BIOMEMBRANES

6

By
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BACTERIAL MEMBRANES AND THE RESPIRATORY CHAIN

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Foreword

The most valuable service Dr. Gel'man and her colleagues have performed for the many investigators of bacterial membrane systems in producing their first excellent monograph on "The Respiratory Apparatus of Bacteria" in 1966 has been continued and expanded in the preparation of this volume. The authors have brought together in a single volume much of the detail of investigations of bacterial membranes at the ultrastructural level and the chemical and biochemical organizational levels. The approach in bringing together this rapidly increasing volume of discovery has been both comprehensive and systematic, with a constant awareness of the importance of the molecular and functional properties and relationships existing in various bacterial membranes.

The monograph naturally reflects the authors' interest and their own intimate involvement in the elucidation at the molecular level of the respiratory chains organized in the prokaryotic bacterial membrane system. It is entirely appropriate that the chapter devoted to this topic should occupy a substantial proportion of this monograph. Indeed, had this volume been prepared at this very moment, that proportion would have been even greater, as the work in this area has literally exploded in the past two or three years, with the isolation of ATPase and respiratory mutants by Butlin and Gibson and their colleagues, and now also in many other laboratories. This more recent aspect of the respiratory apparatus localized in the bacterial plasma membranes unfortunately emerged too late for this volume. However, many of the questions unanswered in this monograph will continue to be unanswered until the biochemical analysis of the many "respiratory" mutants is complete.

As the authors have realized from their introductory remarks, the work in this field has progressed and will progress even more rapidly in the future. The present volume gives us a balanced perspective of the progress of the work on bacterial membranes and the excellent and abundant bibliography will be a great resource for all of us, not only for the prokaryotic but also for the eukaryotic "membraneologists."

It is a pleasure to be able to see the continuation of the monograph into its present form. It will be greatly appreciated by students for its lucidity and by research workers for its detailed presentations of the various facets of the multifunctional bacterial membrane.

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Introduction

The structure and function of biological membranes are fundamental problems in modern biology. The reason for the exceptional interest shown in this problem is that processes of the greatest importance to life take place in the membranes of all cells starting from bacteria and ending with the cells of the human brain. Among the most universal processes connected with biological membranes are the exchange of ions and metabolites between cell and environment and the transformation of energy in electron transport chains during oxidative and photosynthetic phosphorylation. The membrane apparatus of the cell divides it into distinct "compartments," thereby giving the biochemical processes a spatial distribution and creating partition surfaces which play an important role in a number of enzyme reactions. The membranes exercise control over metabolic systems in the cell by regulating the permeability of substrates and of reaction products, as well as of ions as activators or inhibitors of the enzymes. Other specific functions determined by the physiology of the corresponding cell types are also connected with biological membranes. One very important feature is that membranes and, in particular, the enzymes linked together in them are the site of action of cell drugs, poisons, hormones, and some antibiotics.

The fact that the basic physiological functions of membranes are the same in cells at different levels of evolution naturally suggests that membranes are the most primitive biological formations and that the principle of their molecular organization must be the same in different cells.

Oparin (1957, 1960, 1966) expressed the view that in the remote ancestors of modern organisms, which can be imagined as condensations of protoplasm surrounded by a lipoprotein membrane, it performed a number of functions which subsequently were distributed among the different organelles of the cell. In particular, enzymes of the respiratory chain and enzymes of oxidative phosphorylation were evidently located in what at that time was the single external membrane of the most primitive aerobic cells.

It seems to the writers of this book that the prime purpose of membrane biochemistry is to investigate molecular organization. An understanding of the action of energy transformation systems and the discovery of the mechanism of transport of materials and other membrane processes are dependent on the solution of this problem.

The term "molecular organization of membranes" must be taken to mean the arrangement and interaction of the proteins and lipids which, together with molecules of water and cations, form the supramolecular structure of the membrane. When the molecular organization of the membranes is studied the choice of test object is most important. Despite the basic similarity between the functions of biological membranes, the morphology of the membrane system differs in cells standing at different levels of the evolutionary ladder. Whereas in bacteria we find a cytoplasmic membrane and an undifferentiated membrane system, in the cells of plants and animals we find differentiated membrane systems consisting of organelles and a network of inner membranes. Naturally because bacterial membranes are the least differentiated, but are functionally closely related to membranes of the cells of higher organisms, they have attracted special attention. Bacterial membranes also are the richest source of material for the study of several processes taking place simultaneously in the same structure, and also for the study of the biogenesis of membranes. Finally, the study of bacterial membranes is interesting from the point of view of evolutionary chemistry, for it can help with the development of ideas regarding the evolution of structure of the cell as a whole.

There are thus weighty arguments in support of the study of bacterial membranes. The number of investigations in this field in fact grows steadily. Modern approaches to the study of the molecular organization of biological, including bacterial, membranes are extremely varied. They include the development of methods of isolating membranes, of determining the composition of their proteins and lipids, of studying the properties of the isolated proteins, and elucidating the nature of the interactions which stabilize the membrane. Considerable attention is being paid to fragmentation of membranes and the study of the properties of the fragments in the hope of reconstructing the membrane in its original native state and of determining the arrangement of its individual zones. Despite much research in these directions, precise results from which a theory of membrane structure and organization could be formulated have not yet been obtained. The main difficulty is that in order to break up the membrane (a system of lipoprotein) into fragments, and in order to obtain individual proteins, powerful solubilizing agents have to be used. Treatment of the membrane in this way gives rise to artefacts which complicate evaluation of the results. For this reason, physical and physicochemical methods have been applied to the study of membranes in order to assess the state of their components *in situ* during

their natural interaction. In the last few years, some degree of specialization of these methods has begun to take place. For instance, nuclear magnetic resonance and differential thermal analysis are used to study lipids, while optical rotatory dispersion, circular dichroism, and infrared spectrophotometry are used chiefly to study the state of proteins. Meanwhile, introduction of methods using paramagnetic and fluorescent probes and labels has proved very useful both in the study of the state of lipids and proteins separately and in the elucidation of their interaction.

Electron-microscopic investigations of membranes, especially with the use of new techniques of specimen preparation, are also very important because ultimately they will enable the biochemical data to be correlated with membrane structure.

The purpose of this book is to examine the molecular organization of bacterial membranes. Particular attention will be paid to the properties and organizations of enzymes of the electron transport chain (the respiratory chain) of heterotrophic and chemoautotrophic bacteria. The process of electron transport has been studied comparatively thoroughly, and it can therefore act as "marker" for the study of the topography, if not of the membrane as a whole, at least of those parts of it which contain the components of the respiratory chain.

CHAPTER I

The Membrane Structures of Bacteria

Morphology of the Bacterial Membrane System

The membrane structures in the cells of about 70 species of bacteria have now been studied.* The results obtained before 1966 have been collected in several surveys (Glauert, 1962; Murray, 1963; Salton, 1964; Van Iterson, 1965; Lascelles, 1965; Gel'man et al., 1966; Kushnarev, 1966a). In recent years the membrane structures of many bacteria, which are listed below, have been studied: *Bacillus anthracis* (Avakyan et al., 1967), *Bacillus licheniformis* (Highton, 1969), *Bacillus fastidiosus* (Leadbetter and Holt, 1968), *Bacillus stearothermophilus* (Walker and Baillie, 1968), *Sarcina ventriculi* and *Sarcina maxima* (Holt and Canale-Parola, 1967), *Sarcina lutea* (Cherni, 1967), *Micrococcus denitrificans* and *Micrococcus halodenitrificans* (Kocur et al., 1968a,b), *Chondrococcus columnaris* (Pate and Ordal, 1967), *Pseudomonas* sp. and *Achromobacter* sp. (Wiebe and Chapman, 1968a,b), *Leptospira* sp. (Kats and Konstantinova, 1966), *Franciscella tularensis, Pasteurella pestis,* and *Bordetella abortus* (Kats, 1966; Avakyan et al., 1967), *Bacteroides insolitus* (Ushijima, 1967), *Haemophilus vaginalis* (Reyn et al., 1966), *Halobacterium halobium* (Cho et al., 1967), *Thermus aquaticus* (Brock and Edwards, 1970), thermophilic sulfur bacteria (Brock et al., 1971), *Caulobacter crescentus* (Cohen-Bazire et al., 1966), *Chloropseudomonas ethylicum* (Holt et al., 1966), *Rhodoteca pundens* and *Rhodopseudomonas* sp. (Cherni et al., 1969), *Ectothiorhodospira mobilis* Pelsh (Remsen et al., 1968), *Ferrobacillus ferrooxidans* (Remsen and Lundgren, 1966a), *Thiobacillus novellus* (Kocur et al., 1968a,b), *Thiococcus* sp. *nov. gen.* (Eimjellen et al., 1967), methane bacteria (Langenberg et al., 1968; Proctor et al., 1969; Davies and Whittenbury, 1970), *Methylococcus capsulatus* and *Methanomonas methanooxidans*

(Ribbons et al., 1970), hydrogen bacteria (Repaske, 1966), *Clostridium tetani* and *Clostridium botulinum* (Takagi et al., 1965; Pavlova and Sergeeva, 1969), *Clostridium pectinovorum* (Hoeniger and Hedley, 1968), *Clostridium perfringens* (Hoeniger et al., 1968; Pavlova and Larina, 1969), *Lactobacillus corinoides* (Schotz et al., 1965), *Lactobacillus plantarum* (Kakefuda et al., 1967), *Lactobacillus casei* (J. Brown et al., 1968), and *Lactobacillus acidophilus* and *Lactobacillus bifidus* (Lickfeld, 1967).

Analysis of the electron-microscopic data lies outside the scope of this book; they have been examined in the surveys of Ryter (1969), Van Iterson (1969a,b,c), and Kats (1971). What is important in the present context is that membrane structures, although differing in their complexity, have been found in all bacteria investigated, and membranes are evidently an essential component of the bacterial cell.

The membrane system of bacteria consists of the cytoplasmic membrane and the internal membranous structures. Judging from the electron-microscopic data, the cytoplasmic membrane is similar in shape in all bacteria. Morphological differences between the membrane systems are exhibited at the level of the internal cell structures which are usually formed by invagination of the cytoplasmic membrane.

As Salton (1967b) points out, the membrane system of bacteria exists in two principal forms, lamellar and vesicular, which differ from one another in the arrangement of the Robertson's membrane revealed by fixation with osmic acid. Discrete membranous structures of vesicular or lamellar type in heterotrophs are usually called mesosomes and in photoautotrophs they are called chromatophores. Mesosomes of vesicular type are clearly defined in members of the Bacillaceae family. Mesosomes of lamellar type are observed more frequently in cocci and in certain other bacteria. In many photosynthesizing and chemosynthesizing bacteria there are no discrete membranous structures whatever, but numerous membranes arranged parallel to each other penetrate throughout the thickness of the cell such as, for example, in *Rhodospirillum molishianum* (Giesbrecht and Drews, 1962) and *Nitrosocystis oceanus* (Murray and Watson, 1965). The possibility cannot be ruled out that the arrangement of the membrane in the mesosomes of bacteria is determined to some extent by the conditions of fixation of the cells (pH, fixative, ionic strength, bivalent cations). Burdett and Rogers (1970) describe modification of the structure of the mesosomes in this way under the influence of fixation in the case of *Bacillus licheniformis*. In their opinion the native mesosome consists of a system of vesicles and tubules. The lamellar form of mesosomes observed in the same bacteria and under the same conditions of fixation (Highton, 1970) can possibly be explained by differences in the age of the cells and in the conditions of cultivation. Replacement of vesicular mesosomes by lamellar mesosomes has also been observed during the development of a culture of *Micrococcus lysodeikticus* (Skopinskaya et al., 1972).

Membranous structures are poorly developed in the cells of most Gram-negative bacteria. The exceptions are the cells of the photosynthetic and of some chemosynthetic bacteria. The Gram-positive bacteria, on the other hand, have a well developed system of internal membrane.

The morphological features of the membrane system can be most conveniently described by combining the bacteria into physiological groups: aerobic and anaerobic heterotrophs, photosynthesizing and chemosynthesizing bacteria, as well as special groups such as halophiles, thermophiles, and pathogenic bacteria.

It was naturally expected that the membrane system would be poorly developed in anaerobic bacteria, which belong to the most primitive forms of life on earth (Oparin, 1957; Hall, 1971), particularly because anaerobic fermenters are unable to carry out oxidative phosphorylation, for the organization of which membranes are essential. However, membrane structures in bacterial anaerobes are very well developed indeed, and in some cases they are actually superior to those found in aerobes. Sections through cells of *Lactobacterium pentoaceticum* and *Clostridium oedematiens* are illustrated as examples (Fig. 1).

Membrane structures of strict anaerobes, unlike those in the aerobic bacteria, do not contain oxidoreductases (Tordzhyan and Kats, 1970; Avakyan et al., 1971).

In aerobic bacteria and in facultative anaerobes possessing enzymes of the respiratory chain and generating energy by oxidative phosphorylation, the membrane apparatus is highly variable. In some aerobic bacteria the membrane systems are very well developed, although of different forms. The mesosomes of a *Bacillus subtilis* cell (Granboulan and Leduc, 1967; Ryter, 1968, 1969) illustrated in Fig. 2 or the mesosomes of other bacilli and, in particular, of *Bacillus megaterium* (Ellar et al., 1968) are good examples. Meanwhile the membrane system of *Azotobacter vinelandii* is much less well developed and consists only of diffusely arranged membranes (Tchan and Webber, 1966), although the cells of *Azotobacter* are among those with the most intensive respiration and contain a complete respiratory chain of enzymes. In bacteria of the enteric group (*Escherichia coli* and *Aerobacter aerogenes*), which also contain a respiratory chain of enzymes, the internal membrane systems also are poorly developed (Steed and Murray, 1966; Cota-Robles, 1966; Kennell and Kotoulas, 1967; Nanninga, 1970a,b). Small mesosomes have been found in only a few strains of *E. coli* (Schnaitman and Greenawalt, 1966; Pontefract et al., 1969). The membrane systems of *Proteus vulgaris* are poorly developed (Leene and Van Iterson, 1965).

A striking example of the variety of forms which can be assumed by the membrane system is given by the photosynthesizing bacteria. Whereas the membrane systems of *Rhodospirillum rubrum* and *Chloropseudomonas ethylicum* consist of vesicular structures joined to the cytoplasmic mem-

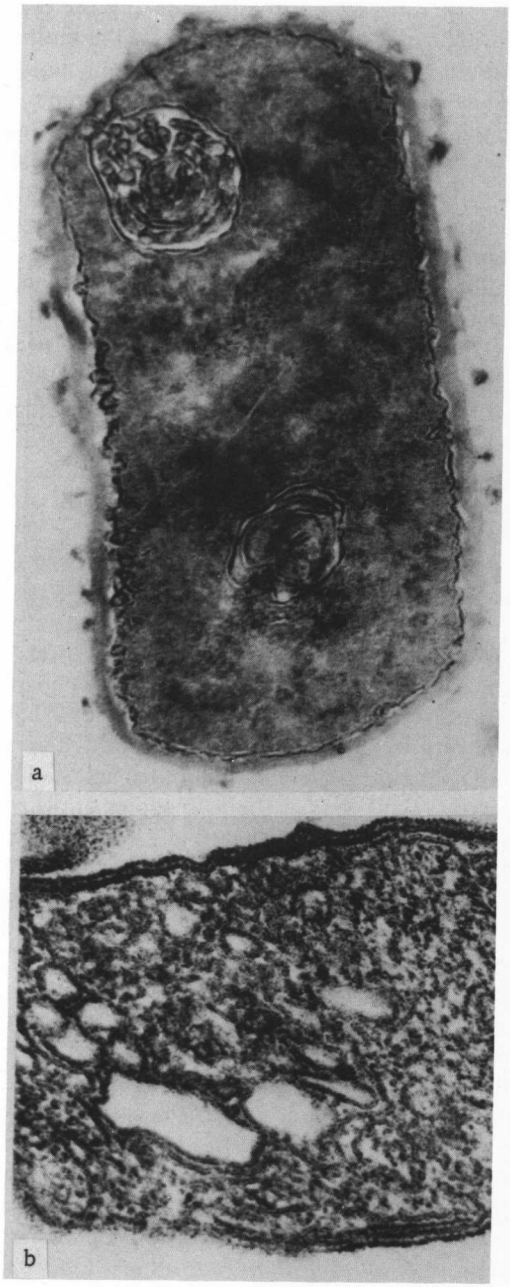

Fig. 1. The membrane system of anaerobic fermentation bacteria: a) *Lactobacterium pentoaceticum,* 140,000 × (Kharat'yan et al., 1967); b) *Clostridium oedematiens,* 90,000 × (Avakyan et al., 1970).

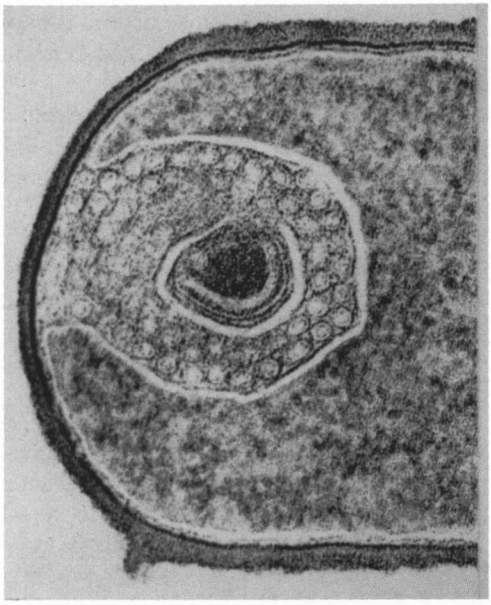

Fig. 2. Mesosomes of *Bacillus subtilis,* 95,000 X (Ryter, 1969).

brane (Cohen-Bazire and Kunizawa, 1963; Boatman, 1964; Holt, et al., 1966), in *Rhodopseudomonas viridis* and *Rhodopseudomonas palustris* complex lamellar systems of membranes exist, resembling the thylacoids of chloroplasts (Drews and Giesbrecht, 1965; Giesbrecht and Drews, 1966; Tauschel and Drews, 1966; Tauschel and Drews, 1967). An exceptionally complex membrane system is found in the photosynthesizing bacterium *Ectothiorhodospira mobilis* Pelsh (Remsen et al., 1968).

The lamellar membrane system in some chemosynthesizing bacteria (*Nitrobacter agilis, Nitrosomonas europea, Nitrosocystis oceanus*) is extremely well developed (Murray, 1963; Murray and Watson, 1965; Remsen et al., 1967). Complex membrane systems have also been found in *Nitrobacter winogradskii* (Tsien et al., 1968). Another group of chemosynthesizing bacteria — the ferro-oxidizing and thio-oxidizing bacteria — usually do not contain complex membrane systems (Remsen and Lundgren, 1966a,b; Mahoney and Edwards, 1966; Kocur et al., 1968a, b), and small mesosomes have been found only in some strains of the thiobacilli (Shively et al., 1970; Avakyan and Karavaiko, 1970). A distinctive system of internal membranes has been found in the halophilic bacterium *Halobacterium halobium* (Stoeckenius and Rowen, 1967; Cho et al., 1967) and in a marine pseudomonad (Backmuire and MacLeod, 1965). The thermophilic bacterium *Bacillus stearothermophilus* possesses well-developed mesosomes (Walker and Baillie, 1968).

The membrane system of the pathogenic bacteria is poorly developed, as has been shown for *Shigella flexneri* (Pavlova and Pershina, 1966) and for certain other organisms (Avakyan et al., 1967).

On the whole, the size, shape, and structure of the membrane formations in bacteria are extremely varied and their morphology could be used as a taxonomic feature were it not for the fact that the internal membrane structures of the bacterial cell vary with the age of the cells, the composition of the nutrient medium, and certain other factors (Ryter, 1969; Kats, 1971).

Although the internal membrane structures are connected with the cytoplasmic membrane there is no proof that the two are identical. On the contrary, if these two types of membranes are separated it is possible to demonstrate differences in their protein, and, in particular, in their enzymic apparatus (see page 16).

Some extremely interesting electron-microscopic pictures showing differences in the structure of the mesosomal and cytoplasmic membranes have been given by Ryter (1969) (Fig. 3). Evidence of differences in the structure of the cytoplasmic and mesosomal membranes is given by electron-microscopic studies of *Bacillus subtilis* cells, using freeze-etching. The cytoplasmic membrane is covered with numerous small granules, whereas the mesosomal membranes are smooth (Nanninga, 1968, 1970a). Indirect evidence of differences between the two types of membranes is presented by Bertsch et al. (1969). Diphosphatidylglycerol is extracted from *B. megaterium* cells only after their destruction, i.e., evidently from their internal membranes, whereas phosphatidylglycerol and phosphatidylethanolamine are readily extracted from whole cells, i.e., from the cytoplasmic membrane.

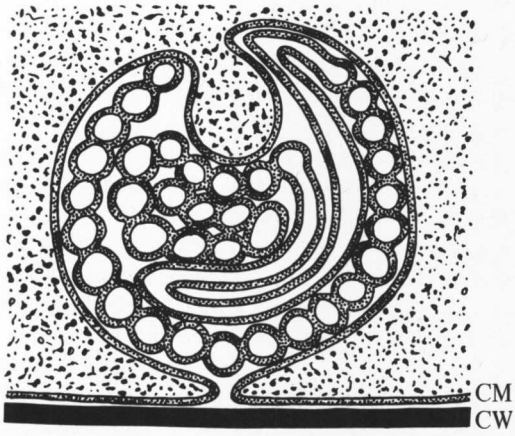

Fig. 3. Diagram of the structure of mesosomes (Ryter, 1969). CM) Cytoplasmic membrane; CW) cell wall.