laboratory to the Radcliffe Infirmary in Oxford. Although the MRC continued to support his research and that of a somewhat smaller unit, many senior members of the MRC unit began to move to positions elsewhere. While he was on leave in Boston, Ken was offered, and accepted, the Chair of Biochemistry in the University of Newcastle upon Tyne with the opportunity of forming a new department there.

Department of Biochemistry, University of Newcastle upon Tyne, 1966–89

In starting the new department at Newcastle, Ken had to decide whether he wanted a department that specialized narrowly in one area or was more diverse. He chose the latter course and sought to recruit staff whose interests varied but who would not become too isolated. Funds were scarce, and he decided that it was unrealistic to expect that it would be possible to provide animal house facilities or to purchase (let alone replace) specialist equipment for areas such as X-ray crystallography, electron microscopy or nuclear magnetic resonance unless an outstanding user of such techniques was recruited. Equipment for sequencing and synthesizing proteins needed to be more generally available, but also needed to be frequently updated and consequently had to be well-used to be justified.

The new department, opened by Krebs, began with Roger Pain (later Professor of Physical Biochemistry) and Richard Virden as lecturers and Adrian Allen as demonstrator. Valuable help was received from the MRC in the form of a Research Group on the Biosynthesis of Macromolecules. In the Unit were Brian Hooton (who worked with Richard Virden on creatine kinase isozymes) and John Midgeley, who examined the kinetics of ribosomal RNA and transfer RNA synthesis *in vivo*. Hooton transferred to a lectureship in the department a year later. Basil Smith, Ken's former DPhil student at Oxford, returned from a postdoctoral position in Renato Dulbecco's laboratory at the Salk Institute in La Jolla as a NATO Fellow working independently on polyoma in the Unit. Ken's initial research aim for the Unit had been to use chemical probes to look at the structure and function of ribosomes, but this never really materialized and he was side-tracked by tRNA purification and a curious, rapidly labelled, phosphorus-containing macromolecule in *E. coli*, possibly in the cell wall. Looking back later, Ken considered that his original idea was misdirected, because the real advances in the field of ribosomal structure came from the purification of individual ribosomal proteins, the preparation of antibodies against them and their localization by high-resolution electron microscopy—an approach that he would not have been equipped to follow.

He did, however, successfully take the osmium tetroxide reaction a step further. He had realized that thiouridine was one of the sites of attack by OsO_3NH in tRNA and, using osmium tetroxide in [^{14}C]methylamine, he was able to convert 4-thiouridine in *E. coli* $tRNA_{phe}$ into *N*-[^{14}C]methylcytidine. This allowed him to establish convincingly the actual sequence around 4-thiouridine in that $tRNA_{phe}$ and provide useful support for the sequence proposed by Barrell and Sanger that was in conflict with that suggested by others.

In 1969 Basil Smith left for a post with the Imperial Cancer Research Fund in London, and Brian Hooton took a position with the British Council. Peter Emmerson (later Professor of Molecular Biology) joined the staff in Hooton's place. With his concentration on the study and cloning of the RecB, RecC and RecD proteins of *E. coli*, Emmerson and his group became a focus of strength in the department. Adrian Allen made much headway with his study of gastric mucus and went on to be appointed Professor and Head of Physiological Sciences at Newcastle. Ken turned his attention to the versatility of purine nucleotides in metabolism: as energy carriers, as precursors of nucleic acids and as components of many enzyme systems. He reasoned that the utilization of ATP and GTP in nucleic acid synthesis must compete with other functions that they have in the cell and began studies of the regulation of various aspects of purine nucleotide metabolism and their effect on nucleic acid synthesis that were to dominate his research efforts for the remainder of his working life.

Studies involving PhD student Gillian Thomas and technician Neil Varney indicated that limited RNA synthesis could be facilitated by hypoxanthine alone in an *E. coli* mutant that requires adenine but cannot use hypoxanthine in place of adenine. Hypoxanthine is apparently converted to GTP; the cell tries to make RNA with this and thus depletes the adenine pools. NAD^+ does not act as a reservoir of adenine. Derepression of histidine biosynthesis when external adenine is required but not supplied can similarly deplete the pool of adenine nucleotides. Glycolysis is also inhibited, probably by a deficiency of ADP at the phosphoglycerate kinase step. The conversion of [2-^{3}H]adenine to [2-^{3}H]histidine provided a way of determining the total extent of RNA turnover in *E. coli* (14), and further work with technician June Ramsay led to the conclusion that ribosomal RNA degradation is not as important in controlling ribosomal RNA accumulation as had been proposed by others (16).

In 1974, the year in which he was elected to the Fellowship of the Royal Society, Ken published the last of his papers on thermodynamics. He measured the equilibrium values of the reactants in the NAD^+-linked conversion of propan-2-ol to acetone catalysed by yeast alcohol dehydrogenase over the temperature range 9–42 °C and calculated the enthalpy change for the reduction of NAD^+. In a separate line of study, Ken thought that it might be interesting to study enzyme synthesis in a eukaryote, such as *Neurospora crassa*, that forms heterokaryons. Mike North, a former PhD student who went on to become a lecturer at Stirling, had previously discovered that the glycerol kinase of *Neurospora* was inducible by glycerol, deoxyribose and galactose, and also simply by being kept overnight at 4 °C (North 1973). The mechanism of the cold induction was still obscure. There seemed to be at least two enzymes, and Ken took advantage of the fact that Gunnar Kølmark from Uppsala, who had isolated a mutant of *Neurospora* that was deficient in the inducible enzyme, was making a brief visit to Durham and collaborated with him in examining glycerol kinase activity in his strains (15).

A body of experimental data including Ken's own work on RNA turnover had initially indicated a high-affinity uptake system into *E. coli* for nucleic acid bases such as adenine, but this was in contrast to some work published in the 1970s that indicated that adenine transport was of low affinity and involved group transfer catalysed by phosphoribosyltransferases. Ken used several mutant strains of *E. coli* to clarify the relation between energy coupling and metabolic conversions in the transport of adenine, hypoxanthine and uracil. High-affinity transport seemed to require a protonmotive force and metabolism of the transported bases to nucleic acids (17); with Malcolm Page (a PhD student) Ken found no evidence that phosphoribosyltransferases were located in the cell wall to act in group transfer (18). Between 1976 and 1978, with the movement of Adrian Allen to the Physiology Department, the departure of John Midgeley to Amersham Radiochemicals and the creation of a new post, it became possible to restructure the department. Megs Rogers (interested in low-density lipoprotein), Ian West (who studied the bioenergetics of lactose transport into *E. coli*) and Steve Yeaman (an enzymologist interested in covalent modification, particularly of decarboxylases) were appointed as lecturers.

In 1978–79 Ken spent six months in New Zealand as a visiting professor in my own department at the University of Otago and returned briefly to his earlier studies with osmium tetroxide. Although his results remain unpublished, he found that osmium tetroxide could augment the Maxam–Gilbert method to distinguish between cytosine and thymine.

Once back in Newcastle, Ken worked virtually single-handedly on the adenine transport problem. He isolated a mutant of *E. coli* (which he designated *purP*) that was defective in high-affinity adenine transport, and he was able to place this mutant approximately on the *E. coli* gene map. Competition experiments with the *purP* strain of *E. coli* indicated that at least two other systems are used to transport guanine, xanthine and hypoxanthine (19). This paper was his last publication before his retirement in 1989. However, he persisted with the problem and published a final paper to round off the study in 1994 (20). Ken stepped down as Head of the Department of Biochemistry in 1983 to become Dean of the Faculty of Science (1983–86), and Professor Roger Pain took over as head of the department.

At Newcastle, all of Ken's teaching was to science students. Each year, the department developed a new course until, by 1975, they had a single honours degree and a first-year course (with genetics) taken by almost all biology students. Ken's own courses included sizeable ones on transcriptional control and the cell biology of growth. For about 10 years, Ken and other members of the Biochemistry Department gave courses in genetics until the new Department of Genetics could cope. Ken favoured the closer integration of relevant bio-

medical departments and, after his retirement, the Biochemistry Department was merged with Genetics and then transferred into the School of Medicine while continuing to teach science degrees. Subsequently, the university merged all departments into multidisciplinary teaching schools or research institutes—a move that Ken considered to be a good idea in principle.

Looking back after his retirement, he saw that a drawback of the career that he had chosen was that he was continually torn between research, teaching, and departmental and university administration. With characteristic modesty he felt that he reacted to events and chances rather than creating them and that he was unduly willing to accept opinions of other people and inclined to be slow to develop his own. He recognized that some of the problems that he encountered were historical. Newcastle University had started life as a division of Durham University and the departments that had existed on site when the two divisions separated in 1963 retained structures that they had developed, often competing for funding for similar activities. He was pleased that, in partial response to some of his misgivings about the *status quo*, some reorganization had already occurred. It is not surprising that, as the first Professor of Biochemistry and head of a brand new department, he suffered at first from a degree of obstruction from heads of longer-established departments who saw the newcomer as something of a threat. But there is no doubt that he succeeded in passing on a successful and well-founded academic department to his successors.

Ken was left seriously incapacitated by a severe stroke and took early retirement in 1989. In time, he taught himself to walk again using a self-propelled cylinder mower, and largely recovered his mobility and speech. He was able to return to the laboratory bench to finish off his study of adenine transport. To be closer to their children and grandchildren, he and Hilda moved in 1996 from Stocksfield, Northumberland, to Alfriston in East Sussex, where they lived quietly until his death from cancer on 22 November 2010. He was survived by his wife, two children and six grandchildren.

External activities

Ken served at the national level on several scientific and editorial committees: Editorial Board, *Biochemical Journal* (1959–64), Biological Research Board, MRC (1967–71), Chairman of Grants Committee 1, MRC Biological Research Board (1969–71), joint MRC/CRC Committee on Support of Cancer Research (1970–72), Science Research Council Research Group 2 (grant committee) of Biological Sciences Committee (1974–77), Science Research Council Representative on MRC Subcommittee of Molecular Biology (1975–76), Biochemical Society Committee (1975–78), British National Committee for Biochemistry (1978–82), EMBL Users' Committee (1979–81), and Associate Editor, Royal Society (1979–82).

Personal qualities

Ken's paternal ancestors included staunch Wesleyans and his mother's family were devout members of the Roman Catholic Apostolic Church. Ken's father's experiences in World War I had disillusioned him about human behaviour and Christianity, but Ken received a Christian upbringing and attended college chapel as an undergraduate. However, increasingly he came to think that things accounted for by religion were explained better by science, 'especially now

that molecular biology can help us understand how mutations and development occur', and he called himself an 'unrequited atheist'. Describing his experience of a cardiac arrest while he was in hospital in 1987 when he thought that his life was about to end, he wrote that he was untroubled by any thought of an afterlife but very concerned about what would happen to his wife and family. He was able to call for assistance before he lapsed into unconsciousness and, as he later wrote, 'fortunately I was resuscitated'.

Perhaps not surprisingly given his family background, Ken was angry at the downtrodden state of working people before the war. He was active in the small Labour Club at Cambridge, but the much larger Socialist Society seemed to him to be too dominated by communists. Ken was not politically active in his later life and expressed his disgust with some of the activities of both the Labour and Conservative parties. However, he took an active interest in the development of his own community and served as secretary for many years, and as chairman for six years until shortly before his death, of the Alfriston District and Amenity Society.

Music was one of Ken's passions. His mother's parents gave her a piano (which Ken later inherited) as a wedding present. Ken learnt the piano as a child and, although he did not claim to be a great performer, was sufficiently proficient to play for relaxation and enjoyment. He also learnt to play the clarinet in later life but was unable to continue this after his stroke. He took full advantage of his time at King's College to strengthen his knowledge of classical music and enjoyed the Hallé Concerts under John Barbirolli at Sheffield, the concert scene in Oxford and the opportunities to listen to chamber music and orchestral concerts in the Newcastle area. There were excellent concerts in Alfriston where he retired and, of course, Glyndebourne was nearby. In Chicago he enjoyed the traditional jazz clubs. He regularly listened to recorded music but preferred to collect chamber music rather than orchestral records because the reproduction was better.

In 1945 Ken joined the Cambridge University Youth Hostels Association (YHA) group and he was later active in the local YHA. Life during the war had been constraining, and hostelling trips to country areas such as the Lake District, Scotland, Wales and Ireland and the Yorkshire dales were a new experience to him. In later life he remained an enthusiastic walker, particularly in hilly country, and was especially fond of Northumberland and the Lake District, although he found the Sussex Downs to be better suited to his walking abilities in his retirement. He enjoyed gardening, especially in his retirement.

A devoted family man, Ken was a kind, generous, gentle person with a ready smile. He had an impish sense of humour and was totally devoid of pomposity or egotism. He is remembered by his former staff and students as a wise leader, a concerned supervisor and completely fair. He was passionate about science and spent many hours at the bench—reflected in his sole authorship of an unusually large proportion of his publications. He was a competent mathematician and had an enviably broad knowledge of chemistry, physics, biology and biochemistry that, coupled with a remarkable facility for lateral thought, he used to maximum effect in the design and analysis of experiments. He was meticulous in his scientific writing and insisted that his graduate students follow his example.

Kenneth Burton contributed significantly to our knowledge in a range of subjects: enzymology, biochemical thermodynamics, bacteriophage biochemistry and microbiology and, of course, DNA chemistry. His work on methods for studying DNA primary structure has tended to be downplayed in the excitement of later developments, but he was a true and successful pioneer in what seemed at the time to be an impossible field and through these studies made an important contribution to the history of science.

Acknowledgements

In preparing this memoir I have drawn heavily on a set of autobiographical notes and an account of the Burton family history that Ken prepared before his death. I also had available a set of notes that he had prepared for me in advance of a videotaped interview that I made with him as a contribution to the oral history archive of the Biochemical Society, London ('Conversations with Eminent Biochemists. K. Burton in conversation with G. B. Petersen', 9 February 1990). I am grateful to Ken's son, Dr James Burton, and to Dr Ian West and Dr Basil Smith for their comments and their help with additional material to fill in gaps.

The frontispiece photograph was taken in 1974 by Godfrey Argent and is reproduced with permission.

References to other authors

Krebs, H. A. & Veech, R. L. 1969 Equilibrium relations between pyridine nucleotides and adenine nucleotides and their roles in the regulation of metabolic processes. *Adv. Enzyme Regul.* **7**, 397–413.

North, M. 1973 Cold-induced increase of glycerol kinase activity in *Neurospora crassa*. *FEBS Lett*. **35**, 67–70.

Rudner, R., Shapiro, H. S. & Chargaff, E. 1962 Distribution of 5-bromouracil among the pyrimidine clusters of the deoxyribonucleic acid of *E. coli*. *Nature* **195**, 143–146.

Shapiro, H. S. & Chargaff, E. 1960 Severe distortion by 5-bromouracil of the sequence characteristics of a bacterial deoxyribonucleic acid. *Nature* **188**, 62–63.

Tamm, C., Shapiro, H. S., Lipshitz, R. & Chargaff, E. 1953 Distribution density of nucleotides within a desoxyribonucleic acid chain. *J. Biol. Chem*. **203**, 673–688.

Tomizawa, J. I. & Sunakawa, S. 1956 The effect of chloramphenicol on deoxyribonucleic acid synthesis and the development of resistance to ultraviolet irradiation in *E.-coli* infected with bacteriophage T2. *J. Gen. Physiol*. **39**, 553–565.

Weart, S. R. & Szilard, G. W. (eds) 1978 *Leo Szilard: his version of the facts.* Cambridge, MA: MIT Press.

Bibliography

The following publications are those referred to directly in the text. A full bibliography is available as electronic supplementary material at http://dx.doi.org/10.1098/rsbm.2011.0014 or via http://rsbm.royalsocietypublishing.org.

(1) 1951 The stabilization of D-amino-acid oxidase by flavin-adenine-dinucleotide, substrates and competitive inhibitors. *Biochem. J*. **48**, 458–467.

(2) The L-amino-acid oxidase of *Neurospora*. *Biochem. J*. **50**, 258–268.

(3) 1955 The relationship between the synthesis of deoxyribonucleic acid and the synthesis of protein in the multiplication of bacteriophage T2. *Biochem. J*. **61**, 473–483.

(4) 1956 A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J*. **62**, 315–323.

(5) 1957 (With H. A. Krebs & H. L. Kornberg) A survey of the energy transformations in living matter. *Ergebn. Physiol*. **49**, 212–298 (Appendix: Free energy data of biological interest, by K. Burton, pp. 275–292).

(6) 1958 Energy of adenosine triphosphate. *Nature* **181**, 1594–1595.

(7) 1959 (With T. Benzinger, C. Kitzinger & R. Hems) Free-energy changes of the glutaminase reaction and the hydrolysis of the terminal pyrophosphate bond of adenosine triphosphate. *Biochem. J*. **71**, 400–407.

(8) 1960 (With G. B. Petersen) The frequencies of certain sequences of nucleotides in deoxyribonucleic acid. *Biochem. J*. **75**, 17–27.

(9) Frequencies of nucleotide sequences in deoxyribonucleic acids. *Biochem. J*. **77**, 547–552.

(10) (With L. I. Fessler & M. V. Kelemen) Synthesis of protein in a purine-requiring *Escherichia coli* infected with bacteriophage T2. *Biochem. J*. **77**, 558–563.

(11) 1962 Sequences of nucleotides in deoxyribonucleic acid containing 5-bromouracil. *Biochim. Biophys. Acta* **55**, 412–415.
(12) 1966 (With W. T. Riley) Selective degradation of thymidine and thymine deoxynucleotides. *Biochem. J.* **98**, 70–77.
(13) 1967 Oxidation of pyrimidine nucleosides and nucleotides by osmium tetroxide. *Biochem. J.* **104**, 686–694.
(14) 1976 Analysis of RNA turnover in bacteria using histidine as a radioactivity trap for [2-^{3}H]adenine nucleotides. *J. Mol. Biol.* **102**, 333–348.
(15) (With K. Holm, E. Nilheden & H. G. Kølmark) Genetic and enzymatic analysis of a glycerol kinase deficient mutant in *Neurospora crassa*. *Mol. Gen. Genet.* **144**, 11–15.
(16) 1977 (With J. Ramsay) The stability of nascent ribosomal nucleic acid in growing *Escherichia coli*. *Biochem. Soc. Trans.* **5**, 459–462.
(17) Transport of adenine, hypoxanthine and uracil into *Escherichia coli*. *Biochem. J.* **168**, 195–204.
(18) 1978 (With M. G. P. Page) The location of purine phosphoribosyltransferase activities in *Escherichia coli*. *Biochem. J.* **174**, 717–725.
(19) 1983 Transport of nucleic acid bases into *Escherichia coli*. *J. Gen. Microbiol.* **129**, 3505–3513.
(20) 1994 Adenine transport in *Escherichia coli*. *Proc. R. Soc. Lond.* B **255**, 153–157.