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Tribute

Contributions of Henrik Lundegårdh

Anthony William Derek Larkum
School of Biological Sciences, University of Sydney, NSW 2006, Australia (e-mail: alark@mail.usyd.edu.au; fax: +61-2-93514119)

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Abstract

Henrik Lundegårdh made major contributions in the field of ecology and plant physiology from 1912 to 1969. His early work at Hallands Väderö in the Kattegat pioneered quantitative approaches to plant ecology and laid the understanding of carbon dioxide exchange in natural communities which is still useful today in global carbon accounting. Very early on in this work he invented the *flame photometer*. In trying to understand salt respiration of plants, he started to formulate hypotheses for the relationship between respiration and ion movement, including protons, hypotheses that were forerunners to the *Chemiosmotic Hypothesis of Peter Mitchell*. Necessarily, this involved work on plant cytochromes. He invented several early recording spectrophotometers and made many early discoveries in the field of plant cytochromes, including the photo-oxidation of cytochrome *f* in photosynthesis.

Abbreviations: ABC – ATP-binding cassette; ATP – adenosine triphosphate; Cyt – cytochrome

Life and work

Henrik Lundegårdh was born in 1888 to a rich and well-established Stockholm family. He was a gifted student who played the violin and had a lifetime interest in the visual arts. His early studies were in Stockholm and he obtained his first degree (1907) and Phil Dr (1912) from Stockholm University, Sweden. In these earliest studies he was inspired by Otto Rosenberg and took up the microscopic investigation of plants and cells, resulting in the publication of some 12 papers on cell division. However, as early as 1910, he turned his attention to plant physiology and the permeability of roots to various salts. This led to two periods abroad, in the laboratories of Georg Klebs in Heidelberg (Germany) and Wilhelm Pfeffer in Leipzig (Germany) in 1912–1913. In 1915 he moved to the University of Lund, Sweden, where he stayed until 1926. There he set up (financed to a great extent from personal funds) an experimental station, for ecology and physiology, on the island of Hallands Väderö in the Kattegat (between Sweden and Denmark). This work was characterized by the application of quantitative techniques to measure the exchange between plants and their environment. Here Lundegårdh's genius for inventing instruments, where no adequate instruments existed, first came to the fore. This work led to several books, the most famous of which was 'Klima und Boden in Ihrer Wirkung auf dass Pflanzenleben' ['Climate and Soil and Their Effect on Plant Life'], (first German edition, 1925, fifth edition, 1957; English edition, 1930 (translated by Eric Ashby); Russian edition, 1940; Japanese edition, 1968). In addition, 'Der Kreislauf der Kohlensäure in der Natur' ['The Carbonic Acid Cycle in Nature'] was published in 1924 and 'Die quantitiative Spektralanalyse der Elemente' ['The Spectral Analysis of Elements'] in 1929.

In 1926 Lundegårdh was appointed Professor and Head of the Botany Division of the Central Institution for Agricultural Research at Experimemntalfältet, near Stockholm. There he carried out a comprehensive

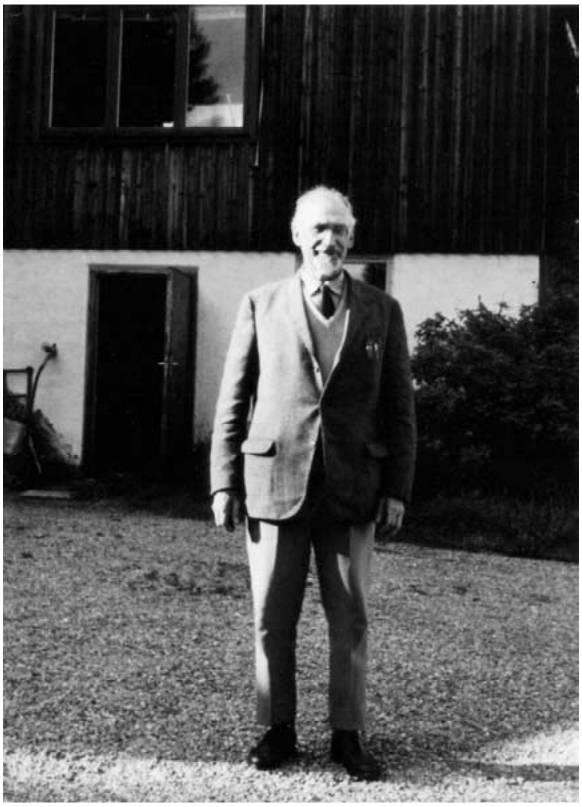

Figure 1. Henrik Lundegårdh in front of his private laboratory at Penningby in 1962. Photograph by the author (A.W.D. Larkum).

program on the absorption of salts by plant roots, specializing particularly in wheat roots. This work was carried on when he moved to the Lantbrukhögskolan, just south of Uppsala, as professor of plant physiology in 1935, where he stayed until he retired in 1955. It was during these two periods that he developed the flame photometer, which is described, inter alia, in his book 'Die Blattan Analysis' first published in 1944 (English edition, 'Leaf Analysis,' 1951), and forerunners of modern autoanalyzers. From 1933 onwards, he worked with Hans Burström, and others, on the relationship of respiration and salt uptake in roots, work which strongly influenced the fields of membrane transport, respiration and energy metabolism and was cited by Peter Mitchell in the development of the chemiosmotic hypothesis (Mitchell 1978).

In 1947, Lundegårdh built a private laboratory at Penningby, 70 km from Stockholm and Uppsala, on the Baltic Sea, near Norrtällje and in the beautiful Stockholm Skärgård (see Figure 1). There he built a number of spectrophotometers and after his retirement, in 1955, he made many discoveries on the role of cytochromes in plant roots and in photosynthesis.

From a very early stage Lundegårdh had had an interest in cytochromes. During the 1930s he entered into a prolonged correspondence with David Keilin in Cambridge (UK), which centred on the operation of cytochromes, which Keilin had discovered. This correspondence is partially contained in the Keilin papers at the University Library, Cambridge, UK (http://www.bath.ac.uk/ncuacs/cambio.htm). In 1943 Lundegårdh was elected to the Royal Swedish Academy of Science. He subsequently led the proposal to get David Keilin awarded the Nobel Prize in Physiology and Medicine. In 1955 both David Keilin and Axel Hugo Theorell were proposed for the award. Since Theorell was Swedish this led to a difficult decision on the part of the Royal Swedish Academy of Science, which eventually made the award to Theorell alone. Thus ended the hopes of many admirers of David Keilin that he would get the ultimate award which it was felt he justly deserved, as the father of cytochromes; David Keilin died in 1963. It was therefore fitting that Peter Mitchell in his Nobel Award Lecture (1978) should entitle it 'David Keilin's Respiratory Chain Concept and its Chemiosmotic Consequences.' Lundegårdh told me, in Penningby in 1960, that he was so upset with the result in 1955 that he had nothing more to do with the Royal Swedish Academy of Science. This and the somewhat autocratic attitude that Lundegårdh took toward his colleagues may account for the lack of suitable appreciations of his life and work, upon his death in 1969. The other reason of course is that he outlived most of his peers.

Carbon dioxide exchange and global carbon accounting

In his quantitative ecological investigations of plants at the research station on Hallands Väderö from 1915 to 1926, Lundegårdh obtained quantitative data on the CO_2 exchange of a wide variety of plants and soils, from marsh to forest associations. F.F. Blackman had incorporated the law of limiting factors into plant growth, in which at any instant a single factor was seen to control the integrated response of a plant (see Rabinowitch 1951). In studying photosynthesis, Lundegårdh found that the rate of photosynthesis might be dependent on several factors at the same time, which he saw as a modification of the law (Lundegårdh 1924;

Rabinowitch 1951). Lundegårdh made many of the first observations of Rate of Photosynthesis (P) vs Light Intensity (I) curves for many natural plants (e.g., *Oxalis* and *Stellaria*). In particular he defined soil CO_2 flux as simply the sum of all soil metabolic functions in which CO_2 is involved, such as root respiration and decomposition, litter decomposition and soil respiration, etc. (Lundegårdh 1927). This work is still quoted in recent attempts to model CO_2 exchange and in global CO_2 accounting studies (see e.g., Peng et al. 1998). Lundegårdh devoted a whole book to the subject of the carbonic acid cycle, 'Der Kreislauf der Kohlensäure in der Natur' in 1924, and a chapter of his book 'Klima und Boden' (1925, 5th edition 1957) dealt with carbon dioxide exchange of vegetation and soils. It should also be noted that these books also contained some of the first quantitative measurements of the factors (light, temperature, CO_2 concentration and chlorophyll content) affecting photosynthesis in a wide range of plants.

Discovery of the flame photometer

As early as 1915 Lundegårdh developed automated instruments for the detection of environmental variables. In his laboratory studies at Lund he also developed cinematographic techniques for the study of tropic movements in plants and the action of auxins. These initiatives led on to attempts to solve what Lundegårdh regarded as one of the reasons for the stagnation of research on the uptake of salts by plants, the lack of sensitive and rapid chemical methods for quantitative determinations of salts, especially cations. He now turned his attention to the spectral emissions of cations in hot gases. In 1859 G. Kirchoff and R.W. Bunsen were the first to demonstrate that elements have both characteristic absorption and emission spectra and H. Kolrausch had developed a method for semi-quantitative assessment of cations in gas flames. In the early part of the 20th century, attempts had been made in France and England to stimulate the spectral emission of elements and salts under electrical discharges, but this did not prove effective as the accuracy was low. Lundegårdh now set about developing a sensitive and accurate flame spectrophotometer based on an acetylene-air flame and an aqueous atomiser, by which the cations were introduced into the flame (Lundegårdh, 1929, 1934). The analysis system was automated so that many samples could be processed automatically in a single day. Many refinements were made to this approach during 1930–1940. Attempts were also made to measure, simultaneously, K^+, Ca^{2+} and phosphorus in various arcs. However, the technique to measure all three together was never really successful (Lundegårdh 1951). Nevertheless, Lundegårdh came very close in these attempts to developing an effective atomic absorption spectrophotometer (later developed by Alan Walsh in 1955). The modern flame photometer was developed by Hilger and Watts Company (London) by the simplification of the technique to use specific filters rather than a spectrophotometer for individual cations. It was this company that published the English edition of 'Leaf Analysis' in 1951 (translated by R.L. Mitchell).

A forerunner to Peter Mitchell and the chemiosmotic hypothesis

While at Lund before 1926, Lundegårdh had already carried out many investigations of the uptake of anions and cations by roots aided by his development of the flame spectrophotometer. However, at the Central Institution for Agricultural Research and at the Lantbrukhögskolan he led a team effort which preoccupied him and his students for the next 30 years: the uptake of ions by plant roots, using wheat as a model plant. His aim here was to understand the uptake mechanisms of roots and to be able to detect and correct nutrient deficiency in plants. A key in this understanding was to understand the role of root respiration and to this end he investigated the relationship between respiration and salt uptake. His book in 1932 'Die Nährstoffaufnahme der Pflanze' ['Nutrient Uptake by Plants'], took into account for the first time the colloidal properties of plant cell walls and their cytoplasm. In the same year he was the senior author on the first of a series of papers entitled 'Untersuchungen über die Salzaufnahme der Pflanzen' ['Investigations of Salt Uptake in Plants'], co-authored with Hans Burström and others (e.g., Lundegårdh et al. 1932a, b; Lundegårdh and Burström 1933).

Lundegårdh and coworkers discovered that there was a component of active uptake of salts, irrespective of any passive processes. This active process was induced in the presence of salts. Salts stimulated high rates of salt uptake and the active component was found to stimulate respiration and was sensitive to cyanide and carbon monoxide. There followed a period of intense investigations to find the causal relationship between the active salt uptake and the cyanide sensitive respiration (for a discussion of these results and the results of others, see Briggs et al. 1961).

As part of the work on salt respiration, the respiratory enzymes were investigated in detail. It was shown that cytochrome oxidase (Cyt a + Cyt a_3), cytochrome b and succinic dehydrogenase were present in plants and, in roots, they participated in the cyanide-sensitive salt uptake process. This was at a time when the role of mitochondria in respiration and energy production was unknown. Furthermore, the role of the tonoplast membrane around the large vacuoles of plant cells was unknown and it was widely assumed that the major process of salt accumulation lay at the plasmalemma, the outer cell membrane. It is not surprising therefore that Lundegårdh concentrated on mechanisms at the plasma membrane.

Lundegårdh proposed that the uptake of ions was driven by the vectorial flow of hydrogen ions across the plasmalemma (Lundegårdh 1939, 1942, 1946, 1954, 1960): the hydrogen ions were liberated by respiratory redox reactions which split H atoms from the substrate into H^+s and electrons, and the H^+s acted electrostatically to drive the uptake of Cl^- and other anions by plant root surface cells. Lundegårdh proposed that cations were carried passively (Lundegårdh 1937, 1946, 1954). Some of these insights are remarkably similar to those used by Peter Michell in his chemiosmotic hypothesis. However it was proposed for the wrong membrane and did not include a mechanism of ATP formation. With hindsight it is not difficult to see that Lundegårdh and subsequent workers in the field (e.g., Robertson and Wilkins 1948) could not easily have reached the solution to the problem *at that time*: now it is more easy to see that salt-stimulated respiration was due to the induction of ATP binding cassette (ABC) ion transporters in the tonoplast membrane (as well as in the plasmalemma) which were fed by ATP from mitochondria; and that cyanide inhibited electron transport in the mitochondria and hence the supply of ATP to the cytoplasm of root cells. It was not until the central role of mitochondria in ATP supply was established in the 1950s and 1960s (see, e.g., Briggs et al. 1961) that the situation was ripe for an explanatory hypothesis of proton-linked vectorial energy generation across the inner membrane of the mitochondrion (and the thylakoid membrane in the case of photosynthesis) (Mitchell 1961).

It is ironic that Lundegårdh was too preoccupied with the cell membranes of roots and with photosynthesis in the later period of his life to factor in the developments in mitochondria, which would have provided him with the answer to salt respiration and perhaps to the chemiosmotic hypothesis. This is all the more poignant because in the case of chloroplasts he did consider the possibility that a process similar to that which he proposed for roots took place in the membranes of chloroplasts (Lundegårdh 1954b, 1961). Peter Mitchell recognized Lundegårdh as a forerunner in the field of chemiosmosis, in his Nobel speech (see Mitchell 1978): however, although both scientists contributed articles to the Society of Experimental Biology (SEB) (of UK) Symposium volume VIII in 1954, which came out in succeeding chapters, and Lundegårdh's was perhaps the most clear statement of his views, it was unfortunately not quoted by Mitchell in his Nobel address. However, Mitchell later realized this shortcoming and in his third grey book (Mitchell 1991) gave much greater historical backing to the place of Lundegårdh as a forerunner of the chemiosmotic hypothesis.

Major discoveries in the field of photosynthetic cytochromes

Lundegårdh's interest in the role of cytochromes became his overriding interest in retirement at Penningby. Here, he developed to the full his genius for inventing instruments, in this case largely spectrophotometers. His aim was to investigate both the cytochromes of roots (James and Lundegårdh 1959) and of leaves and chloroplasts (Lundegårdh 1954). Interestingly, he did not have the capabilities to isolate mitochondria and therefore did not make progress in this direction. However he could easily isolate chloroplasts and this led him to make some of the most sophisticated investigations on cytochromes of chloroplasts in the 1950s and 1960s. This was possible due to his development and perfection of a range of very sensitive spectrophotometers. One was a very sensitive single-beam instrument with a wavelength control designed by himself (Figure 2) (Lundegårdh 1954) using a large Bausch and Lomb monochromator. Another was a rapid recording single-beam scanning instrument that could scan at rates of less than 1 s (Lundegårdh 1961, 1964). Previous work by Robin Hill and colleagues had already shown that chloroplasts possessed Cyt f (a c-type cytochrome) and one or more b-type cytochromes (see, e.g., Hill and Scarisbrick 1951).

As early as 1954, Lundegårdh found that Cyt f was photo-oxidized in *Chlorella*, in wheat

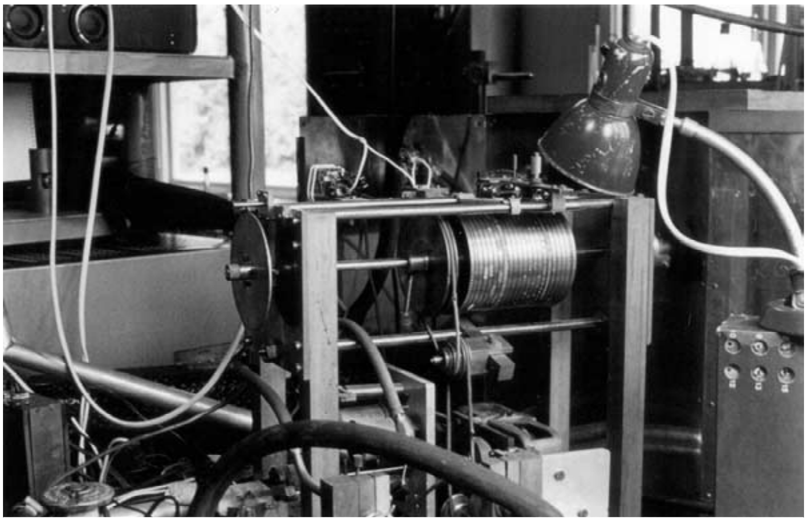

Figure 2. A partial view of the wavelength drive and other parts of the very sensitive single beam recording spectrophotometer that Henrik Lundegårdh built in the 1950s. Photograph by the author (A.W.D. Larkum).

leaves and in homogenates of leaves (Lundegårdh 1954b). In *Chlorella*, the peak of the change was at 556 nm as opposed to that found by H.E. Davenport and R. Hill (1952) of 555 nm, and in the leaves and homogenates it was at 556–557 nm. Nevertheless Lundegårdh was confident that it was Cyt *f*. Later in the same year L.N.M. Duysens (1954) published a similar finding (and cited Lundegårdh's paper) for photo-oxidation of Cyt *f* in the red alga *Porphyridium cruentum*, using a more sensitive system and found a peak at 555 nm. Despite the fact that Rabinowitch (1956; see pp. 1859–1862) discussed these findings in detail their importance was not recognized by others in the field. It was also not followed up by Lundegårdh himself until much later. In 1961 he published a paper in Nature (Lundegårdh 1961) that dealt in detail with similar observations in chloroplasts. However, this was totally overshadowed by the publication in the previous year of the 'Z' scheme of photosynthesis (Hill and Bendall 1960) in which they proposed that cytochromes *f* and b_6 lay between the two photosystems, without citing the earlier crucial Lundegårdh/Duysens evidence. In the hectic rush to test the 'Z' scheme there were many proposals of light-induced oxidation of Cyt *f* and Lundegårdh's and Duysens' contributions were overlooked (as in some articles in Kok and Jagendorf 1963).

Undeterred, Lundegårdh carried on publishing articles on the role of cytochromes, especially the *b*-type cytochromes, and carotenoids to near the time of his death, aged 81, in 1969 (Lundegårdh 1964, 1966, 1969). In all, Lundegårdh had 18 papers published in the journal *Nature* over his lifetime, the last of which, fittingly, was published in his last year (Lundegårdh 1969).

Concluding remarks

This article has concentrated on the photosynthetic work of Henrik Lundegårdh. In this alone his discoveries were very influential. In the field of energy metabolism, too, he had a great influence, especially as a forerunner to Peter Mitchell in generating hypotheses of energy coupling across membranes. However this is by no means the whole story. Henrik Lundegårdh influenced several generations of workers in the fields of plant ecology and physiology. His genius for development of instruments also lives on in the flame photometer, which is still used in laboratories around the world. His achievements must place him as one of the foremost plant biologists of the early twentieth century.

Acknowledgments

I thank Anne Wiktorsson (Archivist, Royal Swedish Academy of Science) for supplying biographical material and Malcolm Ricketts for help with the photographs. I also wish to state the gratitude that I have for Henrik Lundegårdh, who took on a raw gradu-

Figure 3. Tony Larkum in his laboratory in Sydney in 2002 preparing spinach chloroplasts for measurements of thylakoid pH using NMR spectrometry. Photograph by Min Chen.

ate student as an assistant for one year in 1961–1962 (Lundegårdh and Larkum 1965). This experience had the greatest influence on my subsequent endeavours and approach to science.

I was greatly aided in biographical details by an unauthored ms supplied by Anne Wiktorsson and a five-page article in Svensk Biografiskt Lexicon (Stockholm), Vol 24 (1982–1984), pp. 256–260, (editor: B. Lager Kromnow). This article lists all of Henrik Lundegårdh's publications, but without titles or page numbers.

This paper was invited and edited by Govindjee. At his request, a recent photograph of the author is included in Figure 3.

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