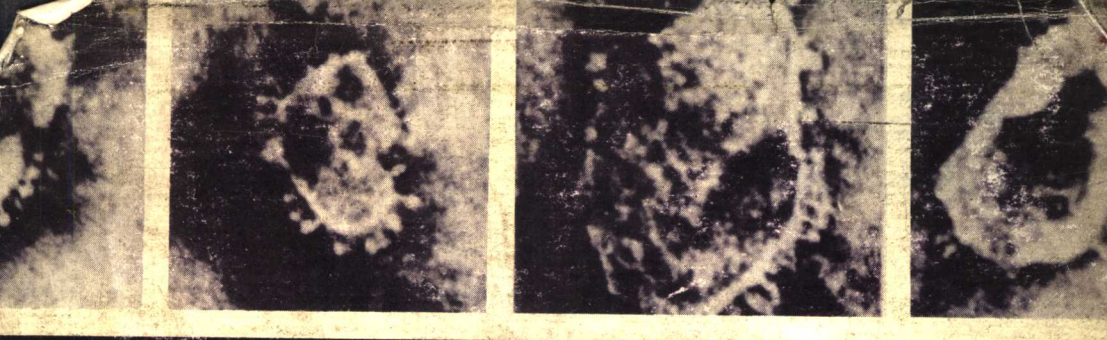

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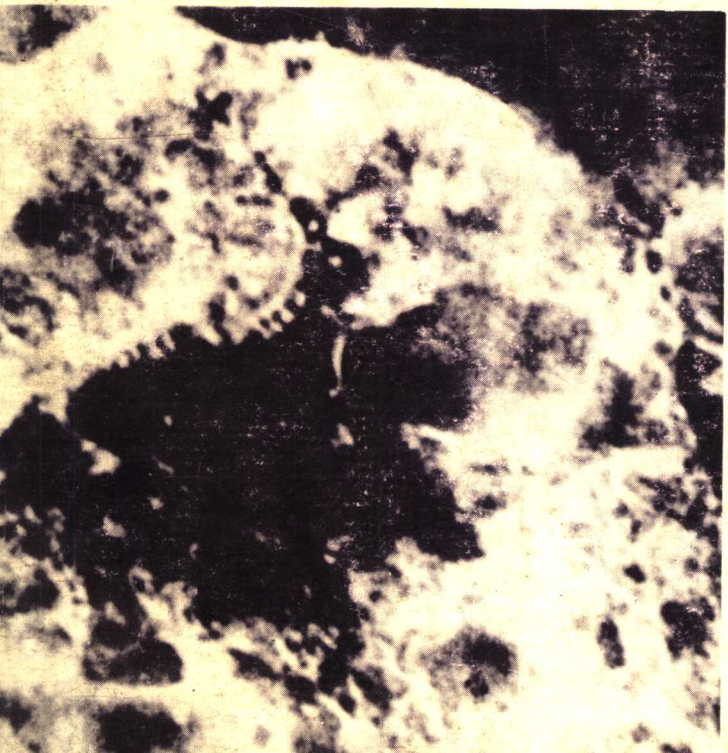

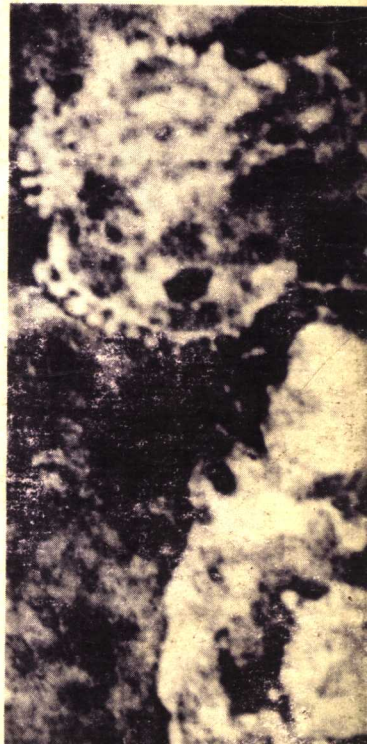

Respiration and Phosphorylation of Bacteria

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Foreword

This book is obviously of great interest to bacteriologists and all those interested in bacterial metabolism and especially to those interested in bacterial energy metabolism. It will also appeal to a much wider audience for two reasons. First, bacteria because of their primitive position on the evolutionary scale can't help but tell us a great deal about how life began on earth. Second, the use of bacteria in biochemical studies has often, in the past, opened up new areas and provided new knowledge that was later confirmed in mammalian tissues. There is every indication that this will continue to be true in this field.

This book is particularly timely and important in that it provides the only thorough and up-to-date attempt to collect and evaluate the literature on bacterial respiration and oxidative phosphorylation. While there have been a great many reviews of mitochondrial metabolism this is the only one that covers the bacterial literature with any degree of completeness. Making these data available in one place is a very valuable service, which not only provides insight into bacterial mechanisms but also points up similarities and differences between bacterial and mitochondrial structure and metabolism. This book will certainly provide a stimulus for new and exciting research.

Gifford B. Pinchot

Baltimore
June, 1967

Preface to the American Edition

This book is an attempt to sum up the literature on respiration and phosphorylation in bacteria. The topic of discussion is the morphology and molecular organization of the membranous structures, the enzymatic composition of the respiratory chain, the mechanism of oxidative phosphorylation, and possible pathways of evolution of the respiratory apparatus.

Since the progress of research in this field is very rapid there is a danger that the state of the problem as given in the book may lag behind the information which has appeared by the time that the book is published. Nevertheless, we hope that the book will be of use to research workers in the field of respiration, bacterial energetics, and the molecular organization of bacterial membranes.

We take this opportunity of thanking the authors whose diagrams and tables are given in the book.

The Authors

Contents

Introduction

Intensive research over the last ten years has shown that many of the metabolic pathways, enzyme systems, and intermediate products common to all modern organisms are also found in bacteria, thus confirming the concept of the common origin of all forms of life in earth (Oparin, 1924, 1957, 1960). Although the common nature of several metabolic processes has weakened the sharp distinction between bacteria and highly organized cells, bacteria still occupy a special position among living creatures as regards morphology and biochemical characteristics. Bacteria are unique and extremely interesting organisms. They cannot be regarded as ordinary cells—like animal or plant cells, only smaller. The special nature of bacteria is so pronounced that they sometimes appear to be free-living nuclei or mitochondria.

An interesting feature which distinguishes bacteria from multicellular organisms is their ability to develop in the partial or complete absence of air. This ability indicates the antiquity of their origin and, perhaps, even their association with the anaerobic period of the earth's atmosphere (Gaffron, 1964).

The comparatively simple cellular organization and metabolism of bacteria and their ability to live anaerobically suggest that they lie somewhere at the base of the evolutionary ladder. Hence, investigations of bacteria can contribute to theories of the evolution of cell structure and function on the basis of a comparison of bacteria with highly organized cells.

Oparin (1957, 1960, 1966) has suggested that the remote ancestors of present-day organisms can be pictured as blobs of protoplasm surrounded by a single lipoprotein membrane. This membrane had to perform several functions, which were later distributed among the different cell organelles. In particular, this single outer membrane of the earliest aerobic cells must have carried the enzymes of the respira-

tory chain and oxidative phosphorylation. The situation is rather similar in the most simply organized organisms living today, such as, for instance, the smallest living creatures (down to 0.1 μ in diameter), belonging to the group of pleuropneumonia-like or coccoid bacteria (Pirie, 1964; Gale, 1962). Hence, an investigation of the structure and physiology of bacteria, particularly small forms, is of very great interest for clarification of the central problems of evolutionary biochemistry. For instance, the study of bacterial nucleic acids can throw light on the origin and development of the genetic apparatus, since the genetic apparatus of bacteria is similar in its abilities to that of the smallest living organisms. The lipoprotein membranes of bacteria provide an abundance of material for the study of polyfunctionality—the combined performance of several processes such as photosynthesis, respiration, active ion transfer, etc., by one structure.

In accordance with the widely accepted concept of "biochemical" unity, one of the most significant features of the living state is the continuous and directed movement of electrons in the cell (Kluyver and Van Niel, 1959). The formation of phosphorylated high-energy compounds is the main purpose of this process, which is common to all living things.

Electron transport and oxidative phosphorylation are effected in the respiratory apparatus, which is an assembly of enzyme systems, spatially organized in the structural elements of the cell. In the cells of animals, plants, and most microorganisms, this apparatus consists of a system of enzymes localized in the mitochondria. The cells of bacteria and blue-green algae have enzyme systems similar in composition to those of all other cells, but they possess no mitochondria; hence, the structure of the respiratory apparatus and the spatial organization of the enzymes of electron transport and oxidative phosphorylation are different. The system of enzymes responsible for the transfer of electrons to oxygen is called "the respiratory chain" or "the electron-transport chain." We prefer the first name, since it expresses the nature of the process more concisely.

Our concepts of the nature and functions of the respiratory chain have been obtained mainly from mitochondria of animal tissues. According to Chance and Williams (1956), the respiratory chain of mitochondria has three main functions: (1) to transport electrons from the substrate to oxygen and, in particular, to maintain the necessary level of oxidized nicotinamide adenine dinucleotide

within the cell; (2) to provide at least three sites for energy conservation, where ADP is converted to ATP; and (3) to regulate metabolism in accordance with the levels of control substances (e.g., ADP, NAD, or hormones).

The respiratory chain of mitochondria includes NADH dehydrogenase, succinic dehydrogenase, and cytochromes of groups *A, B,* and *C.* These enzymes are assembled in a definite order in the structure of the mitochondrial lipoprotein membranes. Ubiquinone appears to be a component of the system.

In contrast to the situation for animal and plant cells, the respiratory chain of bacteria has not been adequately investigated, although there is now a large body of facts which show that, in the aerobic and facultative anaerobic bacteria that have been investigated, the composition of the respiratory chain is similar in many respects to that of other cells. Another similarity is that the respiratory chain of bacteria is associated with membranous elements of the cell, i.e., is spatially organized. Yet there are no mitochondria, and oxidative phosphorylation in preparations of bacterial membranes is very inefficient. The regulation of oxidative metabolism in bacteria is very inferior to that in mitochondria. There is no respiratory control mechanism in bacteria.[1]

The aim of this book is to characterize the respiratory apparatus of bacteria, i.e., the composition of the enzyme chains and their structural organization. We were interested in the special features of the enzyme composition (the assortment and properties of the components, their number, behavior in regard to inhibitors, methods of fractionation and purification) and the molecular organization of the respiratory chain (the ultrastructure of bacterial membranes, the composition of lipoprotein complexes of membranes, and the role of lipids in maintaining the structure of the respiratory chain and in enzyme activity). In the treatment of this problem we have tried to bring out the similarity and differences between the respiratory apparatus of bacteria and mitochondria, structures with similar functions, which are much more advanced morphologically and evolutionally younger.

[1] Editor's note: This statement is no longer true. See: Ishikawa, S., and Lehninger, A. L., *J. Biol. Chem.* 237, 2401, 1962; and Scocca and Pinchot, *Fed. Proc.* 24, 544, 1965.

Chapter I

Membranous Structures of Bacteria

STRUCTURAL FEATURES OF THE BACTERIAL CELL

Most bacteria are extremely minute organisms. Their average size is about 5 μ (Ierusalimskii, 1963; Zarnea, 1963; Morowitz and Tourtellotte, 1964), and the smallest, the coccoid bacteria for instance, have a diameter of only 0.5–1 μ. Thus, cocci are a thousand times smaller than the average animal cell and are smaller even than such cell organelles as mitochondria, which can attain a length of 10 μ with a diameter of 0.5 μ. (Marr, 1960). A very significant index for illustration of the size of bacteria is the number of free hydrogen ions (H^+) at pH 7 in a volume of liquid equal to the volume of the cell. In this case the cell of a coccus contains 5–50 hydrogen ions (Chen and Cleverdon, 1962). This does not mean that there are always 5–50 H^+ ions in a cell in which the pH of the cytoplasm is 7. Different metabolic processes can alter the H^+ concentration very considerably for certain short intervals of time, but the over-all effect due to these variations corresponds to the H^+ concentration at pH 7. The small size of the bacterial cell is of very great significance for the biochemical processes occurring in it. The size of the cell is particularly important when it is reduced to a few tenths of a micron, only slightly exceeding the theoretically permissible minimum (0.05 μ), as in the case of some forms of mycoplasma (diameter 0.1 μ) (Pirie, 1964; Morowitz *et al.*, 1962; Morowitz and Tourtellotte, 1964).

Calculations show that the bacterial cell can contain a very limited number of protein and nucleic acid molecules. This is reflected in the specificity of bacterial metabolism. For instance, Bresler (1963) points out that the DNA of *Escherichia coli* contains about 1 million units of information. This is sufficient for the synthesis of only 2000

different protein molecules, while *Dialister pneumosintes* can synthesize only 600 types of protein molecules (Chen and Cleverdon, 1962), whereas the DNA of the animal cell carries 1000 times more information. It has been suggested (Gale, 1959) that bacterial proteins must each perform several functions, since he estimates that there are only 400,000 to 500,000 protein molecules per *Staphylococcus aureus* cell.

It has been shown that polyfunctionality is a feature of the few membranous structures (the cytoplasmic membrane, for instance), which must each carry several enzyme systems. These systems are localized in specialized organelles in higher organisms.

The wall of the bacterial cell is about 20% of its weight (27–38% in *Streptococcus faecalis*), but there is no evidence yet that it takes any active part in cell metabolism (Salton, 1964). The relatively large dimensions of the cell wall are attributed simply to the need for mechanical protection of the protoplast (Weidel and Pelzer, 1964). The lipoprotein cytoplasmic membrane plays a much greater role in the life of the bacterial cell. In some bacteria it is the only membranous structure of the cell. It is of interest that the ratio of the area of the cytoplasmic membrane to the volume of the cell is ten times the corresponding value for the animal cell.

The nuclear apparatus of bacteria differs greatly from that of higher organisms. Bacteria do not have a typical nucleus with a distinct membrane and nucleolus (Brieger, 1963; Stanier, 1964). The DNA of bacteria forms a "nuclear body" or "zone" (Van Tubergen and Setlow, 1961; Juhacz, 1961), which lacks a lipoprotein membrane, but can be isolated in fairly pure form (Godson and Butler, 1962; Spiegelman *et al.*, 1958; Echlin and De Lamater, 1962). Specialists believe that this type of nuclear apparatus—a compact endosome according to Dillon's terminology (1962)—is intermediate between the apparatus of blue-green algae, on one hand, and that of protozoa, on the other, although some investigators (Stanier, 1964) put bacteria into an independent evolutionary series.

The photosynthetic apparatus in photosynthetic bacteria has a distinctive structure. The bacteriochlorophyll in them is usually contained in spherical granules, or chromatophores (diameter about 600 Å), which are small in comparison with chloroplasts (Kamen, 1963; Marr, 1960). There are other forms of organization of the photosynthetic apparatus. As distinct from plant chloroplasts, the chromatophores do not contain the enzymes required for CO_2 fixation. Morphologically, as Boatman (1964) recently showed for *Rhodospirillum*

rubrum and Cohen-Bazire *et al.* (1964) for *Chlorobium sp.*, the chromatophores are formed by invagination of the cytoplasmic membrane.

Until very recently, no internal membranes or membranous structures resembling the endoplasmic reticulum or mitochondria had been found in the cytoplasm of bacteria (Bradfield, 1956; Luria, 1960). It was believed that the ribosomes lay freely in the cytoplasm and that the respiratory enzymes were localized in the cytoplasmic membrane—the only membrane which could be observed in the bacterial cell (Hughes, 1962). The first reports of membranous structures in bacteria were met with disbelief and were even disputed. However, the presence of membranous structures of various kinds in most bacteria can now be regarded as proven, and attention is being concentrated on their function. If they play the part of an endoplasmic reticulum, they will carry the ribosomes and resemble the canals and cisternae of the reticulum, but will not contain the phosphorylating respiratory chain. If they are equivalent to mitochondria, their structure should show some elements of similarity with that of mitochondria, and they will have to carry the respiratory chain, i.e., the cytochromes and dehydrogenases. It is obvious that obligate anaerobes, which lack a respiratory chain, will not contain membranous structures.

The membranous structures in bacteria are of interest in the study of their respiratory apparatus (structure, molecular organization, and function). For comparison we will later mention information relating to the structure and function of mitochondria, particularly the mitochondria of the mammalian heart, which have been the most thoroughly investigated.

Great progress in the technique of obtaining ultrathin sections and the development of special fixing and staining methods led to the discovery of membranous structures inside the bacterial cell. Representatives of various groups of bacteria and of related microorganisms, such as actinomycetes and blue-green algae, have been investigated. In most investigations, intact cells were the object of study, but the membranous structures have been isolated from some bacteria, and their structure and composition investigated.

The membranous structures investigated in intact cells will be described separately for each bacterial species studied. In view of the great polymorphism of the membranous structures and the impossibility of reproducing numerous photomicrographs we have attempted

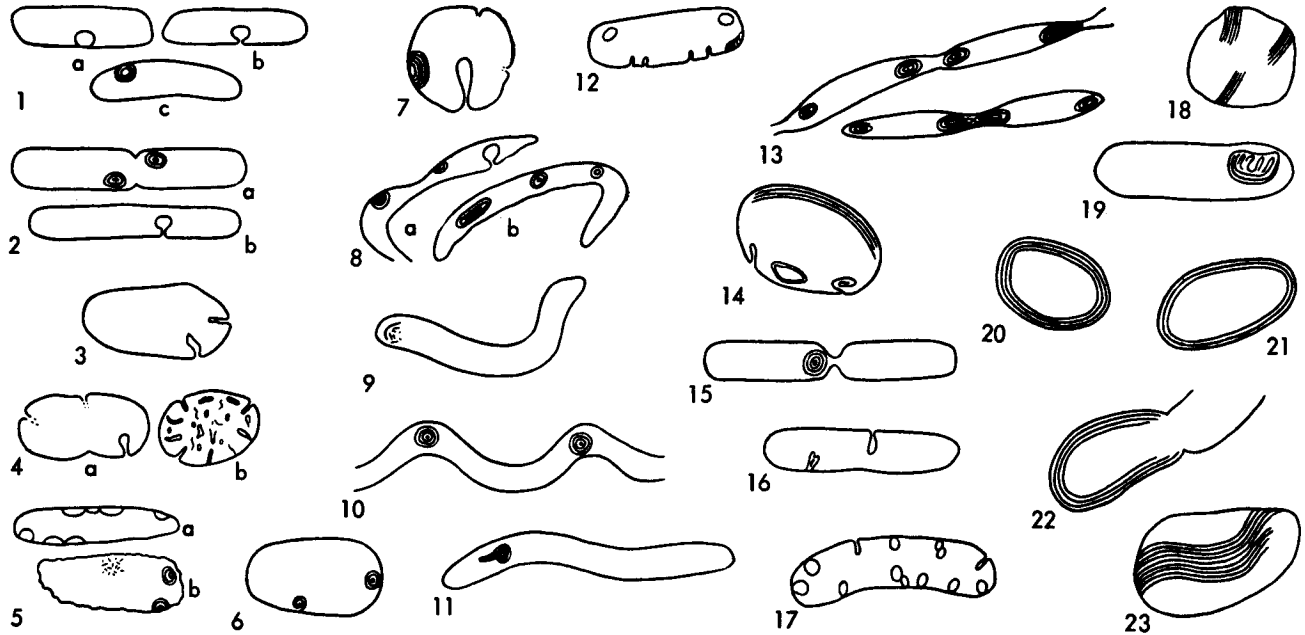

Fig. 1. Membranous structures in gram-negative bacteria. 1. *Escherichia coli* (*a*—Niklowitz, 1958; Biryuzdva, 1960; *b*—Kran, 1962; *c*—Vanderwinkel and Murray, 1962); 2. *Spirillum serpens* (*a*—Vanderwinkel and Murray, 1962; *b*—Murray and Birch-Anderson, 1963); 3. *Acetobacter suboxydans* (Claus and Roth, 1964); 4. *Azotobacter sp.* (*a*—Wyss *et al.,* 1961; *b*—Pangborn *et al.,* 1962; Van Iterson, 1963); 5. *Proteus. vulgaris* (*a*—Nermut and Rýc, 1964; *b*—Van Iterson and Leene, 1964*b*); 6. *Rhizobium sp.* (Dart and Mercer, 1963*b;* Dixon, 1964); 7. *Neisseria gonorrheae* (Fitz-James, 1964*a*); 8. *Treponema Reiter* (*a*—Ryter and Pillot, 1963; *b*—Kawata and Inoue, 1964); 9. *Treponema microdentium* (Listgarten *et al.,* 1963); 10. *Leptospira pomona* (Ritchie and Ellinghausen, 1965); 11. *Borrelia recurrentis* (Ludvik, 1964); 12. *Brucella abortus* S and R (De Petris *et al.,* 1964); 13. *Caulobacter bacteroides* (Stove Poindexter and Cohen-Bazire, 1964); 14. *Hyphomicrobium sp.* (Conti and Hirsch, 1965); 15. *Bacteroides ruminicola* (Bladen and Waters, 1963); 16. *Fusobacterium polymorphum* (Takagi and Uejama, 1963); 17. *Rhodospirillum rubrum* (Cohen-Bazire and Kunizawa, 1963; Boatman, 1964); 18. *Rhodospirillum molishianum* (Giesbrecht and Drews, 1962); 19. *Chlorobium sp.* (Cohen-Bazire *et al.,* 1964); 20. *Rhodomicrobium vannielii* (Boatman and Douglas, 1961; Conti and Hirsch, 1965); 21. *Nitrosomonas europea* (Murray, 1963; Murray and Watson, 1965); 22. *Nitrobacter agilis* (Murray, 1963; Murray and Watson, 1965); 23. *Nitrosocystis oceanus* (Murray, 1963; Murray and Watson, 1965).

to schematize their structure (see Fig. 1 for gram-negative and Fig. 2 for gram-positive bacteria). Classification by Gram staining is a rather formal approach, particularly since the nature of the reaction has not yet been explained (Salton, 1964). It is known, however, that division of bacteria into these two groups is correlated in some way with some physiological and biochemical characters and with structural features of the cell wall. All spore-forming bacteria are gram-positive, whereas gram-negative bacteria never produce spores. Autotrophic bacteria are gram-negative. For some unknown reason, the cells of gram-negative species usually contain ubiquinones, while gram-positive cells contain naphthoquinones. An examination of the membranous structures also shows a definite correlation with Gram staining.

To explain the diagrams shown in Figs. 1 and 2, we will recall that absolutely all bacterial cells have a similarly constructed cytoplasmic membrane, 75–80 Å thick, enveloping the protoplast. Structurally, internal membranous formations differ considerably in different bacteria and in some bacteria they are not found at all. The structure of the cytoplasmic membrane was first shown by means of a special fixing technique by Kellenberger and Ryter (1958), using *E. coli.* The ultrastructure and molecular organization of the cytoplasmic membrane and membranous structures will be discussed in special sections. For convenience in Figs. 1 and 2, the cytoplasmic membrane is depicted as a single line and the internal membranous structures, which are connected with the cytoplasmic membrane and are constructed from membranes which resemble it when fixed in the corresponding manner, are also depicted as single lines. In other words, a 75-Å thick membrane of the "unit membrane" type is shown as a single line.

MEMBRANOUS STRUCTURES IN GRAM-NEGATIVE BACTERIA

In most gram-negative bacteria, the membranous structures are less well developed and simpler than those in gram-positive bacteria. The specialized cells of photosynthetic and chemosynthetic bacteria are an exception (Fig. 1). Simple membranous structures in the form of invaginations of the cytoplasmic membrane were first observed in *Spirillum serpens* and unidentified bacteria (Chapman and Kroll, 1957; Chapman, 1959). Differentiated lipid-rich regions were found

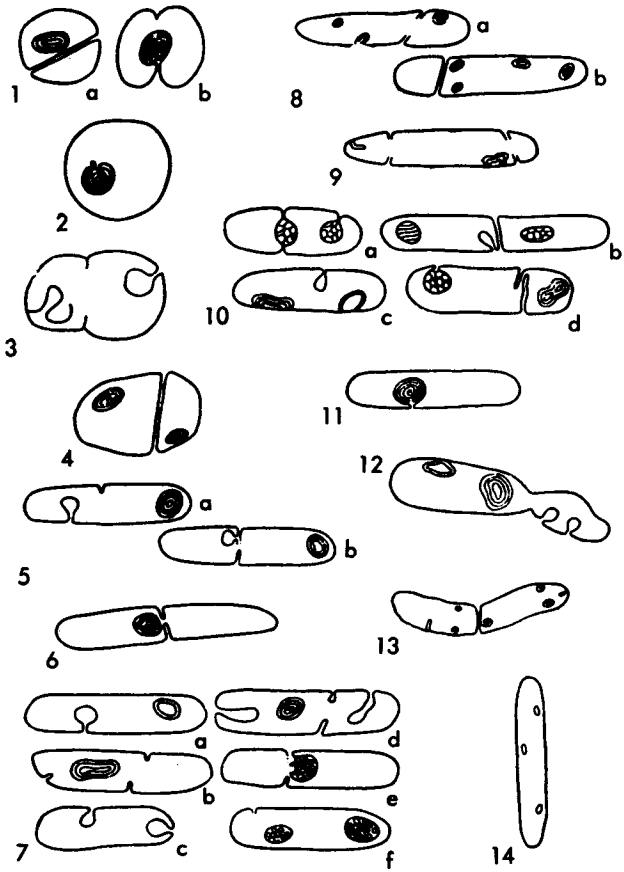

Fig. 2. Membranous structures (mesosomes) in gram-positive bacteria. 1. *Micrococcus lysodeikticus* (*a*—Salton and Chapman, 1962; *b*—Murray, 1963); 2. *Micrococcus roseus* (Murray, 1963); 3. *Diplococcus pneumoniae* (Thomasz *et al.*, 1964); 4. *Dermatophilus congolensis* (Gordon and Edwards, 1963); 5. *Bacillus megaterium* (*a*—Giesbrecht, 1960; *b*—Fitz-James, 1960); 6. *Bacillus coagulans* (Ohye and Murrell, 1962); 7. *Bacillus subtilis* (*a*—Van Iterson, 1961; *b*—Glauert *et al.*, 1961; *c*—Ryter and Jacob, 1963; *d*—Van Iterson and Leene, 1964*a*; *e*—Eiserling and Romig, 1962; *f*—Kawata, Inoue, and Takagi, 1963; 8. *Bacillus mycoides* (*a*—Tikhonenko and Bespalova, 1964; *b*—Malatyan and Biryuzova, 1965); 9. *Bacillus cereus* (Avakyan *et al.*, 1965); 10. *Listeria monocytogenes* (*a*—Grund, 1963; *b*—North, 1963; *c*—Kawata, 1963; *d*—Edwards and Stevens, 1963); 11. *Mycobacterium* sp. (Imaeda and Ogura, 1963); 12. *Corynebacterium diphtheriae* (Pavlova, 1964); 13. *Lactobacillus acidophilus* (Kodicek, 1963); 14. *Clostridium pectinovorum* (Fitz-James, 1962).