DAVID KEILIN

Ramsey and Muspratt

Obituary Notice

DAVID KEILIN

(1887–1963)

While attending a meeting of the General Board of the Faculties of Cambridge University, and during a discussion of the future of the Molteno Institute, David Keilin died suddenly on 27 February 1963, at the age of 75.

He was born in Moscow on 21 March 1887, and one of his earliest memories was the return of the family to their home in Warsaw. His health was poor during childhood and it was not until he was ten years old that he went to school, where he showed promise in mathematics, literature and languages. Following the tradition of the times he sought higher education abroad and in 1904 he enrolled at the University of Liège. There he followed a broadly based course in the natural sciences which fired an ambition to take up medicine. He was persuaded, however, that his asthmatic attacks would be too serious a handicap in such a calling. Thus he arrived in Paris in 1905 with no clear sense of vocation. He read extensively in the Bibliothèque Ste Geneviève, he deepened his artistic sensibilities in the museums and art galleries of the capital, and he attended lectures by Henri Bergson, then professor of philosophy at the Collège de France. After leaving one of these lectures he took shelter from the rain in a building near the Panthéon where a lecture was in progress. The building was the Laboratoire d'Évolution des Êtres Organisés; the lecturer was Maurice Caullery, later to become a member of the French Academy of Sciences and a foreign member of the Royal Society. With the training that he had received at Liège, Keilin found the lecture to be so interesting that he attended the rest of the course and entered into the discussions which followed the lectures. Caullery was clearly impressed by this young man, for he invited him to join in the work of the Laboratory.

This was the stimulus that Keilin needed. With apparently limitless energy he read geology, botany, zoology and embryology at the Sorbonne, began research under Caullery on parasitic and free-living larval stages of Diptera, and during vacations he studied at the marine stations of Banyuls, Roscoff and Wimereux. His work on Diptera was recognized in 1914 by the award of the Prix Passet of the Société Entomologique de France; it also formed the subject of a thesis which he presented in 1917 for the degree of Doctor of Science. In 1915 he accepted an invitation to work in Cambridge as research assistant to G. H. F. Nuttall, the first Quick Professor of Biology.

Keilin was elected to a Beit Memorial Research Fellowship in 1920, he became Lecturer in Parasitology in 1925, and in 1931 he succeeded Nuttall as Quick Professor and Director of the Molteno Institute.

In 1902 Frederick James Quick bequeathed to the University of Cambridge a fund the income from which was to be used to endow a research professorship, the Quick Professorship of Biology. The Quick Laboratory, which had been improvised for Nuttall in 1907, consisted of one large room in an unfinished part of the Medical Museum (a site now occupied by the Zoological Laboratory). This 'temporary accommodation' was occupied for 14 years until, through the generosity of Mr and Mrs Percy Alport Molteno, the University was able to provide an institute for parasitology. When Keilin became Quick Professor the subject of research was changed from Parasitology to Biology of the Cell. In 1934 the Molteno Institute for Research in Parasitology became the Molteno Institute of Biology and Parasitology.

I am not qualified to give an appreciation of Keilin the entomologist and parasitologist and I am indebted to Dr P. Tate, Reader in Parasitology in the University of Cambridge, for information on which the following summary is based. [A complete account of Keilin's contributions to entomology and parasitology will be found in an obituary to be contributed by Dr Tate to *Parasitology* (vol. 54).] In the years up to 1931 Keilin published about 70 papers on entomological subjects. These deal mainly with parasitic insects and with protista that parasitize insects. His outstanding contributions on the life cycle of parasitic and free-living Diptera established him as a world authority. Of the protists that he described eleven were placed in new species or new genera. He would always seek to interpret morphology on an evolutionary basis and he became increasingly interested in the adaptations of dipterous larvae to the parasitic mode of life and in the respiratory problems that arise. This in turn stimulated his interest in the physiology of insect respiration, in the pursuit of which he was led to the discovery of cytochrome. Although his parasitological publications became less frequent after 1931 his stature as a parasitologist remained undiminished, largely owing to his influence as editor of *Parasitology*, a position which he held from 1934 until his death. A fitting culmination to this part of his career was the monograph 'Respiratory Systems and Respiratory Adaptations in Larvae and Pupae of Diptera' (*Parasitology*, 1944, 36, 1). Originally written in French, this paper

remained unpublished for many years. He eventually translated it into English as recreation during a convalescence.

Yet this was the man who, in 1925, changed the face of biochemistry. Apart from those who have been in close touch with him, few people were aware that this small figure, far from robust in health and continually harassed by asthma, had attained in two spheres of scientific endeavour a supremacy that few of us can hope to achieve in one.

Between 1922 and 1924 Keilin was studying the life cycle of the horse bot-fly *Gasterophilus intestinalis*, the female of which attaches its eggs to the hairs on horses' legs. The larvae develop within the egg and emerge when the horse licks the affected parts. When the larvae are swallowed they attach themselves to the stomach wall by mouth hooks. As they develop they become bright pink owing to the formation of a specific intracellular haemoglobin. Keilin was curious about the fate of this haemoglobin during metamorphosis since the adult fly, resembling a large honey-bee, shows no sign of pink colour. When he examined the tissues of the fly with a microspectroscope (a direct-vision spectroscope that can replace the eyepiece of a microscope) he could not detect the pair of absorption bands which are characteristic of oxyhaemoglobin. The thoracic muscles, however, showed a spectrum with four bands, none of which corresponded to those of haemoglobin or oxyhaemoglobin. In attempting to relate this thoracic pigment to the larval haemoglobin Keilin examined the adult forms of insects which are devoid of haemoglobin at the larval stage. He saw the identical four-banded spectrum in thoracic muscles of the blowfly (*Calliphora erythrocephala*), but the larvae had been fed on meat and so the suspicion remained that ingested muscle haemoglobin was the source of the new pigment. Having cultured *Bacillus subtilis* as a possible nutrient for *Gasterophilus* larvae *in vitro*, Keilin examined a thick mass of the cells spectroscopically and again detected the four-banded spectrum. Since the bacillus had been cultured on broth it was again not possible to exclude haemoglobin as the precursor of the new pigment. However, the appearance of the same four absorption bands in the spectrum of thoracic muscles of the wax-moth, *Galleria mellonella* (the larvae of which had been bred out of contact with material containing haemoglobin) and of the honey-bee, as well as in the spectrum of a pellet of baker's yeast, finally convinced him that the pigment was not a transformation product of haemoglobin. In fact, as he very soon found, it was far more widely distributed than haemoglobin, being detectable in many animal and plant tissues as well as in a wide range of micro-organisms.

One day, when Keilin was examining baker's

yeast, not as a pellet but as a suspension prepared by shaking it vigorously with water in a test tube, he was surprised to find that he could not see the four bands. He received a greater surprise when, within a few seconds, the bands quite suddenly appeared. If the suspension were again shaken and immediately re-examined the bands were not visible, but they appeared when the yeast suspension remained undisturbed for a brief period. This cycle of changes could be repeated many times. The same cycle could be observed by spectroscopic observation of the thoracic muscles of a living intact moth (*Galleria*): the spectrum bands were strong while the wings were vibrating, but faded when movement ceased. These dramatic observations convinced Keilin that the new spectrum was that of a substance which, by reversible oxidation and reduction, was playing a vital role in cell respiration. His belief was confirmed by the effects of respiratory inhibitors. When cyanide was added to a yeast suspension no amount of shaking in air would cause the spectrum bands to fade. Narcotics (urethane), however, had the opposite effect: they delayed the reappearance of the bands after the suspension had been aerated. From observations that the bands neither appear nor disappear simultaneously he deduced that the four absorption bands—*a* (604 m μ), *b* (564 m μ), *c* (550 m μ), *d* (521 m μ)—represent a mixture of three haemochromogen [ferrohaemochrome]-like compounds of which *a*, *b* and *c* are the respective α -bands, while the more diffuse band *d* consists of overlapping β -bands. To the mixture of pigments Keilin gave the name 'cytochrome' ('pending the time when its composition shall have been properly determined') and he designated the three components as cytochromes *a*, *b* and *c*. A search of the literature disclosed that in 1884 C. A. MacMunn, a medical practitioner in Wolverhampton, had detected these four absorption bands in the spectra of many animal tissues and had convinced himself that they represent a pigment with respiratory functions. MacMunn's discovery had, however, become buried under the confusion that arose from unjustified criticism of his work.

In his first paper in this new field ('On Cytochrome, a Respiratory Pigment, Common to Animals, Yeast, and Higher Plants', *Proc. Roy. Soc. B*, 1925, **98**, 312) Keilin suggested that the components of cytochrome are haematin compounds which act as oxidation-reduction catalysts in respiring cells, being alternately reduced by dehydrogenases and oxidized by oxygen through the agency of an enzyme. How revolutionary was this concept can be appreciated if we view it against the background of the preceding decade. Thunberg and Wieland had elaborated a dehydrogenase theory of cell respiration according to which metabolites are each activated by specific enzymes. This

activation facilitates transfer of hydrogen atoms from metabolites to hydrogen acceptors. Artificial acceptors (e.g. methylene blue) were known, but the mechanism by which the hydrogen was transferred to oxygen remained uncertain. Warburg, on the other hand, argued that the dehydrogenase concept was misleading and unnecessary. He postulated the existence in all cells of an iron-containing respiratory enzyme, *Atmungsferment*, which catalyses the reaction of oxygen with all metabolites. In effect, the fundamental reaction of respiration is activation of oxygen. According to Warburg's theory this activation, together with non-specific surface forces, would suffice to explain cell respiration. The disagreement between the two schools was complete and further progress was impossible until Keilin produced the key to the problem. Both hypotheses were correct in that they represented the initial and final stages of a complex respiratory pathway. Cytochrome was the link between a multiplicity of dehydrogenases and a single 'oxidase'.

The discovery of cytochrome established two new principles which transformed the experimental approach to biological oxidation. One was that haematin compounds function as oxidation-reduction catalysts in aerobic cells. Hitherto their only established role was that of oxygen carrier (e.g. haemoglobin). The first consequence of the new discovery was the work from Warburg's Laboratory on light-reversible inhibition of cell respiration by carbon monoxide, which led to the conclusion that *Atmungsferment* is a haematin compound. The other principle was the respiratory chain. The primitive enzymology of the preceding era had established the existence of numerous intracellular enzymes but could provide no clear evidence for their ordered sequential operation *in vivo*. Far-sighted men like Gowland Hopkins argued that the efficiency of cell metabolism could only be based on a high degree of organization of catalysts: only that could ensure the rapid sequence of catalysed reactions that is characteristic of the cell's economy. It was Keilin's observations that furnished evidence of such integrated reactions in living cells.

High-precision spectrophotometry has provided a wealth of information on interactions in the respiratory chain, and the essential outline formulated by Keilin is now a rich mosaic. Keilin's original observations were made with a spectro-scope small enough to be carried in the pocket, and there is no doubt that this low-dispersion instrument was the ideal one for his purposes. Had today's commercial spectrophotometers been standard laboratory equipment in 1925 it is most unlikely that Keilin would have discovered cytochrome—apart possibly from cytochrome *c*, which can be extracted in soluble form. Certainly the function of cytochrome would have escaped detec-

tion. The truth of this becomes evident to anyone who attempts to verify Keilin's early observations on cytochrome with a standard spectrophotometer. (It was in 1925 that Hans Fischer concluded, after most thorough investigation, that yeasts contain free porphyrin but no haematin.) Until 1940 all Keilin's contributions to knowledge of cytochrome were based on the use of this simple spectro-scope and it remained his basic instrument for all subsequent experimental work. Keeping the entire visible spectrum under observation he could follow and unravel the intricacies of respiratory activity within living cells; only later would he bring in other techniques to confirm his conclusions. He continued to find much aesthetic satisfaction in visual spectroscopy and was sorry for those to whom cytochrome is no more than a line drawn by a servo-operated pen.

The six years beginning in 1925 were very busy ones. As university lecturer Keilin was responsible for teaching and direction of research in parasitology. However, he found time to publish eight papers on cytochrome and as many on parasitological subjects, and this despite initial financial stringencies which, in later years, he would recall with amusement. There was the one-man-power centrifuge, and the borrowed Barcroft manometers which had to be held and shaken, one in each hand, throughout the experiment. In three subsequent papers in the *Proceedings of the Royal Society*, B (1926, 100, 129; 1929, 104, 206; 1930, 106, 418) Keilin produced further evidence for the nature and function of cytochrome. Extensive studies on the nature and spectral characteristics of haemochromogens established their relationship to cytochrome. It was found that the factor responsible for the oxidation of cytochrome is the enzyme characterized earlier as indophenol oxidase and now shown to be identical with *Atmungsferment*. Cytochrome *c* was extracted in soluble form from yeast and shown to have the same catalytic properties as the intracellular form, being oxidized by 'oxidase' and reducible either chemically (with cysteine) or enzymically through the agency of succinate dehydrogenase. In a letter to *Nature* (1927, 119, 670) Keilin described experiments with a 'slightly modified' Thunberg tube. In his 1929 paper the slight modification was illustrated; it was a stopper with a side arm, a device which allowed the tube to be evacuated before enzyme and substrate were mixed. With typical modesty he continued to describe this as a Thunberg tube as indeed everyone else has since done. The only published acknowledgement of this brainwave that I can trace appears in catalogues of scientific apparatus published by A. Gallenkamp and Co. Ltd. in the 'thirties.

After 1931 Keilin became increasingly interested in other aspects of biological catalysis. Thus it was

not until 1938–39 that evidence for the nature of the ‘oxidase’ (now cytochrome oxidase) was brought forward. Critical spectroscopic examination of a colloidal extract from washed and ground heart muscle led to the conclusion that what had hitherto been regarded as a single haemoprotein, cytochrome *a*, consisted of two components whose absorption bands in the visible spectrum were superimposed. One of these components, cytochrome *a*₃, is autoxidizable and it was suggested that this component is the oxidase which catalyses the oxidation of the other components by oxygen. A simpler and more reproducible method of isolating cytochrome *c* was devised, this time from heart muscle, and in 1955 the existence and function of cytochrome *c*₁ (whose bands are normally masked by those of cytochrome *c*) were finally established. Studies on the oxidation of succinate by heart-muscle preparations focused attention on the vital importance of the spatial organization of catalysts within the colloidal particle for the efficient oxidation of succinate via the dehydrogenase–cytochrome-oxidase pathway. Keilin’s last publication (and his 191st) was a critical review of the reactions and spectral properties of cytochrome *c* (*Proc. Roy. Soc. B*, 1962, **156**, 429).

Of Keilin’s other contributions to cell respiration and enzymology there is space for only brief mention. After cytochrome, his chief interests were the haemoprotein enzymes catalase and peroxidase, with methaemoglobin serving as an enzyme model. Spectroscopic study of their reactions with peroxides and with inhibitors of enzyme activity threw much light not only on the mechanism of inhibition but also on the nature of the enzyme–substrate reaction. Major advances were the establishment of the haemoprotein nature of peroxidase and the spectroscopic observation of a compound between peroxidase and hydrogen peroxide; also the demonstration of ‘coupled’ oxidation of alcohols in the presence of catalase and a system which generates hydrogen peroxide (e.g. the aerobic oxidation of uric acid by urate oxidase). The work on coupled oxidation led to a generalization that the physiological function of catalase is a peroxidatic one. One development from this work was the introduction of azide as an inhibitor of respiratory enzymes; another was comparative studies of haemoglobins, especially those in root nodules of legumes and in *Gasterophilus* larvae, which have properties intermediate between those of blood haemoglobin and oxidases. Extensive purification of laccase proved that its active metal is not manganese, as had long been believed, but copper. A physiological role for zinc was established when carbonic anhydrase was shown to be a zinc protein. Work on the nature and specificity of notatin, otherwise glucose oxidase from *Penicillium notatum*, established the nature of the prosthetic

group and led to a highly specific enzymic method for the determination of glucose. An enzyme that catalyses mutarotation was discovered in the same mould. The sensitivity of spectroscopic methods was increased as much as 20 times by developments in low-temperature spectroscopy. In addition to the well-established sharpening of absorption bands at the temperature of liquid air, a new phenomenon of intensification was observed which is associated with solidification of the solvent to a microcrystalline form.

In 1946 Keilin came across sealed tubes of sterile blood which had been prepared by Nuttall 40 years previously. Though the erythrocytes were lysed, the haemoglobin was indistinguishable from the haemoglobin of fresh blood. Similarly, the activities of several endoerythrocytic enzymes had scarcely changed during storage. These results stimulated Keilin’s interest in wider implications of the longevity of biological materials: the stability of whole organisms under conditions where metabolism is reduced to an imperceptible level. This condition, anabiosis, is favoured by low temperatures or by desiccation.

From the formative years of his training in Paris, Keilin had acquired a profound feeling for the history of science and a keen appreciation of its potential contributions to contemporary thought. He would never depend on the writings of scientific historians—too frequently they perpetuated the errors of their predecessors. The original papers that he sought were, unfortunately, often in volumes which, having long remained undisturbed, would shed a dust to which he was peculiarly sensitive. After his retirement from the Quick chair in 1952 he devoted much time to the history of anabiosis, bringing to light a dramatic conflict of scientists, philosophers and theologians whose beliefs on the nature of life and death had to accommodate such startling facts as revival of primitive animals (rotifers) after prolonged desiccation followed by heating at 100°. A notable outcome of this research was Keilin’s Leeuwenhoek Lecture to the Royal Society in 1958 (*Proc. Roy. Soc. B*, 1959, **150**, 149).

Keilin’s long association with Magdalene College, Cambridge, began in 1915 when he registered as a research student. He was later elected to a Professorial Fellowship, and in 1957 he was given an Honorary Fellowship, a distinction that he greatly valued. He was elected to the Royal Society in 1928, receiving its Royal Medal in 1939 and the Society’s highest honour, the Copley Medal, in 1952. He retained a sincere affection for France and, among the honours that came to him from overseas, the rare distinction of election as Membre Associé Étranger de l’Académie des Sciences brought him much happiness. Generous support from the Rockefeller Foundation between 1934 and 1952 permitted expansion of facilities for research

and acceptance of an increasing number of research students and visiting scientists from all parts of the world.

The esteem in which we—at once his pupils, colleagues and friends—held David Keilin was compounded of far more than admiration for his scientific achievements. In one who had well earned the right to assert himself we found, above all else, the humility of greatness. This quality we saw not only in his attitude to the complexities of the living world but also in his complete devotion to the progress of learning without thought of personal advancement. It was expressed by his pleasure in developments elsewhere of his pioneering work on numerous aspects of biological catalysis, and again by his pride in the achievements of those who, thanks to his active guidance, found their feet set more confidently on the road to success. Balancing this humility were the determination of his scientific approach and the candour of his criticism in the face of injustice, misrepresentation or bombast. We found also a transparent integrity, a remarkable insight and an exceptional ability to give the right judgement in all matters. These attributes, together with his modest charm and ever-warm welcome, led to his advice being widely sought; and it was always freely given. There was no outer office with the customary Amazonian guard; he was accessible to all and far too indulgent of interruption. After his frugal lunch in the Laboratory he would settle in an easy chair for his 'snooze'. After nights disturbed by asthma he would sometimes sleep soundly and many white lies were told to keep would-be visitors from his door at such times. One instance of his judgement may be mentioned. In 1947 a Unit was set up in the Cavendish Laboratory to study X-ray diffraction by protein crystals as a possible approach to protein structure. When the Unit could not find funds for permanent support Keilin suggested an application to the Medical Research Council. The Council was told by Sir Lawrence Bragg that chances of success were remote and that, even if success were attained, useful applications to medicine would still lie many years ahead. Keilin had been actively encouraging Dr M. F. Perutz in this work by providing facilities at the Molteno Institute for crystallizing haemoglobin. He now strongly supported the application and the Medical Research Council took the risk. Thus was Molecular Biology launched.

Keilin's approach to research was essentially pragmatic. Keeping any preconceived ideas firmly disciplined he would patiently amass experimental facts until he was able to discern the pathway to the crucial experiment. Slow to formulate a theory himself, he could be censorious of those who trimmed their facts to fit some Procrustean frame-

work of conjecture and speculation. He inspired both respect and affection in students who attended his courses. His lectures on respiratory pigments and intracellular respiration were always profusely illustrated by demonstrations in the preparation of which he would spare no pains. He showed great consideration for students, often preferring to talk to a student society than to more learned and austere scientific bodies. With its undertones of French and Polish, his English retained an agreeable yet incisive accent that was all his own.

All biological sciences came within his orbit and he could speak with authority on many of them. It is thus hardly surprising that he had little time for outside interests. Fluent in Russian, Polish, French and English he found relaxation with writers of the nineteenth century, from Tolstoy to Dickens and Thackeray. During sleepless nights or illness he would descend to modern detective stories which, once read, would be rapidly forgotten. Neither is it surprising that he sometimes showed detachment from mundane matters and tiresome routine. The Quick Professorship is a three-year appointment. It was not unknown for the Quick managers, at their triennial meetings, to find no applicants for the shortly-to-be-vacated chair. However, after a hasty message to the Molteno Institute and a brief adjournment they received a note which enabled them to confirm the current occupant of the chair for a further three years.

His zest for the day ahead, his eagerness to get to the bench and the spectroscope, his confidence that his plans for the day's work contained the key to one of his current problems, all these were epitomized in the sound of his lively, purposeful step along the corridor to his room as he arrived at the Laboratory in the morning. He is survived by his wife, Dr Anna Keilin, whom he met and married when they were both students in Paris. Their daughter Joan shared her father's interest in tetrapyrrole pigments and is known for her work on haematin and haemoproteins. Thanks to their watchful care he remained actively working until the last, which is, without doubt, what he would have chosen to do. The foundation of his greatness was a mind untrammelled by specialization; by the man-made boundaries and dividing lines that obscure the ultimate unity of biology.

His scientific spirit finds an epigraph in the words of Joseph Priestley:

Let others reason better from the facts with which I supply them if they can. I shall listen to them with attention. But I cannot forbear observing, that I should be more obliged to them for the discovery of more facts from which to reason. Speculation is a cheap commodity. New and important facts are most wanted, and therefore of most value.

E. F. HARTREE