Book Reviews

History of Cell Respiration and Cytochrome

One attribute of a living thing is that it must have a source of energy to maintain itself and to make more of itself. The source of this energy is cellular respiration, a process in which foodstuffs are broken down to short-chain molecules that are burned in a very special, well-controlled, and orderly fashion. Cell respiration has often been compared to a burning candle. In the burning candle, long-chain paraffin molecules are first broken down to short-chain molecules and these are then burned. In this burning, oxygen is used and carbon dioxide and water are formed. But in the burning candle all the energy released appears as heat and light, whereas in the cells a major part is stored in packets of energy which take the form of molecules called ATP.

Only within the last 50 years have we begun to learn the details of this intricate burning process which goes on in the cells of all living things. The process begins with the release of electrons from certain small organic molecules derived from foodstuffs. This release is caused by special enzymes, the dehydrogenases. Then, these electrons migrate down a "potential hill" through a series of reddish proteins, the cytochromes, toward a special cytochrome, cytochrome oxidase, which activates oxygen to accept and combine with these electrons to form water. Thus, the oxygen comes into the burning process only at the end of a long line of enzymic reactions. The carbon dioxide, however, is released by the early enzymic steps; indeed the oxygens in the carbon dioxide are not derived from oxygen but from the original foodstuffs and water. In this electron flow through the cytochromes, toward oxygen, energy is set free which is converted into ATP units, the universal energy units of all protoplasm. These ATP units serve as energy sources of the cell to form new molecules, to tie small molecules together, to transport special molecules across semipermeable membranes, to cause contraction of muscle and to transmit impulses along nerves.

How most of the concepts of this remarkable burning process, called cellular respiration, came to be discovered is told in this book, **The History of Cell Respiration and Cytochrome** (Cambridge University Press, New York, 1966. 436 pp., $17.50), by the late Professor David Keilin of Cambridge University. The book is one of the major monographs of biochemistry of the 20th century, and it ranks in importance with Warburg's 1949 monograph *Heavy Metal Prosthetic Groups and Enzyme Action*. Keilin's book has been edited and brought up-to-date by the skillful and loving hands of his daughter, Dr. Joan Keilin. Biochemistry is greatly indebted to her for the successful and knowledgeable accomplishment of this task.

The first chapters of Keilin's book present a historical survey of the subject of cellular respiration up to the 20th century. These chapters should prove to be profitable reading for general biology and chemistry students. The main part of the book is a review of Keilin's own outstanding work on the discovery and properties of the cytochromes, and also includes a summary of more recent studies on cytochromes made by various investigators. It represents a critical compilation of the properties of the cytochromes and will be most appreciated by the specialist of cellular respiration and by advanced students of biochemistry. The book will be of special value to students who seek to do research in this field because it is written by a great experimentalist who makes clear what is known and what further problems there are for investigation.

In the first five chapters Keilin authoritatively reviews the history of circulation, of respiration, and of the concepts that gradually led to the theories of cellular oxidations and reductions. One is thrilled by the ingenuity of the experiments and the flashes of insight of the great investigators. Even during the second century A.D., Galen must have compared the burning of oil with the respiration of mammals, whereby air is used, heat is generated, and an injurious "smoke of combustion" is given off. However, it was some 1600 years later, when chemistry had developed into a systematic science in the hands of Lavoisier, that further advances were made. Then respiration could be considered as a burning in which oxygen was used and carbon dioxide was formed. Advances in biology were also taking place. In 1668 Leeuwenhoek's remarkable observations on the circulation in the tail of the tadpole demonstrated the capillary circulation which Harvey had postulated 25 years before. Here was an early example which showed biologists that a difficult problem could be solved by a suitable choice of biological material.

In considering the contributions of Justus von Liebig, Keilin credits him with the suggestion, made in 1843, that oxygen formed a loose compound with the iron of the blood. This brilliant guess was, however, overbalanced by a seriously wrong guess—that yeast was not a living organism but an organic sediment produced by enzymes of fermentation. Because of Liebig's great authority as a chemist, Keilin believes that this wrong guess "retarded by at least a quarter of a century, all further progress in the study of fermentation." The moral is that authoritarianism has no place in science. Its use has lessened men, starved a state, and even humbled a Pope at Pisa. Yet the lesson is never fully learned.

The connection between cellular respiration and oxidation-reduction reactions had to await the development of the concept of enzymes. Observations on reversible redox dyes played a more important role than one may gather from Keilin's résumé. In 1884 Ehrlich observed that dyes such as methylene blue could be reversibly reduced to a colorless form by tissues and then reoxidized by air to the colored form. Some dyes were reduced more readily than others. Then followed Thunberg's experiments (1930) on methylene blue reduction with dehydrogenases, the development of the concepts of redox potentials, and (not mentioned by Keilin) the discovery by Leonor Michaelis of organic free radicals which led to the principle of the transfer of single electrons via flavins to the cytochromes and finally to oxygen.

MacMunn's discoveries (1884) of the absorption bands of the "histohematins" are reviewed in chapter 6. Keilin believes that MacMunn's work was neglected by his contemporaries because of the lack of evidence that the absorption spectrum changes could be correlated with reversible redox reactions in living tissues and the possibility that these bands might be related to myoglobin and its decomposition products.

In a most exciting chapter Keilin tells how, during a relatively few years, around 1925, he came to rediscover the "histohematins" while seeking to find out how the bot fly decomposed ingested hemoglobin. To avoid problems of contamination with hemoglobin or myoglobin, Keilin chose the adult wax moth as biological material for further study. Through the wing muscle of this insect he observed the appearance of a four-banded absorption spectrum when the wings were in active motion and the disappearance of the bands when the wings were at rest. He also found the same absorption bands in suspensions of a bacillus and of yeast when oxygen was used up, and the disappearance of the bands in the presence of air. Then, with the use of the specific poisons, urethane and cyanide, with differential heating of the cells, and with extraction methods, he was able to demonstrate that the four-banded spectrum actually represented three distinct heme proteins within the cells which he designated as cytochromes *a*, *b*, and *c*. His intimate knowledge of the current literature led him to a connection between the dehydrogenases, the cytochromes, and oxygen.

It is worthwhile asking how Keilin came to make all of these fundamental findings in the amazingly brief time that he did. I suggest three reasons: (i) Keilin had an astute, analytical mind; (ii) he was a biologist with a broad base of knowledge of biological material; and (iii) he was an excellent experimenter in biology. Biological phenomena are highly complex. In investigating such phenomena it is important to use simple rapid methods of analysis so that one can make numerous observations with a large number of samples under varying conditions. To make numerous rapid observations of the cytochrome bands Keilin used a small dispersion spectroscope mounted on a microscope. With this device he could examine a clear solution, or an opalescent suspension, or a thick tissue slice within a few minutes. His knowledge of biological material permitted him to survey the cytochromes from the bacteria all the way up to mammalian tissues. Thus, he was able to show that the cytochromes are components of most cells.

In succeeding chapters the experiments that further revealed the properties of the cytochromes are described. At liquid air temperatures, the absorption bands of the cytochromes sharpen and shift in wavelength. In 1955 Keilin and Hartree, using this property, were able to establish the presence of a new cytochrome c_1, which at room temperature overlapped the bands of cytochrome *c*. The studies on cytochrome *a* showed that it was an insoluble complex consisting of two heme protein components *a* and a_3 as well as a copper-containing component. Whether oxygen is activated by cytochrome a_3 or by the copper is still not known.

The last part of chapter 9 is a polemic that I mention merely because it may aid in the sale of this important book. This section contains a criticism of Warburg's criticisms of Keilin's interpretations during the years 1924 to 1932. It is evident that Warburg failed to recognize the significance of Keilin's discoveries on the cytochromes. Humans, even those with a scientific background, will delight in this tiff between two intellectual giants, although, physically, both men were of small stature. Perhaps the lesson we may gain here is that a scientist must think arrogantly, but write with humility. But how inhuman is this combination of qualities!

The great achievements of Keilin and his co-workers at Cambridge have revealed much about cellular respiration. However, a problem early perceived, the interaction of the cytochromes, is far from solution. Actually, this problem evolved into three major problems of present-day biochemistry: the spatial interaction of component cytochromes organized in juxtaposition on a lipoprotein membrane; the coupling of electron transport with the formation of high energy phosphate bonds; and the activation of oxygen to accept four electrons, one at a time, from cytochrome *c*. Here are problems to challenge even new young Keilins and Warburgs.

Biochemistry is changing so rapidly that the useful lifetime of books in this field is only several years. David Keilin's monograph will become a classic of biochemistry and will be read by many generations of students. It is well printed and bound. It costs $17.50 and is well worth the price.

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Oceanography

Marine Geochemistry (Narragansett Marine Laboratory, University of Rhode Island, Kingston, 1965. 380 pp.), edited by David R. Schink and James T. Corless, is a paperback collection of papers presented in a symposium at the marine laboratory of the University of Rhode Island in 1964, dealing primarily with trace elements and isotopes in marine geochemistry. Most of the papers are reports of research in progress rather than completed investigations. The following notes are a sampling of the topics covered, but by no means a complete summary.

Variations in deuterium and oxygen 18 are the subject of a long paper by H. Craig and L. I. Gordon and of a shorter one by A. C. Redfield and I. Friedman. In both studies it is demonstrated that isotope concentrations have characteristic values in the major deep water masses, and hence can serve as tracers for water movements. Craig and Gordon discuss in detail the equilibrium and kinetic factors involved in the partial separation of isotopes during movements of water across interfaces between sea and air and between sea and ice.

F. T. Manheim, in a long article with an 11-page bibliography, describes manganese-iron accumulations in shallow marine environments and relates these to the better known manganese-iron nodules from the deep sea. He concludes that both major and minor metals in the nodules must come largely from the continents, and that the weathering of submarine volcanic products can be important as a source of metal only locally.

Preliminary results of a study of iodine, bromine, and chlorine in the air-sea-sediment system, using neutron activation as a method of analysis, are