THE PATHWAY FOR OXYGEN

Structure and Function in the Mammalian Respiratory System

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Harvard University Press
Cambridge, Massachusetts, and London, England
1984

Printed in the United States of America
10 9 8 7 6 5 4 3 2 1

This book is printed on acid-free paper, and its binding materials have been chosen for strength and durability.

LIBRARY OF CONGRESS CATALOGING IN PUBLICATION DATA

Weibel, Ewald R.
The pathway for oxygen.

Bibliography: p.
Includes index.
1. Respiration. 2. Oxygen in the body. I. Title.
[DNLM: 1. Respiration. 2. Respiratory system—Anatomy and histology. 3. Respiratory system—Physiology.
WF 102 W415p]
QP121.W395 1984 612'.2 83-18622
ISBN0-674-65791-8 (cloth)
0-674-65790-X (paper)

FOREWORD

THIS BOOK is based on a series of lectures delivered by Professor Ewald Weibel in the fall term of 1979, when he was a visiting Alexander Agassiz Professor to the Museum of Comparative Zoology of Harvard University. In those sessions, Professor Weibel provided the basis for a new synthesis between the traditional fields of anatomy and physiology by applying stereological methods to quantify structures and their spatial relationships. He used a comparative approach to examine the quantitative match between structures and their function in the transfer of oxygen from the environmental air to the respiratory chain enzymes in the mitochondria. He tested the idea that there is rationality and symmetry underlying the design of structures. The lectures generated lively discussion, and the audience grew from week to week as the practitioners of the traditional fields challenged assumptions and questioned conclusions. The book stimulates the same intellectual excitement that characterized the lectures—the excitement involved in breaking new ground and formulating a new level of understanding of the relationships between structures and their function.

We hope that this publication will be the first in a series of Agassiz Lectures which preview the forefronts of comparative zoology and provide us with new syntheses. It is fitting that the first book in this Agassiz series is written by a current leader of Swiss science. When Louis Agassiz moved from Switzerland to the United States and founded the Museum of Comparative Zoology at Harvard, he set in motion research in all aspects of zoology and natural history in North

America. To Louis Agassiz, natural history museums were centers for multifaceted studies on the biology of organisms and for the training of the next generation of biologists. This was the stamp he placed upon the MCZ as he nursed it through its infancy. The endowment supplied by the Agassiz family, and in particular Louis Agassiz's son Alexander, made Louis Agassiz's early policies and administrative decisions a tradition of the MCZ. The establishment of the Alexander Agassiz professorships played an important role in the realization of this tradition. This book reflects the tradition of the MCZ's founder, and once again zoology is indebted to the land of Louis Agassiz's birth.

A. W. CROMPTON
C. RICHARD TAYLOR

PREFACE

UNQUESTIONABLY, most of the great advances in the understanding of living organisms have come about through the thorough and imaginative work of investigators who acquired special skills with which to solve a problem at hand: they were either morphologists, physiologists, biochemists, or molecular biologists. The field of respiration has been no different. In this book I have tried to pull together the results of these many approaches to the problems of respiration. My aim has been to explore the mechanisms by which an efficient pathway for oxygen—from the lung through the blood, the tissues, the cells to the molecular respiratory chain in the mitochondria—is established and maintained in man and animals, and how these different mechanisms are dependent on each other. I have had to consider questions of design as well as regulation, of structure as well as function, at the macroscopic as well as the cellular and molecular levels of organization. My bias as a morphologist will be clear throughout this book, but I have made a special effort to give physiology and biochemistry their due weight. The meaning of design in a functional system cannot be understood without a full consideration of dynamic regulatory mechanisms, including all the ancillary functions performed by a multitude of cells that maintain a healthy lung or an adequate distribution of blood flow to the organs of need, to mention only two problems.

The topic is vast and almost unlimited; it was impossible to deal with all aspects in the appropriate depth. Each chapter has therefore been supplemented with a bibliography where the reader can find guidance on how to deepen his understanding of some topic by further reading of review articles and textbook syntheses. But to understand the process of science — which often proceeds in leaps — one must also look at the sources of scientific information: original papers, often highly specialized and focused on a very particular question. Such references are usually large in number; for each chapter I have compiled only a small, rather personal list, from very recent publications to "classics" (marked by an asterisk) which have initiated a major step in our understanding of the system. At the end of the book there is a list of general references which make particularly worthwhile additional reading.

How This Book Came About

In the fall of 1979 I was invited by Harvard University to deliver a series of Agassiz Lectures, "Structure and Function in the Respiratory System." These lectures formed the core of this book, but they have been expanded to include background material and to be more explicit where, in the lecture, I had relied on the audience's intuition.

The lectures were built on a body of experience acquired in two decades of scientific activity. After solid training as a morphologist with Gian Töndury in Zürich and Averill A. Liebow at Yale University, I had the unusual privilege of working in two outstanding centers of respiratory physiology and cell biology: first with André F. Cournand, Dickinson W. Richards, and Domingo Gomez at Columbia University, and then with George E. Palade at Rockefeller University. My experience with these great teachers has deeply affected my thinking. I could not have taken the approach chosen for this book — and for my research — had I not been instilled, in this early period, with the desire to understand the role of structural organization in terms of the functions served, to look beyond my own trade of morphology to physiology and cell biology. I have become convinced not only that structure determines function, but that functional demand also determines structural design, be it through evolution or by modulation of design features.

To shed some light on this interdependence of structure and function has been my constant endeavor. This would not have been possible without the contributions of a large group of superb col-

leagues and collaborators, particularly Hans and Marianne Bachofen, Robert Bolender, Peter Burri, Luis Cruz-Orive, Peter Gehr, Joan Gil, Hans Hoppeler, Odile Mathieu, and Klaus Schwerzmann. For our group a decisive period began some years ago when a collaboration was established with C. Richard Taylor from the Museum of Comparative Zoology at Harvard University. Our scientific interests and special skills are mutually complementary, with sufficient overlap to stimulate close cooperation. This has been a most fruitful association. It has also allowed me to write a good part of this book in the stimulating Harvard environment while spending a sabbatical leave at the Museum of Comparative Zoology. I am grateful to C. Richard Taylor and Alfred W. Crompton for their hospitality.

The burden of writing a book like this must be shared. I am most grateful to my secretary of many years, Gertrud Reber, for her superb and devoted assistance, and also to Karl Babl, who did most of the graphic art for this book, diligently assisted by Marianne Schweizer. Some of the artwork was contributed by Alexandra Sänger and Reinhold Schneider.

Last, but not least, I should gratefully acknowledge the generous and continued support which our research has received, particularly from the University of Berne and the Swiss National Science Foundation.

E. R. W.

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OXYGEN AND THE HISTORY OF LIFE

IF A CANDLE BURNS in a closed container, its flame will soon be extinguished because the O_2 required to burn the wax has been consumed. Living creatures kept in a closed box will die for much the same reason: a continuous flow of O_2 is required for maintaining the "fire of life," a most appropriate term coined by Max Kleiber and used as title for his fundamental treatise on the metabolism of animals, published in 1961.

Life is a complex phenomenon that depends on a high level of activity and on a high degree of order among the many functional units that make up an organism. No such order and no such activity can be maintained without the continuous input of energy, and for most "animals"—protozoa and metazoa alike—this energy is produced by combustion of organic compounds such as sugars, a process which requires O_2.

According to ancient, even medieval, views and beliefs, the heart was considered to be a furnace where the "fire of life" kept the blood boiling. Contemporary cell biology holds that each cell maintains a set of furnaces, the mitochondria—organelles specialized toward supplying the cell with energy by combustion, that is, by oxidation of organic substrates. As we shall see in detail later, the mitochondrion is equipped with a set of enzymes that can break up sugars into CO_2, extracting hydrogen ions in the process which are, in turn, burned with O_2 to make water (Fig. 1.1). For every four hydrogen ions one molecule of O_2 is consumed, and this liberates a lot of energy which can be utilized to drive the vital functions of the cell. This funda-

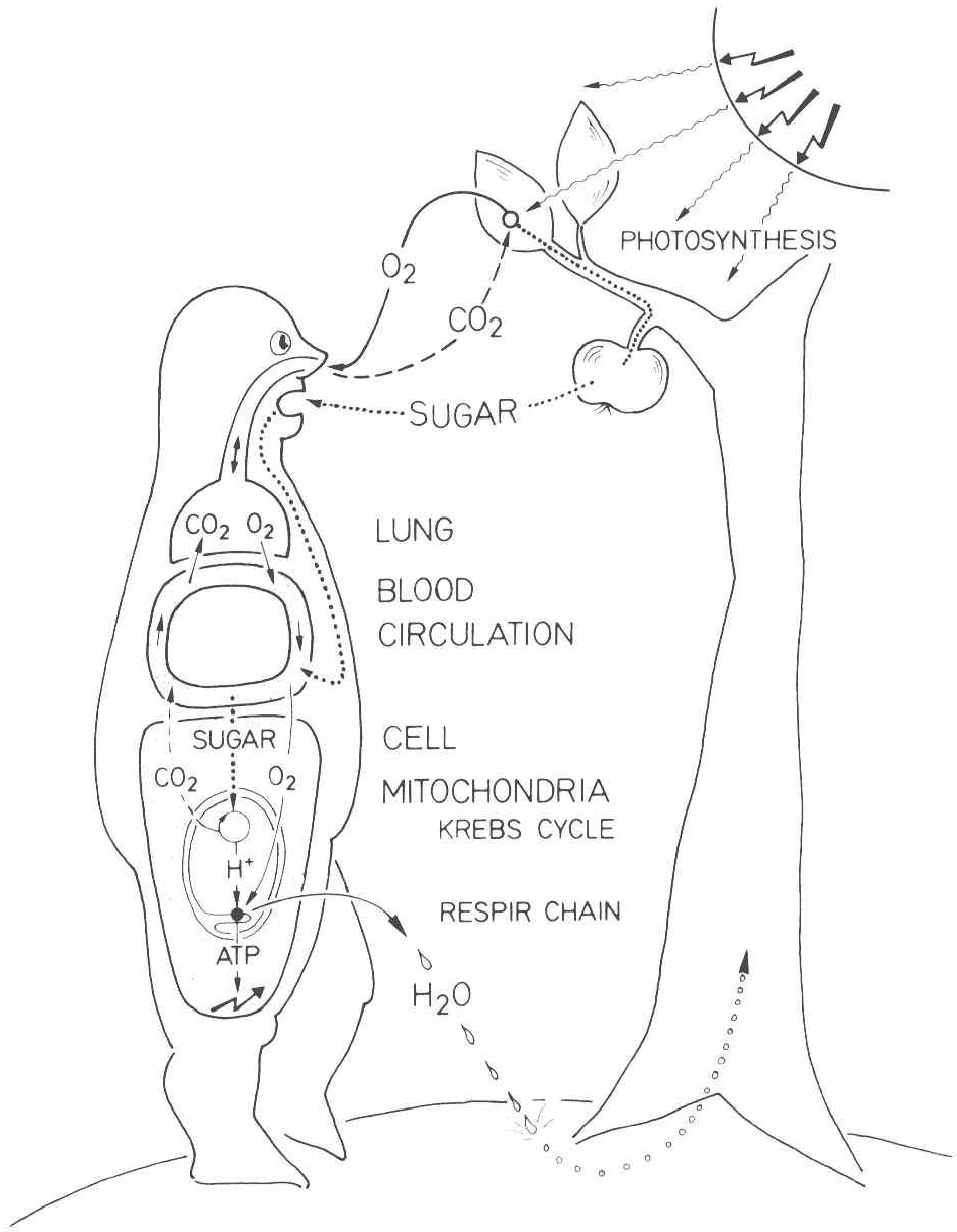

Fig. 1.1 The oxygen cycle: the energetic needs of our body are paid by solar energy.

mental process of energy production by oxidation depends totally on an adequate and continuous supply of O_2. The cells do have other means for covering their energetic needs in the absence of O_2, but they are rather inefficient and, in the long run, they all eventually need to be supported by oxidative metabolism. Thus, O_2 plays a key role in cellular energetics.

Why are O_2 and carbohydrates such convenient sources for covering the energetic needs of cells and living creatures? Why aren't these sources of energy soon exhausted? Indeed, one can easily calculate that the population of a larger city will consume most of the O_2 contained in the air column above the city's grounds in just about one life span of a human being. This does not happen, however, because the O_2 in the atmosphere is continuously replenished by the plants that surround us and, most importantly, by algae in the oceans. Drawing on part of the sun's energy radiation, plants use the CO_2 and H_2O produced by combustion in animal cells as substrates for making carbohydrates through photosynthesis (Fig. 1.1); by the same token they generate molecular O_2 which is released to the air, thus quasi reversing the process. Thus, in a broad sense, animals and plants form a large symbiotic system with O_2 and CO_2 as the two gaseous components that are exchanged in a continuous cycle through the air, a cycle that is driven by solar energy (Fig. 1.1). The energetic costs of life on earth are hence borne by the sun. Remember that all nonnuclear fuels that we burn to keep our houses warm or to run our machines and automobiles—wood, coal, and fossil oils—derive from this same process and are stored solar energy; if they are consumed at a rate greater than that by which they are regenerated, these energy sources will soon be exhausted.

The Cell's Oxygen Sink and Energetics

It is believed that the first elements of life developed when the earth's atmosphere did not yet contain any molecular O_2 to speak of. Indeed, anaerobic conditions were important for the formation of nucleotides by the "first spark"—a process that is now considered to be the origin of life since it led to the development of DNA and RNA, the carriers of genetic information. Under these conditions energy was presumably generated by anaerobic "oxidation," that is, by simple transfer of electrons to some suitable electron acceptor.

Let me explain. It turns out that the essential part of "combustion" or "oxidation" is the liberation of energy as electrons are transferred from an "electron donor" to an "electron acceptor" of higher electron affinity, a point we shall discuss in detail in chapter 4. All this can happen without O_2. But when, in the course of time, O_2 did become available in the earth's atmosphere, it became the ideal *terminal* electron acceptor because of its very high electron affinity, and "combustion" could now be carried to the end, that is, to true "oxidation," liberating as much energy as the substrates can give off and ending up in the formation of water (Fig. 1.1).

Now, the energy liberated in this process cannot be utilized directly, mostly because our system is not equipped to exploit the energy contained in heat; rather, it is necessary to "capture" this energy in a high-energy bond between phosphate groups that are attached to a nucleotide, mostly in the form of *adenosine triphosphate*, ATP. This energy can then be donated to most energy-requiring processes of cells, such as the contraction of muscle proteins or the metabolic activity of enzymes.

One of the fundamental steps in the evolution of cells, therefore, was the development of a complex set of enzymes or catalysts that could transfer the energy liberated by the various steps of oxidation to ATP in an efficient and well-controlled manner, a process called *oxidative phosphorylation*. In principle, it involves three steps (Fig. 1.1). Sugar which contains six carbon atoms is first split into two halves in a process called *glycolysis* (this step is different if other substances such as fats are burned). The fragments are then broken into CO_2 molecules, and this liberates hydrogen ions (H^+) which carry electrons along; this occurs in what is called the *Krebs cycle*. The third step takes place in the *respiratory chain* which ends in the reaction of H^+ with O_2 to make water. This last reaction liberates a lot of energy—remember the explosion that results if hydrogen and oxygen are reacted in the chemical laboratory—and this energy is used to make ATP. As we shall see, six ATP molecules can be generated for every O_2 molecule consumed.

These mechanisms of oxidative energy production are rather ancient in terms of evolution, as evidenced by the fact that all aerobic bacteria contain a set of enzymes by which they can generate, by oxidation of substrates, the energy-rich fuels like ATP that drive the cell's functions. These enzymes are not simply contained in the cell, but many of them are concentrated in a specialized region of the bacterial cell membrane.

In eukaryotes, the enzymes of oxidative phosphorylation, very similar to those found in bacteria, are housed in a specialized organelle, the *mitochondrion*, a small rod-shaped body bounded by two membranes and about the size of a bacterium. As in bacteria, one finds part of the enzyme system, namely the respiratory chain and part of the Krebs cycle enzymes, to be bound to the inner mitochondrial membrane, as we shall see later. Indeed the enzyme systems of bacteria and mitochondria are so similar that it has been speculated that mitochondria evolved from bacterial microorganisms which joined with protoplasmic elements in a kind of endosymbiosis where each part would contribute to and benefit from close cooperation: the protoplasmic elements would perform most synthetic functions, whereas the bacterial endosymbionts would spill off energy-rich compounds produced by oxidative metabolism. This notion is further supported by the fact that mitochondria contain their own genetic material in form of a circular strand of DNA, similar to bacterial DNA which is also circular. Furthermore, they contain their own ribosomes, again similar to those of bacteria, which can perform the synthesis of some mitochondrial proteins.

Mitochondria are fundamental constituents of all living cells beyond bacteria, from simple protozoa to all higher animals and man. When cells multiply by division, their mitochondrial complement is evenly divided between the two offspring. Cells are regarded as units of living matter not only because each contains the full set of genes, but also because each cell is self-sufficient in terms of liberating energy from substrates; when mitochondria are involved this depends on the availability of O_2.

Getting Oxygen to the Sink

During the evolution of the eukaryotic cell a first element of order or design was introduced into the respiratory system when the site of ATP generation was separated from the sites of ATP utilization: the enzyme system supporting oxidative phosphorylation became concentrated in the mitochondria, the cells' furnaces. Mitochondria act as "*O_2 sinks*" where molecular O_2 is removed as water, the end product of combustion, is produced (Fig. 1.1). But the "fire of life" can only be kept burning if there is a continuous inflow of O_2 from the cell's environment. In protozoa such as amoebae or paramecia, the cell's environment is the aqueous medium in which the cell lives and which either picks up its O_2 from the air or receives a continuous local supply from the photosynthetic activity of algae. In higher organisms, made of a large number of specialized cells each equipped with mitochondria, this environment is the intercellular fluid that is found in tissues. The problem is now how to ensure an adequate flow of O_2 from its store in environmental air to all the cells in the body.

The mechanisms by which a flow of O_2 into the tissues can be maintained are (1) *diffusion*, or molecular movement of O_2 through air or fluid, and (2) *convection*, or mass transport of O_2 by moving the medium in which O_2 is contained. Diffusion can be compared to people actively walking in the streets; convection to using a subway for getting a group of people from one point in town to another.

Diffusion of O_2 molecules is related to Brownian motion: in a gas, or in a fluid, molecules are in continuous motion which is random in a stationary state. If the gases are unevenly distributed, however, the molecular motion will show a preferential direction away from the higher concentration until homogeneity is achieved. Let us now see what happens in a situation such as that shown in Figure 1.2: two compartments A and B are separated by another compartment Y. Assume that the O_2 concentration in A is larger than that in B, and that O_2 can easily diffuse into and through Y; molecular motion will then drive O_2 through Y from the side with higher concentration in A to that of lower concentration. The driving force for this O_2 flow across compartment Y is the difference in O_2 partial pressures, P_{O_2} in compartments A and B, also called the P_{O_2} gradient across the barrier Y. In this case one finds that the O_2 flow rate by diffusion across Y, expressed in moles of O_2 per minute, is

$$\dot{M}_{O_2}(Y) = G(Y) \cdot [P_{O_2}(A) - P_{O_2}(B)]. \tag{1.1}$$

This flow rate achieves a constant value if the P_{O_2} in A and in B is kept constant, that is, when the inflow of O_2 into A and the outflow out of B are equal to the flow across Y. Such a situation is called a "steady state."

The coefficient G(Y) is the conductance of compartment Y and depends on a number of its properties. First it depends on the

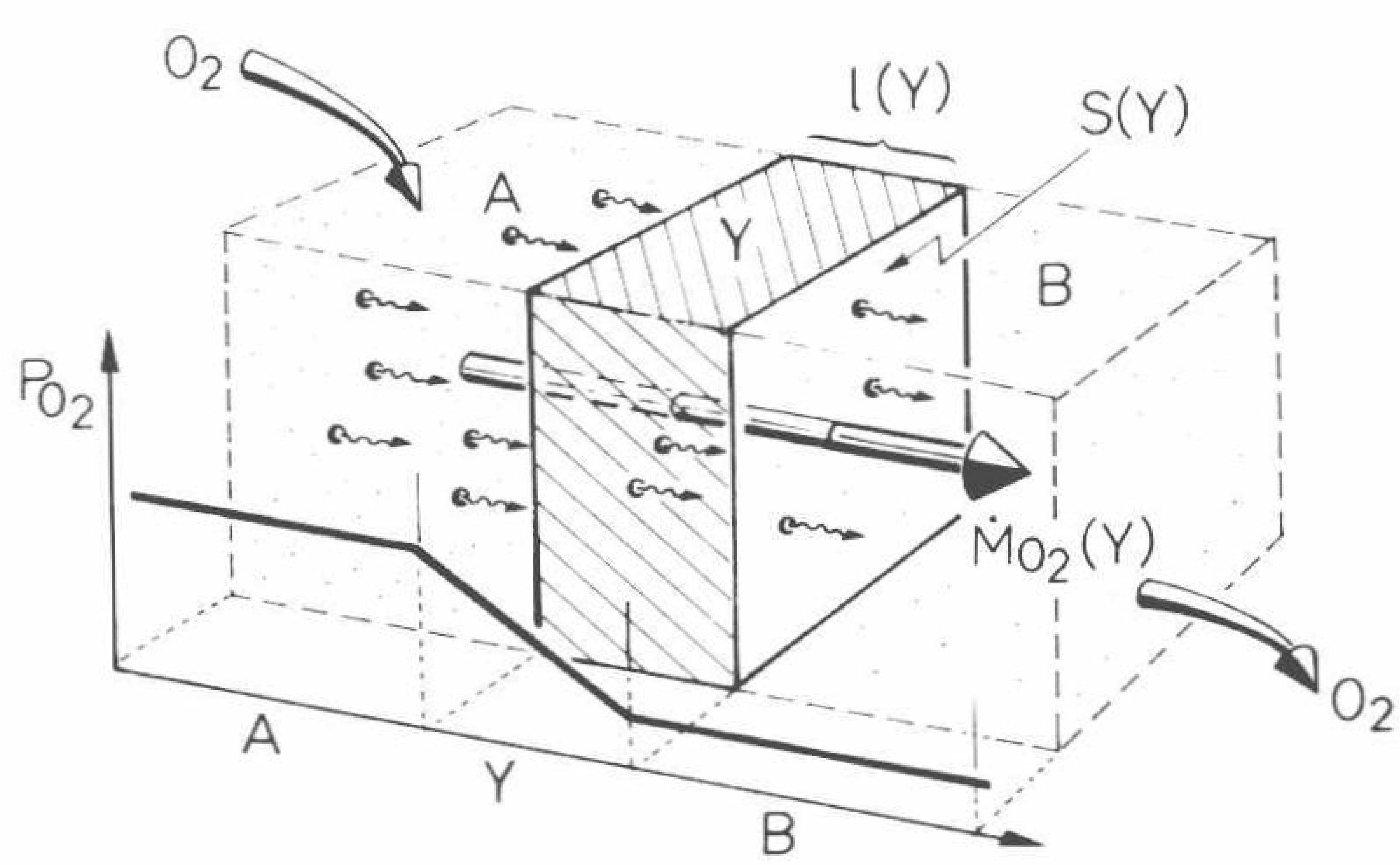

Fig. 1.2 O_2 transport by diffusion is driven by the O_2 partial pressure gradient and determined by the barrier dimensions, S(Y) and l(Y).