BACTERIOLOGICAL REVIEWS, Dec. 1975, p. 405-463 Vol. 39, No. 4
Copyright © 1976 American Society for Microbiology Printed in U.S.A.

Mesosomes: Membranous Bacterial Organelles

JOHN W. GREENAWALT[1]* AND THERESA L. WHITESIDE

Department of Physiological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland 21205, and Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

INTRODUCTION 405
Historical Background 405
Mesosomes as Cellular Organelles 406
Distribution of Mesosomes 406
Purposes of the Review 407
MORPHOLOGY AND ULTRASTRUCTURE OF MESOSOMES 407
Distinctive Features of Bacterial Mesosomes In Situ 408
Morphology of "Extruded" Mesosomes and Mesosome-Enriched Fractions 411
FORMATION OF MESOSOMES IN BACTERIAL CELLS 421
ISOLATION AND CHARACTERIZATION OF "MESOSOME-RICH FRACTIONS" 423
Release of Mesosomes from the Cell 424
Separation and Recovery of Mesosomes 426
Chemical Composition of Isolated Mesosomal Membranes 430
BIOLOGICAL ACTIVITIES OF MESOSOMES: THE QUEST FOR MESOSOMAL FUNCTION 434
Electron Transport, Phosphorylation, and Oxidation-Reduction Reactions 435
Replication and Apportionment of DNA 436
Cell Surface Remodeling: Biosynthesis, Septum Formation, and Partitioning of Cells 440
Possible Role in Secretory Processes 443
Other Functions Attributed to Mesosomes 444
Are Mesosomes Essential for Bacterial Survival? 445
EVOLUTIONARY CONSIDERATIONS OF BACTERIAL AND MITOCHONDRIAL MEMBRANE SYSTEMS 446
SUMMARY AND CONCLUSIONS 451
LITERATURE CITED 452

And is it not true that the comparatively simple act of looking through a microscope presents the eye with pictures that we should all declare fantastic and far-fetched if we happened upon them by chance . . . (Schmidt, 270)
Meditation over an electron micrograph of a bacterial cell wall or the endoplasmic reticulum of a liver cell brings conviction that the position of synthesis within the cell must be as important as the chemical synthesis itself. (Gale, 89)

INTRODUCTION

Historical Background

The development of techniques of cell fractionation (1, 60, 325) and of methods for the electron microscopic analysis of thin sections of whole cells and isolated cell fractions (16, 46, 188, 192) had a profound conceptual impact on biochemistry and cytology. Answers to questions concerning the structural-functional relationships of cellular components could be sought experimentally. For the first time investigators could ask *where* in the cell specific biochemical functions take place, *what* relationships exist between the structural organization and the biochemical compartmentation of cells, and *how* specific biochemical activities of cellular components are integrated and regulated at the molecular, organellar, and cellular levels. As a result, researchers from many disciplines were encouraged to isolate fractions enriched in specific organelles or subcellular structures, not "activities," and to characterize these fractions by determining their biochemical, chemical, and ultrastructural properties. Thin-sectioning techniques also permitted for the first time the comparison of the ultrastructural organization and compartmentation of prokaryotic and eukaryotic cells.

Prior to the development of thin-sectioning techniques, light microscope studies had shown that prokaryotes were constructed of a relatively simple design and lacked not only discrete, membrane-enclosed nuclei but also other well-defined membranous organelles common

[1] Present address: Department of Biochemistry, University of Tennessee, Knoxville, Tenn. 37916.

to eukaryotes (for review, see 314). Considerable interest was aroused when the application of cytochemical stains led to the description of "bacterial mitochondria" (192). However, early electron microscope studies revealed little internal, physical compartmentation in bacterial cells with the possible exception of "peripheral bodies" (46). Furthermore, the enzymatic removal of bacterial cell walls (180, 258, 259, 325) with the subsequent isolation of plasma membranes and soluble fractions provided evidence that major biochemical activities clearly associated with mitochondria in eukaryotes (e.g., tricarboxylic acid cycle enzymes, respiration, oxidative phosphorylation) were distributed, in bacteria, between soluble and particulate fractions. Although the distribution of these activities was somewhat variable depending upon the species and the methodology of fractionation (1, 180, 188), the experimental data then available did not support the concept of bacteria having counterparts analogous to eukaryotic organelles.

As improvements in preparative techniques for electron microscopy were made and exposed finer details of cellular structure, a number of workers observed internal membranes in bacterial cells (see reviews: 248, 256, 314). Various terms which reflected, in part, the individual author's view of the relationship of the internal membranes to the cell as a whole and/or the plasma membrane were used to describe these structures: "mesosomes" (78), "intracytoplasmic membranes" (136), "plasmalemmasomes" (64), and "chondrioids" (313). In each case, these distinctive membranes appeared to be derived from the plasma membrane, and general similarities in the ultrastructure, location, and size of these structures suggest that these different terms probably were applied initially to entities very likely corresponding to the "peripheral bodies" of Chapman and Hillier (46). The initial description of "mesosomes" and the possibility that they played a specific role in cell division and sporulation (78, 247) evoked an immediate and enthusiastic interest; since 1960, this term has been almost universally used although some reservations about this terminology remain (314).

The localization of mesosomes along the plasma membrane in association with septa (46, 78, 136) and also with the nuclear region (98, 313) was interpreted as presumptive evidence for their involvement in the processes of cell division and septation. Inevitably, the discovery of these intracellular bodies with their distinctive ultrastructure revived the question of the existence of mitochondria or their equivalents in bacteria; thus, it was proposed on ultrastructural and physiological grounds (98, 192, 193) that the mesosome was the "bacterial mitochondrion." Cytochemical studies contributed to this concept by revealing that mesosomes in certain species under specified conditions were sites of oxidation-reduction reactions (164, 312, 316, 317). However, inconsistencies were reported between the interpretation of these results and of data obtained from cell fractionation studies in which enzyme distributions were measured. No specific functional role has been shown to be applicable to all structures described as mesosomes in a wide range of bacterial species studied under a variety of conditions. As a result, the bacterial mesosome still must be discussed largely in descriptive ultrastructural terms.

Mesosomes as Cellular Organelles

On the basis of its morphological and ultrastructural aspects, the mesosome can be classified as a membrane-bounded organelle, but the absence of known functional specificity at this time makes this assignment presumptive. It is possible that this structure may be "an extension" of or even a "functionally deficient" region of the peripheral membrane. Specialization is usually accompanied by a reduction in generalized functions. Regardless, the mesosome does, indeed, represent physical compartmentation of the bacterial cell; accordingly, should it be proved that specific enzymatic activities are not localized exclusively or chiefly in the mesosomes, its unique ultrastructural organization still potentially could provide for the concentration and localization of membrane-associated biochemical functions at specific sites within the cell.

Distribution of Mesosomes

The term mesosome is accorded its broadest definition in discussing the distribution of this structure in various bacterial genera. Since this point has been discussed in considerable detail by others (see Gel'man et al. [93], Ryter [249], and Salton [256, 257]), it will be given only brief consideration here. There is now fairly general agreement that mesosomes are present in both gram-positive and gram-negative bacteria. Although mesosomes have been observed more commonly in gram-positive species by virtue of their prominent size and large numbers, mesosomes have also been reported in gram-negative cells grown under normal conditions (32, 228, 229, 252), but they are often inconspicuous and difficult to identify. They are readily seen in some species, notably thiobacilli

(135, 281), *Caulobacter crescentus* (51), *Achromobacter* (334), *Chondrococcus columnaris* (226, 227), and *Chromobacterium violaceum* (246). In certain strains of *Escherichia coli*, well-developed, large intracytoplasmic membrane structures, continuous with the peripheral, inner membrane have been observed under specified growth conditions (4, 130, 191, 208, 209, 273, 328, 329). Gross dissimilarities are sometimes obvious when the ultrastructure of some reported mesosomes is compared with the classical picture described for gram-positive species.

A distinction should be made between the mesosomes of gram-negative bacteria and the extensive, often "stacked" membranous structures found in nitrogen-fixing, nitrifying, and photosynthetic bacteria. These latter structures appear to be related to the unique physiological properties of these organisms and differ from the mesosomes in ultrastructural organization and presumably in biochemical functions (111, 239, 288).

Structures having classical, mesosomal ultrastructure are by no means unique to unicellular eubacteria. Extensive intracellular membranes were reported years ago in *Mycobacterium avium* (280) and in species of streptomycetes (136). More recent studies of Wildermuth (335) indicated the presence of mesosomes in aerial mycelia of *Streptomyces coelicolor*. The existence of well-developed mesosomes has been described in *Listeria monocytogenes* (64) and various diverse genera of corynebacteria (see review by Barksdale [12]), in certain bacilli isolated from cases of human leprosy and grown under different cultural conditions (83), in *Bacterionema matruchotii* (295), in *Nocardia asteroides* (256; Lane Barksdale, personal communication), and in *Myxococcus xanthus* (322).

Purposes of the Review

It seems important in the present review to attempt to analyze the developments which have contributed to the current state of knowledge and, if possible, to indicate avenues, both practical and conceptual, which might lead to new insight. In addition, it would serve some purpose to provide guidelines for the application of the term mesosomes to membrane systems. In view of the fact that no clear function can be attributed specifically to the mesosome, these considerations are of necessity limited primarily to ultrastructural features. Recent developments describing the effects of fixation methods, of the physiological state of the cells, and of the species of bacterium on the presence or absence of mesosomes indicate that some of these problems will be sorted out in the near future (21, 30–34, 57, 120, 121, 127, 198–202, 230, 246, 264, 266, 285, 304, 307, 315, 319, 320, 329). These advances also will be considered here.

It seems appropriate also to discuss the current status of knowledge concerning the physiological and biochemical functions of mesosomes. In our opinion, the data now available do not support any implications that all "mesosomes" within the same cell or having the same localization in different cells are necessarily identical in structure and function. Finally, the observation that, under certain experimental conditions, mitochondria contain membranous inclusions possibly associated with, but distinct from, the typical inner membranes is of interest. These findings have potential significance concerning current hypotheses of how mitochondria evolved (50, 61, 81, 232); it might be expected that if mitochondria have developed from symbiotic bacteria mesosomes would be retained, especially if they function in some vital capacity. These concepts reflect current thinking of workers in the field and indicate certain experimental approaches in need of further pursuit; it seems appropriate to include some brief discussion of these in the present review.

MORPHOLOGY AND ULTRASTRUCTURE OF MESOSOMES

Despite the fact that no clear-cut distinctions have been made as to what constitutes "the mesosome," biochemically or even ultrastructurally, the number of papers reporting the presence of this organelle in bacteria continues to increase. Often these reports occur as the result of investigations not directed toward the study of the bacterial membrane(s) or of mesosomes; rather, the "presence of mesosomes" is reported as the result of casual observation of membranous structures lying within the confines of the bacterial membrane or cell wall. In its broadest sense, of course, "mesosome" (middle-body) encompasses any and all of these forms. Over the years, detailed studies of gram-positive species have described the now "classical" form and locations of mesosomes (78, 249, 276, 313); in our view, these facts impose certain restrictions on the application of this term.

Several criteria can be applied to restrict the indiscriminate use of the term "mesosomes," and they are derived predominantly from the studies of gram-positive organisms, which have been most widely investigated. Accordingly, we propose that "mesosomes" be restricted to structures characterized in the following ways: (i)

they clearly are derived ultrastructurally from the plasma membrane as an invagination (neck or stalk) forming a "pocket" or "sac" within which membranous components are contained, and (ii) upon perturbation, hydrolysis, or removal of the cell wall, or upon plasmolysis, the membranous components within the sac become extruded. Further support for considering certain structures mesosomes would be gained from definitive evidence that they are associated physiologically and/or topographically with (i) septum formation, (ii) chromosome replication and/or distribution, (iii) sporulation, or (iv) hydrolytic activities (68, 69, 77, 78, 142, 248, 249, 251–253; V. M. Reusch and M. M. Burger, Fed. Proc. 31:1098, 1972). It is obvious from studies of gram-negative bacteria that these conditions in most cases cannot or have not been met; e.g., to our knowledge, in only a single case has it been shown that so-called mesosomes of gram-negative cells are extruded upon dissolution of the cell wall (246). In the absence of wide acceptance of the terms "plasmalemmasomes" and "chondrioids" (64, 314), which are perhaps in some ways more appropriate, we suggest that all other structures which are shown *not* to meet these criteria be called intracellular or intracytoplasmic membranes; others which have not been investigated in enough detail to establish these relationships (and yet have some ultrastructural similarities to mesosomes) should be referred to as "mesosome-like" structures or bodies. The micrographs used in the present review have been selected to illustrate the wide diversity of forms described in the literature as mesosomes; therefore, the criteria suggested above are not strictly applied in all of our discussions.

Distinctive Features of Bacterial Mesosomes In Situ

The comparative examination of thin sections of many bacterial cells of different strains and species shows that mesosomes or mesosome-like structures differ widely in size, shape, location, and complexity. A variety of differences have been observed also within the same strain during cellular growth or sporulation (34, 68, 123, 124, 244), but the physiological basis for what often appear to be sequential events has not been firmly established (cf. below, formation and biological activities of mesosomes). On the basis of their ultrastructural appearance in thin sections, mesosomes frequently are distinguished as being one of three types: lamellar, vesicular, or tubular. In some cells the form of the mesosome seems quite reproducible and would appear to be typical of that particular bacterium. The beautiful micrographs of "vesicular type" mesosomes associated primarily with septa but also located peripherally in *Staphylococcus aureus* (230) are a good example of a cell having one particular type of mesosome; however, as the authors point out, the presence of only vesicular mesosomes in staphylococci is not typical (18, 54, 147, 230, 243). Recent evidence suggests that the type and perhaps even the location of the mesosome within the cell may vary depending not only upon the physiology of the cell but also upon the conditions of fixation used for electron microscopy (33, 84, 120, 121, 147, 169, 200, 202, 244, 284, 285). Differentiation of mesosomes based on these descriptive criteria is far from ideal; the likelihood that these are "interconvertible" (244) or in a "transitional state" (314) has been discussed. Furthermore, tubulovesicular (83), lamellar-vesicular, and lamellar-tubular types have been described (34). Mesosomes have been differentiated also according to their cellular location; thus, septum mesosomes, peripheral or plasma membrane mesosomes, and even nuclear mesosomes have been described. Although it may be stated that mesosomes of gram-negative cells typically are lamellar and are fewer in number and less extensively developed than those of gram-positive bacteria, few, if any, other generalizations can be made. For *E. coli* O111$_a$ and other temperature-sensitive strains of *E. coli* (243, 329) it is suggested that even this generalization may not be strictly applicable.

The most detailed studies of mesosomal ultrastructure have been carried out with various species and strains of *Bacillus*, *Micrococcus*, and *Staphylococcus*. Comparisons of the dimensions of mesosomal and plasma membranes in thin sections by microdensitometer measurements indicate that probably no significant differences in dimensions exist (125, 329). A generalized model of mesosomal ultrastructure, growth, and modification most common to gram-positive cells is shown in Fig. 7. Figure 8 represents one possible sequence of events leading to complex lamellar mesosomes or mesosome-like structures more common to gram-negative cells. These models are discussed under the topic of formation of mesosomes (see below).

The variations in profiles of mesosomes and mesosome-like structures seen in thin sections are illustrated in Fig. 1–5. The distinctive mesosomal profile of some gram-positive bacilli is illustrated in *Bacillus subtilis* (Fig. 1). From such a section it cannot be determined whether these are actually vesicular or vesiculotubular

in organization, but it seems clear that more than a single mesosome is present in each cell, associated, in this case, with the peripheral membrane (arrows). (These cells were grown by Mary Kennedy in the laboratory of William J. Lennarz, Johns Hopkins School of Medicine.) *Bacterionema matruchotii,* a pleomorphic, facultative, gram-positive oral bacterium, contains numerous small, pocket-like invaginations of the plasma membrane which enclose what appear to be vesicular elements (Fig. 2). However, vesicular, tubular, and lamellar types of mesosomes have been observed (295). It has been suggested that the large number and different types of mesosomes in this cell may be related to the many diverse membrane-associated functions of this organism: budding, branching, septation, and calcification (295). (We are indebted to Frederick Rothwell for growing and providing samples of this organism.)

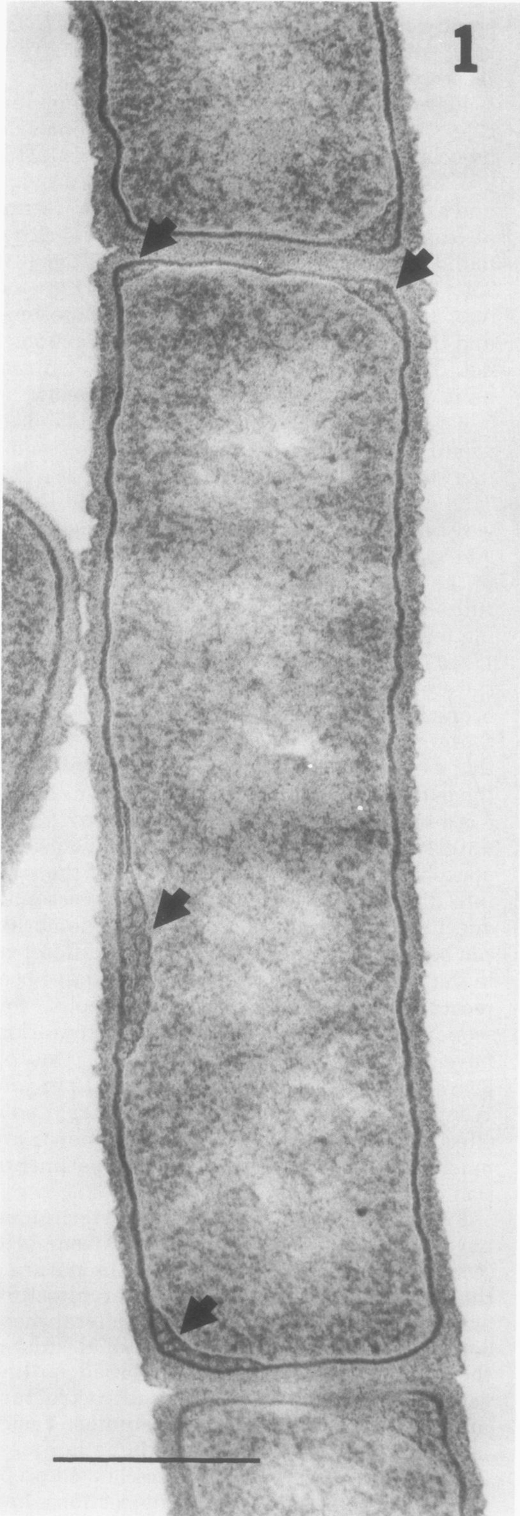
Figures 3 and 4 illustrate simple mesosome-like structures observed in *Thiobacillus denitrificans,* a gram-negative autotrophic bacterium grown in collaboration with Jessup M. Shively, Clemson University (135, 281). These forms resemble, at least in outline, the structures illustrated diagrammatically in Fig. 7 and 8, A and C.

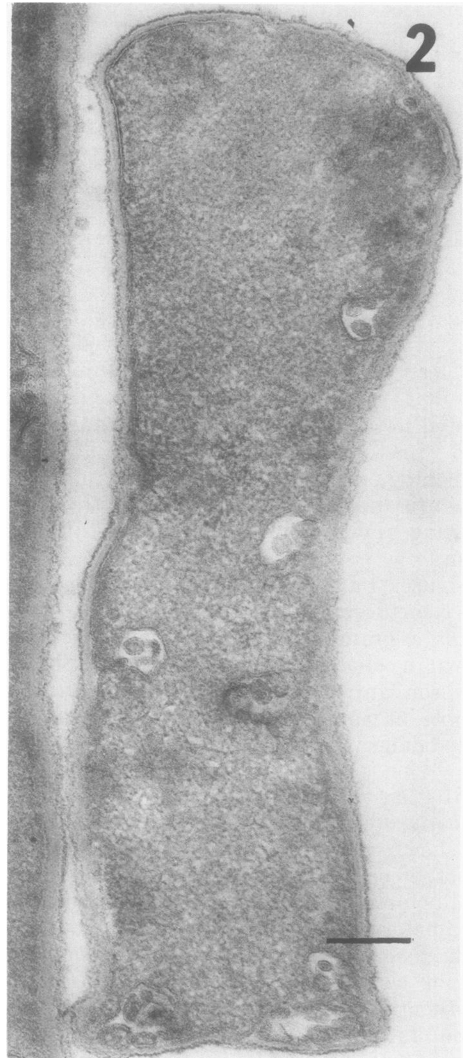
E. coli O111$_a$ grown at 40 C forms vast amounts of intracytoplasmic "extra membrane" as shown in Fig. 5 (4, 103, 273, 328, 329). Whether or not these are, in fact, mesosomes awaits further knowledge as to what functional parameters are assigned to mesosomes. The wide diversity of forms shown in these micrographs clearly demonstrates the difficulty of applying in a meaningful way the term "mesosome" to all of these structures except in its broadest definition. It may be true that the method of fixing cells for electron microscopy may affect the ultrastructural appearance of mesosomes, as we will discuss later. However, the cells illustrated in Fig. 1, 2, and 5 were all double-fixed and processed for electron microscopy in an identical manner in the same laboratory (J.W.G.). It does not seem likely that the great diversity observed can be the result of preparative procedures alone; whether or not the image seen in each case reflects truly the "native state" of the membrane may be questioned. Nevertheless, it seems fair to conclude that the membranes (organelles) of these cells are different, at least to the extent that, when treated in identical fashion, structures widely different in complexity, form, and localization are seen. This argument would seem to hold also for the differences in mesosomes of various staphylococci mentioned above (18, 54, 147, 230, 243), although it is not clear that all strains received identical treatment.

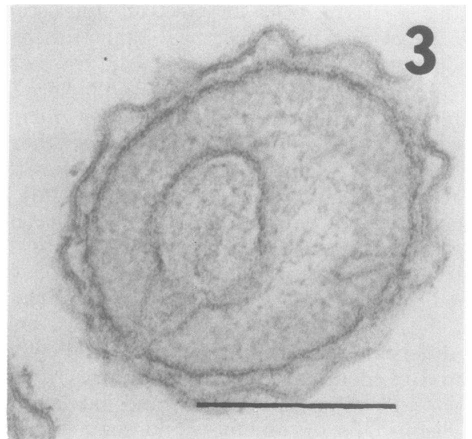
Recent detailed studies of thin sections indicate that structures resembling ribosomes *are* associated with mesosomes in situ (181, 182, 235, 315). This finding contrasts with previous and even recent views (240), and could account, at least in part, for the ribonucleic acid (RNA) found in isolated mesosome-rich fractions (216, 230, 234, 297). This suggests that ribosomes may be functional components of mesosomes and that the RNA measured in the fractions is not there merely as a "cytoplasmic contaminant" entrapped during isolation (see below).

Negative-contrast techniques also have been useful in detecting mesosomes and mesosome-like structures in whole cells (34, 248, 281, 329, 342), and observations of cells prepared in this way have provided additional morphological evidence that the neck of the mesosomes is open to the external milieu (33, 34, 244). The use of ruthenium red in conjunction with thin-sectioning techniques supports this interpretation (33, 171, 172). The presence of "pores" or "plugs" at the site of invagination of the peripheral membrane has been reported (33); what role, if any, these might play in compartmentation within the mesosome or between the mesosome and the external environment is not known. Figure 6 is a micrograph of *Thiobacillus denitrificans,* unfixed and negatively stained with potassium phosphotungstate. The penetration of the stain into discrete regions, presumably mesosomes, due to invagination of the plasma membrane can be seen. In some cases, apparent differences in the numbers of mesosomes per cell have been recorded when negatively stained cells, thin sections, and freeze-fractured preparations have been compared (199–202, 244); this appears to be true in the case of *T. denitrificans.* Whether or not these differences are related to effects of chemical fixation is not known and must await further, controlled experimentation.

Freeze-fracturing and etching techniques have also been used recently in attempts to secure more accurate insight into the nature of the mesosomal membranes, their architecture, and their relationship to the peripheral membranes (5, 134, 198, 199, 202, 307). Results of these experiments support, in broad outline, conclusions drawn from other ultrastructural analyses and cell fractionation studies. Vesicular, vesicular tubular, and/or lamellar mesosomes can be observed in freeze-cleaved preparations of cells which in thin sections have these types of mesosomes (33, 124, 284). The


1


2


3

location, size, and distribution of the mesosomes seen in freeze-cleaved cells are not entirely in agreement with these properties analyzed in cells chemically fixed, dehydrated, embedded, and sectioned (202). However, the inner and outer surfaces of the mesosomal membrane seen in freeze-cleaved cells can be distinguished from corresponding faces of the plasma membrane (see 202 for potential artifacts arising during freeze fracturing). The surface of the envelope (sac) of mesosomes in *B. subtilis* is relatively smooth but marked with blunt protrusions, not detected in thin sections, whereas the inner surface appears smooth with no protrusions. The plasma membrane is marked on its outer surface by densely packed particles and strands about 5 to 10 nm in diameter, whereas the inner surface has similarly sized particles which are more sparsely distributed (198–201).

These conclusions are based upon the appearance and topography of convex or concave surfaces observed to lie in sequence from the cell contents outwards and from the outer cell wall surface inwards. The differences described are attributed to the inner and outer membrane surfaces and not to intramembrane fracture faces. Deep-etching techniques used in conjunction with freeze fracturing have aided in these studies. (For review, see 202.)

Holt and Leadbetter (134), on the other hand, observed 12-nm particles associated with mesosomes in freeze-cleaved cells of *B. cereus*. Near septa and mesosomes, the plasma membrane, which in other regions is covered with particles 12 nm in diameter, was found to be remarkably smooth. The authors do not discuss whether or not this surface represents the outer surface of the mesosomal sac, but this possibility seems likely. Frequently, cross-fractured mesosomes have the "honeycomb" appearance sometimes observed in thin sections and are interpreted to be cross sections of closely packed vesicles or tubules (64, 134). Relatively small smooth-surfaced mesosomes in *B. anthracis* (nonencapsulated strain) undergoing sporulation are shown in Fig. 11. The general lack of structure on the exposed mesosome surface is readily seen, and the general relationships of mesosome to cross wall to endospore observed in thin sections have been confirmed by other freeze-fracturing studies (238). (We are grateful to Homayoon Fanzadegon for this micrograph, Fig. 11.)

Morphology of "Extruded" Mesosomes and Mesosome-Rich Fractions

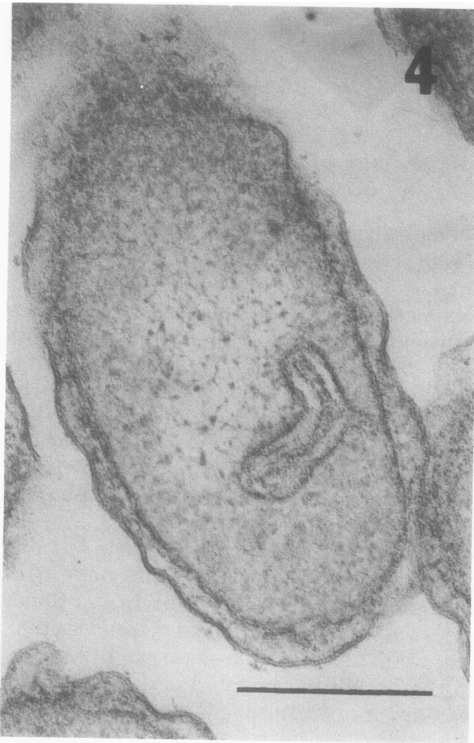
One of the major breakthroughs leading to recent refinements in methods of isolating mesosome-rich fractions was the electron microscopic observation that plasmolysis of gram-positive cells by exposure to hypertonic medium leads to extrusion of the mesosomes into the periplasmic space (250, 252, 313, 326). It was found also that subsequent or concurrent treatment with cell wall-degrading enzymes (e.g., lysozyme) to form protoplasts liberated, in many cases, mesosomal vesicles or tubules into the suspending medium (79, 80, 216, 245). Time-sequence studies of the ultrastructural changes accompanying these treatments have contributed also to a better understanding of the topological organization and the relationship of the mesosomal sac and the mesosomal membranes to the peripheral membrane (230, 254, 326). It appears from a number of studies that, upon the treatments described, the internal mesosomal membranes (tubules or vesicles) only are extruded and released; the enclosing (sac) membrane appears to evaginate to form part of the protoplast membrane indistinguishable, by present techniques, from the rest of the peripheral membrane. However, there is no evidence at present which determines clearly whether or not the mesosomal sac membrane is included in the membranes isolated as the mesosome-rich fractions; the available ultrastructural data suggest that it is not (254, 326).

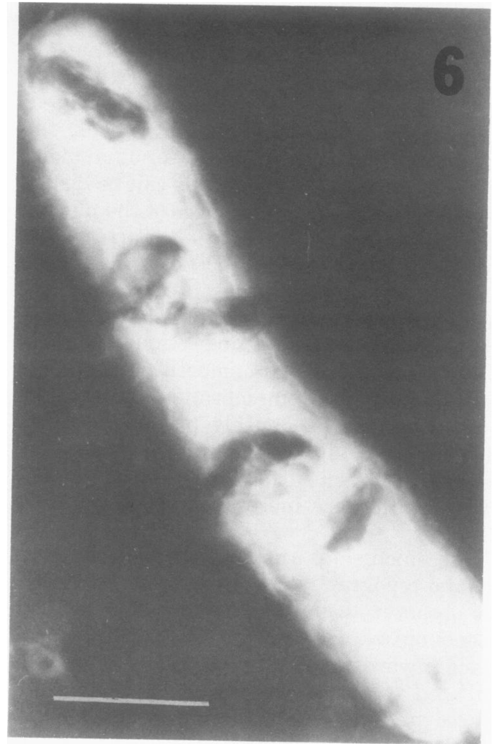
Even though the ultrastructural appearance and organization of the mesosomal membranes may be altered to various degrees by the methods of fixation (33, 284), it seems clear that under some conditions and in certain cells, at least, the "string of pearls" image revealed by negative staining accurately depicts the mesosomal organization (249); these structures can be detected also in unfixed, extruded mesosomes observed as shadowed preparations (248).

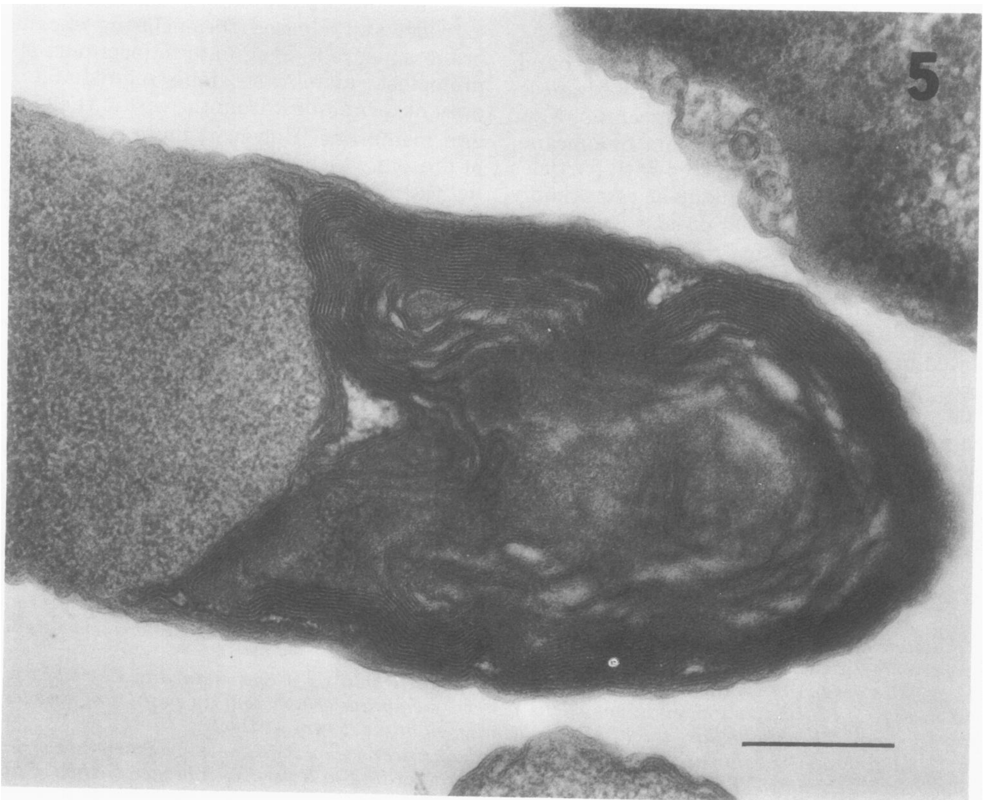
FIG. 1. *Micrograph of a thin section of B. subtilis fixed with glutaraldehyde and OsO_4 and stained with uranyl acetate and lead. Classical profiles of mesosomes can be seen (arrows) as vesicles or vesiculotubules. Bar represents 0.25 μm in this and all subsequent micrographs.*

FIG. 2. *Micrograph of a thin section of Bacterionema matruchotii fixed and stained as described in the legend to Fig. 1. Pocket-like invaginations of the plasma membrane which contain profiles of vesicles are readily apparent. The trilamellar "track" of the plasma membrane is very distinct.*

FIG. 3. *Micrograph of a thin section of Thiobacillus denitrificans showing simple mesosome-like structure and elongated neck attached to plasma membrane. Fixed according to Ryter-Kellenberger method. Stained with uranyl acetate and lead citrate (cf. Fig. 7 and 8).*


4


6


5

Short lengths of straight, more rigid-appearing, cylindrical tubules have been reported by Ryter (248) to occur at intervals along the string of beads. This general structure is consistent with the ultrastructure observed in thin sections of the same cells (230); however, the tubular regions do not appear to be constant features of extruded, beaded mesosomes either. Figures 9 and 10 are micrographs showing the string of pearls appearance of an extruded mesosome of an isolate of *Neisseria gonorrhoeae* (strain Q527) negatively stained with 1% aqueous uranyl acetate. (We are grateful to Paul H. Hardy, Jr., for these micrographs.) The mesosome extends for several micrometers, but no rigid-looking, straight regions can be seen. At higher magnification (Fig. 10), many of the "beads" in the chain appear to have electron-dense centers either due to penetration or overlaying of the stain or possibly due to electron-scattering material enclosed within the vesicle; the latter possibility has been reported by others (17, 315). According to the more or less accepted orientation of a mesosome formed within the sac, this dense material most likely represents cytoplasmic contents (230).

Extruded mesosomes observed as negatively stained preparations have also been described by Fitz-James (79, 80) as being distinguished by their characteristic smooth texture and by Burdett and Rogers (34) as "regularly constricted tubules." The former description is particularly applicable to isolated mesosomal fractions as well (66, 214, 245). There is generally good agreement about the ultrastructure of negatively stained, extruded mesosomes, although Burdett and Rogers (34) reported the presence of membranous sheets distinctly different from protoplast membranes. It was suggested that these possibly represented mesosomal tubules which had opened up and flattened during preparation.

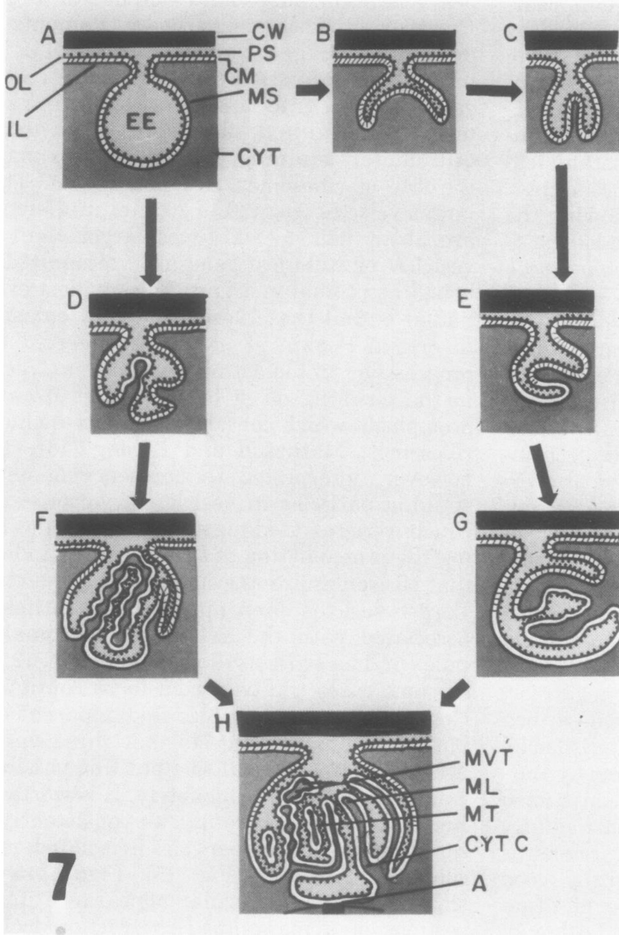
The appearance of extruded mesosomes in thin sections of *B. subtilis* cells suspended in hypertonic medium during treatment with lysozyme is illustrated in Fig. 12. The tubular or tubulovesicular elements between wall and peripheral membrane are consistent with the appearance of the mesosomes seen in situ (Fig. 1). However, other larger vesicular elements are present also (Fig. 12, arrow, and Fig. 13). The nature and derivation of these vesicles, which generally appear to be filled with electron-scattering material and, more specifically, to contain densely staining particles approximately the size of ribosomes, are not known. These larger vesicles (ca. 120 to 200 nm in diameter) are about four to six times larger than the vesicles or tubules generally recognized as being mesosomal in origin. The simplest explanation is that these vesicles are "cytoplasmic" in origin; Popkin et al. (230) suggested that progressive protoplasting of *S. aureus* results in the pinching off of large vesicles from the protoplasts which contain cytoplasm including ribosomes. Matheson and Kwong (181, 182), however, interpreted the presence of dense-staining particles in vesicles of various sizes, which were released together with tubules during the protoplasting of *B. subtilis*, to indicate that ribosomes are associated with mesosomes. Larger vesicles often appear to be intimately associated with the extruded mesosomal tubules in *B. subtilis* as illustrated in Fig. 12, and in some cases the two seem to be contiguous. Consideration of their size and apparent cytoplasmic contents suggests that these vesicles are not the mesosomal sac, but this possibility cannot be ruled out completely. It is worth noting that in our laboratory we consistently observe dense-staining particles in isolated, mesosome-rich fractions (Fig. 15). Figure 15 also shows the tubulovesicular appearance in thin sections of a mesosome-rich fraction isolated from *B. subtilis*. The trilamellar appearance of the enclosing membrane is readily apparent.

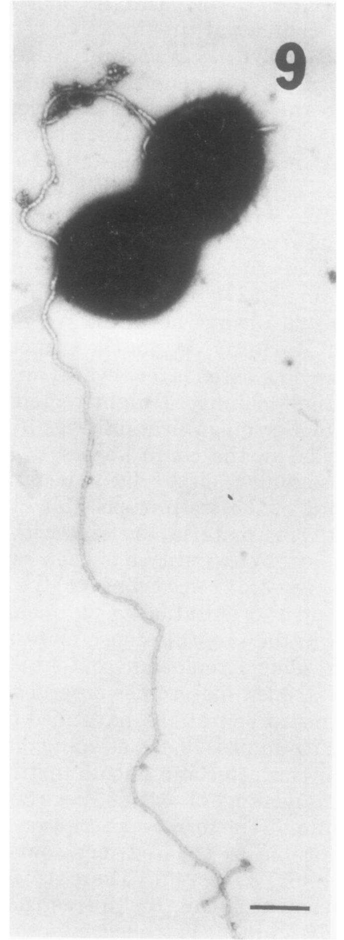
There is little doubt that the mesosomal elements of gram-positive bacteria are attached at one or more sites to the peripheral membrane (244, 248). This is most easily seen during plasmolysis and/or protoplast formation. Characteristically, thin sections reveal mesosomes as a chain of interconnected vesicles attached to the protoplast membrane (34, 230, 248, 249). Burdett and Rogers (34) clearly detected vesicles bounded by a unit membrane when polyethyl-

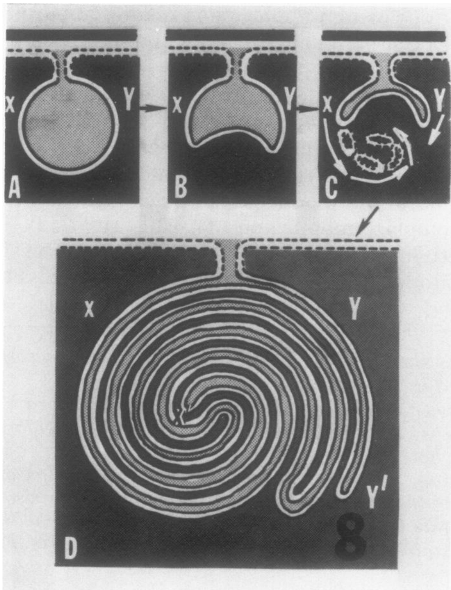
FIG. 4. *Micrographs of a thin section of T. denitrificans showing "inverted Y" form of simple mesosome-like body (cf. Fig. 7 and 8). Fixed and stained as described in legend to Fig. 3.*

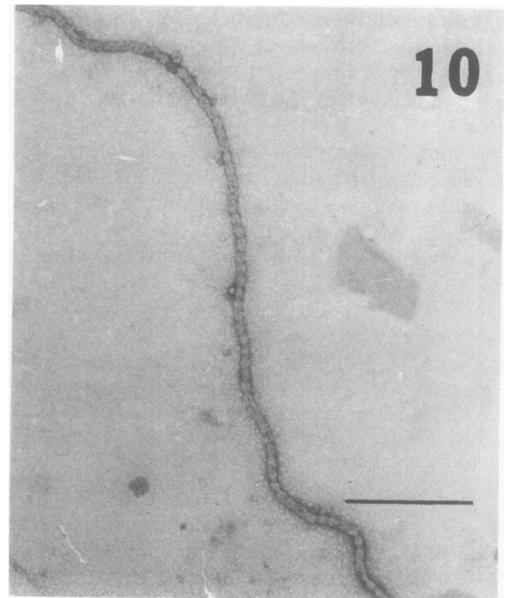
FIG. 5. *Micrograph of a thin section of E. coli O111$_a$ grown at 40 C, fixed and stained as described in the legend to Fig. 1. A huge, polar whorl of "extra membrane" is illustrated. The trilamellar "unit membrane" track of the membranes is visible in large regions of the whorl.*

FIG. 6. *T. denitrificans negatively stained with potassium phosphotungstate, pH 6.5. Penetration of the stain at localized regions represents sites of invagination of the plasma membrane, presumably mesosomes. Apparent size and numbers of invaginations revealed by this technique do not correspond entirely with the impression of only a few small mesosomes of simple organization given by analysis of thin sections (cf. Fig. 3 and 4).*


A
CW
PS
OL
CM
MS
IL
EE
CYT
B
C
D
E
F
G
H
MVT
ML
MT
CYT C
A
7


9


X
Y
A
B
C
D
Y'
8


10

ene glycol was used as a stabilizing agent. The extent to which extruded tubules or vesicles remain linked to the protoplast membrane (Fig. 14) has been shown to be dependent to a high degree upon the concentration of divalent cations present as well as upon the nature of the hypertonic stabilizing medium (67, 216, 235). However, elongated gymnoplasts of *B. subtilis* and *B. megaterium* formed from cells grown in acidic medium (pH 5.5) are more stable and tend to retain mesosomes (213, 318) in both hypotonic and hypertonic media; this effect appears to differ from the stabilizing influence of Mg^{2+} (245). It is of interest that the effective Mg^{2+} concentration appears to vary with different species (216, 235).

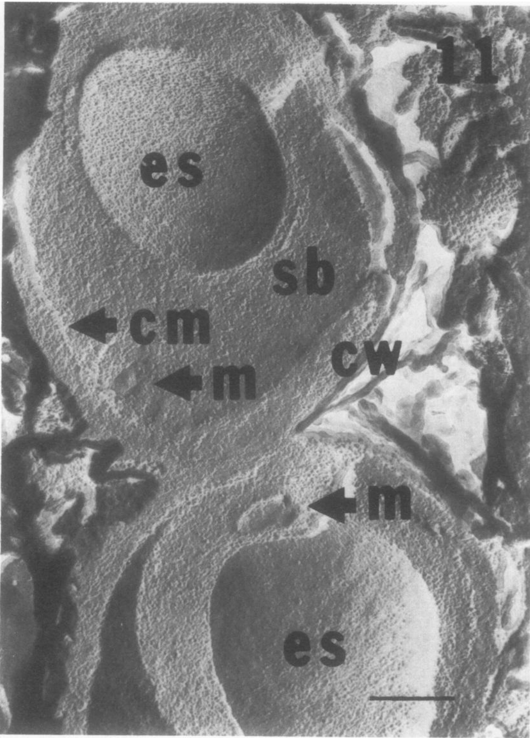
Structural and functional relationships between the mesosome and other components in addition to the plasma membrane need to be considered. No mechanism has been proposed which, in our opinion, adequately explains the extrusion of the contents (vesicles and tubules) of the mesosomal sac into pockets between the wall and the peripheral (or perhaps, sac) membrane upon plasmolysis. Unquestionably, osmotic factors are involved. However, one is forced to ask why the sac contents and the sac membrane itself are displaced toward the cell surface, whereas the peripheral membrane retracts. Is it not possible that mesosomal tubules and vesicles are not so much extruded during plasmolysis but, rather, are restrained from retracting because they are physically attached at some point or points to other components of the cell envelope or to the plasma membrane? Rogers (244) has shown that the mesosomal contents of *B. licheniformis* are in close contact with the cell wall. We have examined random thin sections of *B. subtilis* in a time-sequence study of protoplast formation. The mesosomal elements extruded early during protoplast formation appeared to be linked in some manner to cell wall components as well as being connected to the plasma membrane (Fig. 12). None of the models presented to date give serious consideration to the possible direct, physical association of mesosomes with cell wall components, although it remains a possibility that mesosomes function in cell wall synthesis (see below, biological functions). It would seem that any physical and/or chemical bonds that may occur between mesosomes and cell wall components are broken upon prolonged protoplasting.

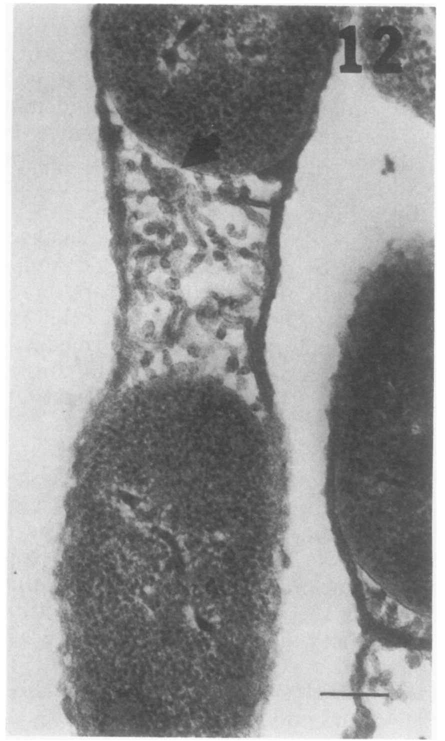
Related to this problem are the differential responses of gram-negative bacteria and different species of bacilli to changes in osmotic condi-

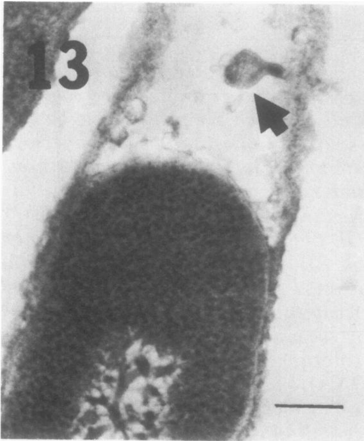
FIG. 7 AND FIG. 8. *Diagrammatic representations of possible models of mesosome formation. Figure 7 illustrates a wide variety of mesosome-like forms seen in both gram-positive and gram-negative bacteria but primarily in gram-positive cells. A through C are simple structures all of which are proposed to be derived from the initial invagination of the plasma membrane to form the sac (A). Collapsing of the spherical sac could result in the forms shown in B and C. D-H and E-H illustrate two of many possible ways in which complex vesiculotubular and lamellar forms could arise by secondary invaginations of the initial sac membrane. Sidedness of plasma membrane is maintained in all structures regardless of complexity. CW = cell wall, PS = periplasmic space, CM = cytoplasmic membrane, OL = outer leaflet of plasma membrane (indicated by dark particles), IL = inner leaflet, CYT = cytoplasm, EE = external environment. Plasma membrane including sac is indicated by hash marks; newly synthesized membrane at sites of secondary invaginations are unmarked (D-H and E-H). MVT = mesosome vesiculotubules, ML = mesosome lamella, MT = mesosome tubule, CYT C = cytoplasmic channel (formed by secondary invagination of sac so that cytoplasmic components are potentially compartmented by these membranes even though the system is not completely closed), A = proposed site at attachment of chromosome (DNA) to mesosomal sac. Figure 8 shows a possible mode of formation of complex, multilamellar whorls observed in such cells as E. coli O111$_a$ (cf. Fig. 5). Panels A through C show forms similar to those in Fig. 7 representing the development of a flattened saccule from the spherical mesosomal sac. X and Y represent end points of saccule and potential sites of extension (growth) of intracellular membranes. For simplification, the diagram illustrates major extension at X by sequence of arrows and vesicles, although some extension is shown at Y also as indicated in panel C. Panel D shows a complex multilamellar structure formed primarily by the growth and infolding of the flattened saccule upon itself by the extension of X to X' and Y to Y'. Such a structure represents a closed system maintaining a distinct outside (light grey) and inside (darker). The potential for concentrating and/or compartmenting constituents of the external milieu and of the cytoplasm through the formation of complex channels separated by membranes which retain original asymmetric topography (sidedness) is an important feature of this model.*

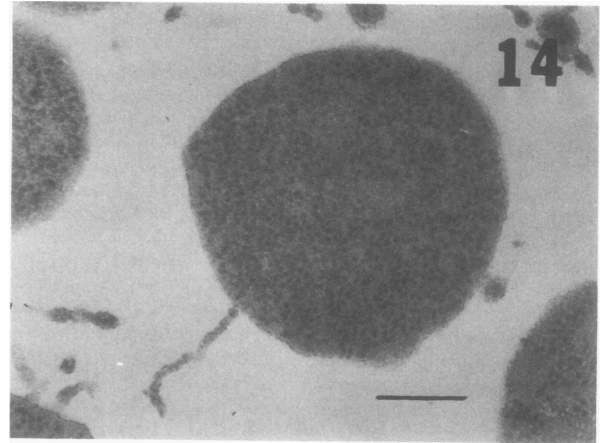
FIG. 9. *Extruded mesosome of Neisseria gonorrhoeae. Fresh isolate (strain Q527, Johns Hopkins Hospital). Cell taken from a 16-h subculture, negatively stained with 1% aqueous uranyl acetate. The extruded mesosome is over 6 μm in length and shows the vesiculotubular "string of beads" substructure consisting of interconnected vesicles. (Micrograph courtesy of Paul H. Hardy, Department of Microbiology, Johns Hopkins University School of Medicine.)*

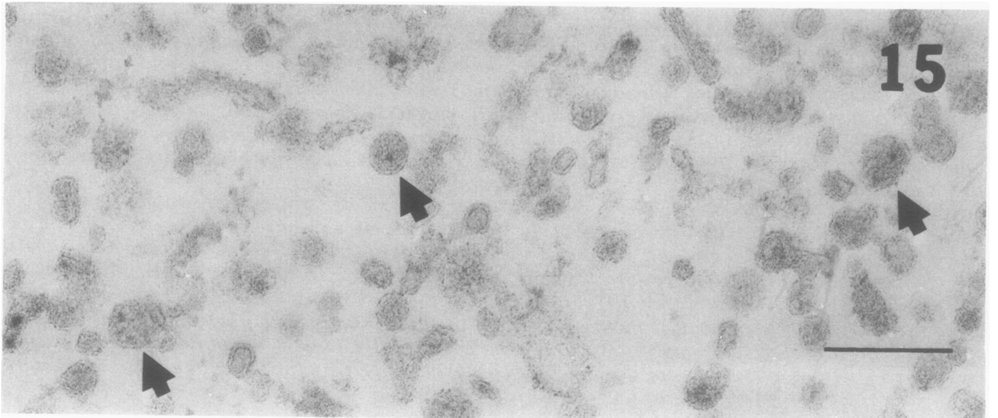
FIG. 10. *A portion of the extruded mesosome shown in Fig. 9 shown at higher magnification. Regular constrictions forming vesicular appearance are seen more readily at this magnification. Electron-dense regions are seen within or on many of the vesicular elements comprising the vesiculotubular mesosome.*


11
es
sb
cm
m
cw
m
es


12


13


14


15

tions and to different plasmolyzing agents (326). The fact that Cota-Robles (55) did not see mesosomes between the wall and plasma membrane in plasmolyzed *E. coli* B cells, whereas this is a common feature of *B. megaterium* and other bacilli, has been interpreted by Weibull (326) to mean that the organelles in these cells are not strictly analogous. The differences in the localization of oxidation-reduction reaction products in cytochemical studies comparing gram-positive and gram-negative cells may reflect functional differences also (312, 316, 317). However, the relative resistance of gram-positive bacteria to plasmolysis compared with gram-negative bacteria has been interpreted as being due to the strong adherence of the plasma membrane to the cell wall in the former cells (326). Such an interpretation is consistent with the idea that such "bonding" is mediated by mesosomes, which are generally more numerous and more highly developed in gram-positive bacteria than in gram-negative cells.

Further investigations along these lines are warranted, but at present alternative explanations cannot be dismissed. For example, "fibrils" and "pegs" have been described in thin sections and in freeze-cleaved cells which may "anchor" two parts of the envelope (membrane-cell wall) together in some unknown manner (244, 245). In addition, Bayer (17) has found that, in plasmolyzed *E. coli* B, duct-like extensions of the plasma membrane remain attached to the cell wall. It was estimated that between 200 and 400 of these localized wall-membrane associations are present per cell. It is not known whether or not these are related to the connections or bridges between plasma membrane and wall reported in other organisms (134). Furthermore, Schnaitman (272) recently presented data which indicate that cell wall-enriched fractions contain fragments of plasma membrane specifically attached to the wall. Extraction of various subcellular fractions with Triton X-100 and comparative analysis of the solubilized proteins by polyacrylamide gel electrophoresis indicated that that part of the membrane attached to the cell wall is quantitatively different in composition from the rest of the plasma membrane. It must be pointed out that the recent findings of Rucinsky and Cota-Robles (246) contrast markedly with the generally accepted differences in mesosomes of gram-positive versus gram-negative bacteria discussed above. These workers found mesosomes of exponentially growing *Chromobacterium violaceum* strain CHRPP to be ultrastructurally very similar to those characteristic of gram-positive cells; the mesosomal sac formed by invagination of the peripheral membrane was found, however, to have multiple invaginations which branched to form tubular mesosomes. The model presented was likened in many respects to that of Burdett and Rogers for *B. licheniformis* (34), but, in this gram-negative bacterium, as many as four mesosomes per cell were observed. Upon plasmolysis in 0.3 M sucrose after a single wash in 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (pH 8.0), the mesosomal tubules were extruded into the spaces between the retracted plasma membrane and the peptidoglycan (R) layer of the cell wall. Thus, this particular gram-negative bacterium, at least, contains membranous structures which conform in ultrastructural appearance and in response to physical changes in environment to the criteria used to define mesosomes.

As discussed below in considerable detail, cell fragmentation studies of several different gram-positive bacteria by a number of laboratories indicate that peripheral (protoplast) membrane-enriched fractions differ enzymatically and in chemical composition from mesosome-enriched fractions. However, with the possible

FIG. 11. *Freeze-etched sample of Bacillus anthracis (noncapsulated strain) undergoing sporulation after growth on nutrient agar in the presence of CO_2 at 37 C for 48 h. CW = cell wall, CM = cytoplasmic membrane, ES = endospore, SB = spore body, M = mesosome.*

FIG. 12. *Thin section of mesosomes extruded between cell wall and plasma membrane during treatment of B. subtilis with lysozyme. Vesiculotubular appearance of extruded mesosomes can be recognized. Larger vesicular elements containing electron-dense particles (arrow) also are present. Fixed and stained as described in legend to Fig. 1.*

FIG. 13. *Thin section showing large nonmesosomal membranous vesicle (arrow) between wall and plasma membrane of B. subtilis treated with lysozyme. Neck apparently connects large vesicle to cell wall rather than plasma membrane. Fixed and stained as described in legend to Fig. 1.*

FIG. 14. *Thin section of protoplast of B. subtilis showing extruded mesosome attached to plasma membrane. Tubular or vesiculotubular appearance is reasonably well preserved, resembling closely extruded mesosomes illustrated in Fig. 12. Fixed and stained as described in the legend to Fig. 1.*

FIG. 15. *Thin section of mesosome-enriched fraction isolated from B. subtilis by the method of Ferrandes et al. (73). Tubular and vesicular profiles enclosed by distinct trilamellar "unit membrane" predominate. In many profiles, small, electron-dense particles (ribosomes?) are present; material of intermediate density presumably is cytoplasmic in origin.*

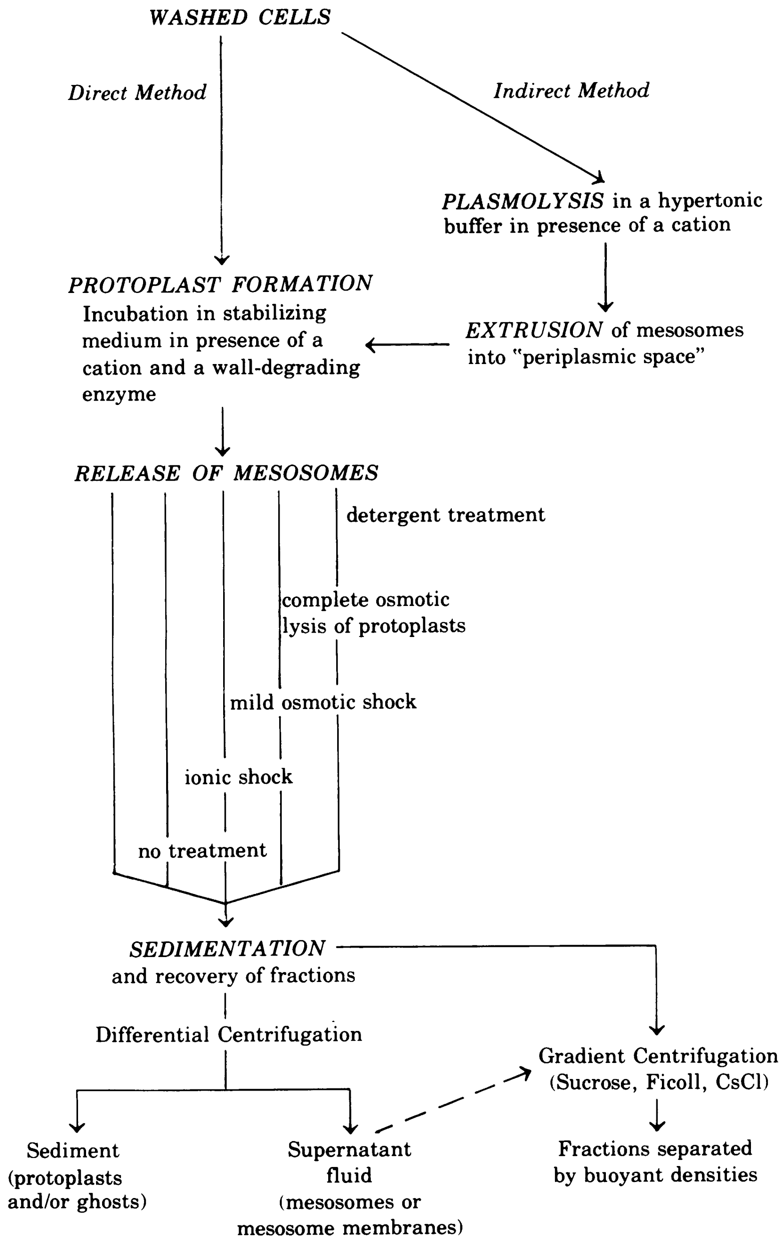


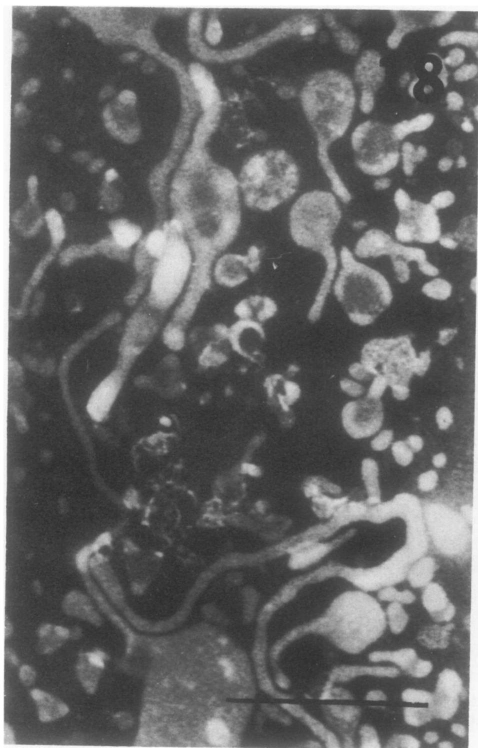
FIG. 16. *Generalized composite of major methods used to isolate fractions enriched in mesosomes from bacterial cells.*

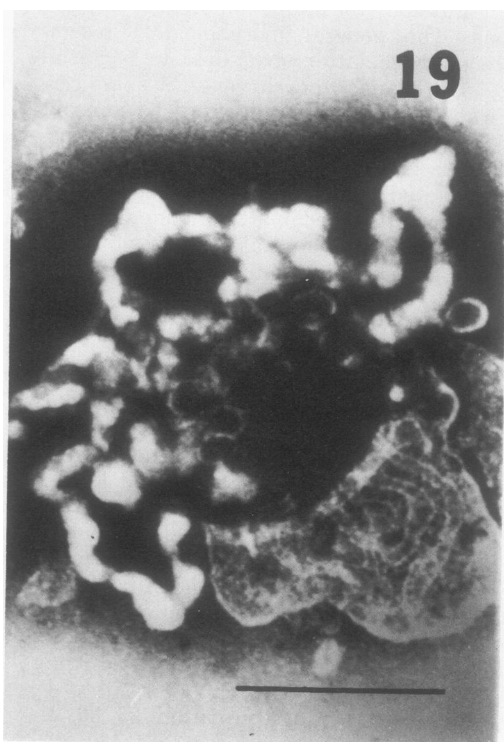
exception of lipoteichoic acid (139), no clear-cut chemical or enzymatic markers have been shown to be associated specifically with mesosomes. Thus, it has been necessary to rely heavily upon electron microscopic analysis to monitor the isolation and to determine the relative purity of mesosome-enriched fractions. Figure 16 outlines the major methods used to obtain mesosome-enriched, subcellular fractions.

Negative staining has been of great assistance in monitoring the fractionation procedures. Comparison of the ultrastructure of fractions as thin sections versus negatively stained preparations reveals some differences; the latter appear to have lost much of the vesicular nature and are more tubular and smoother in outline (95, 214, 216, 235). Also, to some degree in situ morphology is altered (34) upon isolation

when viewed in thin sections (94, 230, 235). Figure 17 is a micrograph of a mesosome fraction isolated from *M. lysodeikticus* negatively stained with ammonium molybdate (214). (We are grateful to J. D. Oppenheim and M. R. J. Salton for this micrograph; reproduced from Biochim. Biophys. Acta **298**: 307 [Fig. 8], with permission of the publisher.) The tubular and spherical structures typical of most negatively stained mesosomal fractions appear filled or inflated and differ decidedly from the flattened, often particle-studded appearance of the protoplast (peripheral) membranes. Figure 18 is a micrograph of isolated "extra membranes" of *E. coli* $O111_a$ (103, 328). The similarity in appearance to the mesosomal fraction in Fig. 17 is obvious. However, the *potential* error inherent in relying only on ultrastructural evidence for identifying subfractions is indicated by Fig. 19. This micrograph is of a negatively stained (potassium phosphotungstate, pH 6.5) "mitoplast" (a mitochondrion stripped of outer membrane) derived from a rat liver mitochondrion by treatment with digitonin (105, 274). Membranous structures of two distinct types can be seen in this micrograph. A flattened portion of the inner membrane revealing cristae is present in the lower right quadrant of the picture, whereas extended tubulovesicular components, unpenetrated by the stain, can be seen in other regions. The general morphological resemblance of these latter structures to bacterial mesosomes is apparent. However, it is *not certain* whether these different profiles represent variations in the same membrane due to preparative procedures, e.g., undisrupted membranes inflated with retained matrix versus flattened, empty inner compartment membranes, or truly different structural entities which together comprise the "inner" mitochondrial membrane. This latter possibility will be considered further in the section on evolutionary considerations of bacterial and mitochondrial membrane systems (below). (See also 330.)

The picture emerging, which is based primarily on the vast literature describing ultrastructural investigations of mesosomes and which is consistent with our extremely limited knowledge of the biochemical and chemical properties of mesosomes, is that these organelles are most likely multifunctional in nature. Difficulties in interpreting the ultrastructural data remain, and some further consideration must be given here to these problems (35, 36, 174, 246). Many conflicting data have been reported concerning the effects of temperature (52, 130, 273, 334), oxygenation (54, 64, 312), nutrition (35, 36, 91, 99, 122, 318), physiological age (57, 68, 120, 282, 322), divalent cations (28, 235, 245), and fixation procedures (33, 124–126, 199, 200, 284) on the presence or absence, size, and ultrastructural appearance of mesosomes in a variety of bacterial cells. Consistent with the variation in the form of mesosomes observed is the idea that this is due greatly to the quality of fixation; this question has been discussed in detail by Nanninga (202) with respect to differences in chemically fixed cells and in cells fixed by rapid freezing for freeze-cleaving. However, detailed studied regarding the various parameters listed above have not yet been conducted on a large enough sample of bacterial species, grown and processed for electron microscopy under a variety of conditions, to permit this to be accepted as a complete and final answer. It may be misguided to expect that mesosomes from all cells and/or those formed under different conditions should be identical in appearance. An analogous situation may be that of the mitochondrion. It is well recognized that mitochondria from different cell types exhibit variations on the basic theme of a two-membrane, two-compartment topology; cristae, for example, vary in morphology from tubular (adrenal cortex or protozoa) to "shelf-like" (rat hepatocytes), and differences in specialized biochemical function are also recognized (165). In addition, "developmental" ultrastructural changes in mitochondria have been reported in numerous in situ studies (13, 62, 104). Also, major morphological alterations can be induced in isolated mitochondria by varying incubation conditions (101, 110, 217, 223). Just as it is now realized that it is a gross oversimplification to refer to "the mitochondrion," it seems likely also that future studies will prove it just as erroneous to refer to "*the* mesosome." In our opinion, a satisfactory solution to the confusing and conflicting interpretations of the effects of various conditions on mesosomal ultrastructure discussed above cannot be achieved until more quantitative approaches are applied which, we hope, will yield answers to specific questions. For example, the optimal Mg^{2+} concentration needed to "stabilize" mesosomal membranes from different bacteria appears to vary (see section on isolation and characterization). However, it has not been ascertained to what extent the effects observed also reflect general osmotic changes. What is the effective concentration with respect to milligrams of cell protein (or even more importantly, membrane protein)? Are the cells being compared grown under similar osmotic conditions? Is the requirement specific for Mg^{2+} or are other ions effective? What percentage of the Mg^{2+} added to stabilize the mesosomes is bound to the structures? Until answers to these and other fundamental questions are obtained, con-


18


19

flicting reports concerning mesosomal ultrastructure can be expected.

FORMATION OF MESOSOMES IN BACTERIAL CELLS

Evidence pertaining to the formation and growth of mesosomes comes mainly from the ultrastructural examination of serial sections in an electron microscope. A generalized sequence of events leading from a simple structure to the formation of a fully developed mesosome may be reconstructed partly on the basis of such studies. Figure 7 is a diagrammatic representation of the origin, growth, and differentiation of a bacterial mesosome. This scheme represents a composite based on the observation of a wide variety of organisms and incorporates some features of earlier models (34, 240, 244, 245, 249). The nature of stimulus or stimuli involved in the initial step of membrane invagination is unknown, and the physiological events leading to changes in size, location, and complexity are not understood.

Basically, this model depicts a series of events that start with infolding of the peripheral membrane to form a mesosomal sac and end with the attachment of the genome (A in Fig. 7H) to a fully developed mesosome. During development, mesosomal lamellae, vesicles, and tubules result from further invaginations of the mesosomal sac. Active membrane synthesis must be a prerequisite not only for mesosomal origin but also for internal mesosomal diffentiation. The mesosomal sac and the invaginations forming the mesosomal membranes proper retain normal membrane asymmetry but are not viewed as static structures. A requirement demanded of this and other models is that they account for the expulsion of mesosomal vesicles and/or tubules into the periplasmic space of cells undergoing plasmolysis (80, 94, 254, 313, 326).

The initial invagination leads to the formation of the mesosomal sac (MS; see 256) which is presumably penetrated by the external environment (EE) and/or periplasmic space (PS) and is connected to the peripheral membrane by a neck or stalk which may vary in length. B and C represent slight modifications of the simple, spherical mesosome shown in A which changes shape slightly only as a result of various degrees of collapsing of the sphere. Two points are of interest in comparing these forms to the more complicated structures proposed in D-H:

1. In the simple forms, A-C, according to this scheme, the entire membrane is part of and identical to the peripheral (plasma) membrane (indicated by cross-hatching). In D-H various amounts of "new" membrane (no cross-hatching), in addition to that forming the sac itself, must be synthesized to account for the increase in membrane surface. The relative amount of new membrane theoretically would increase with the increase in size and complexity of the mesosome. It is presumed that in some cells simple forms of mesosomes are typical whereas in others development from simple to complex may take place as diagrammed in D-H or E-H. It should be noted that as yet no such detailed sequence of changes (simple to complex) has been recorded, although models for mesosomal formation, growth, and division have been reported (34, 68, 78, 93, 120–124, 147, 245, 249, 256). The exhaustive ultrastructural study of synchronously growing cells over a period of time will most likely be needed to determine such sequential events. Alternatively, it remains a very real possibility also that specific mesosomes of distinct types (e.g., different localization within the cell), organization, and complexity are formed within the same cell at different times in response to changing physiological needs.

2. Concomitant with the increasing complexity of mesosomal structure is the potential for a corresponding greater degree of compartmentation of cytoplasmic constituents within the mesosome itself. Figure 7H represents a complex mesosome containing mesosomal vesiculotubules (MVT), lamellae (ML), and tubules (MT). It is clear from such a picture, diagrammatic to be sure, that cytoplasmic material readily could be contained within mesosomal vesicles or tubules or even compartmented merely as a result of the formation of channels (CYT C)

FIG. 17. *Mesosome-enriched fraction isolated from M. lysodeikticus, negatively stained with ammonium molybdate. The smooth nonparticulate outlines of the vesicles and tubules which appear inflated or filled are characteristic of negatively stained mesosomes. Taken from Oppenheim and Salton (214) and printed with permission of the authors and publisher.*

FIG. 18. *A fraction isolated from E. coli O111$_a$ enriched with "extra membranes" present in this cell grown at 40 C. Negatively stained with potassium phosphotungstate, pH 6.5. Similarity in outline, morphology, and staining properties to mesosomes (cf. Fig. 17) is clear.*

FIG. 19. *Mitoplast isolated from rat liver mitochondria (101, 102, 274) negatively stained with potassium phosphotungstate, pH 6.5. Extended tubulovesicular portions of the inner membrane generally resemble negatively stained bacterial mesosomes (cf. Fig. 17).*

formed by the secondary invaginations. Solutes in the external medium and/or the periplasmic space (PS) also could be concentrated because of the channels formed by the mesosomal membranes. Throughout the organelles, however, the "sidedness" of each membrane is maintained; i.e., the outer (OL) and inner leaflets (IL) of both the peripheral and mesosomal membranes remain exposed to the external medium and to the cytoplasm, respectively. The significance of the connections with the environment external to the membrane on one hand, and with the cytoplasm on the other, is not clear. It could be that, by virtue of such connections, the mesosome represents a region of the membrane that is involved in secretory and/or transport activities as yet unknown.

Several aspects of the model presented in Fig. 7 warrant further consideration. A number of reports in the literature (34, 68, 120, 202) comment upon the asymmetry of the plasma membrane. In thin sections, the outer leaflet appears more electron dense than does the inner (cytoplasmic) leaflet. If, as has been postulated here and in other models (240, 244, 249), the mesosome sac and contents are formed by a series of invaginations while the "sidedness" of the plasma membrane is retained, then it might be expected that this asymmetry would be seen in the membranous components of the mesosome; such asymmetry generally has not been reported. This may be the result of a number of effects. First of all, the factors contributing to the apparent asymmetry of the plasma membrane have not been clearly established. Certainly, the *relative contrast* of the inner leaflet is reduced as a result of its being in tight juxtaposition to the cytoplasm. Components of the cell wall very likely contribute to the asymmetric appearance of the plasma membrane but not to the mesosomal sac and contents. Second, even though the various membranes are thought to be contiguous, it is not established that they are of precisely the same chemical composition, especially after fixation for electron microscopy. Third, possible differential effects of chemical and/or enzymatic treatment during preparation for isolation of mesosomal and protoplast membranes are not known in detail. It cannot be determined now whether or not the apparent lack of asymmetry in the membranes of mesosomal elements is of significance with regard to the fundamental structure and function of these organelles. The mesosomal sac and its invaginations (Fig. 7H) represent a hypothetical composite including elements described for different bacterial species; most studies do not show the complexity indicated here. However, Rucinsky and Cota-Robles (246) observed multiple invaginations of the mesosomal sac in *Chromobacterium violaceum*. If this complexity (Fig. 7H) were a common feature of most mesosomes, extrusion should result in bunches of tubules attached to the protoplast membrane. This is not the usual case, but the appearance of the tubules extruded from *B. subtilis* early during protoplasting (Fig. 12) is more complex than generally is reported when the cell wall has been completely removed.

The exact sequence of events culminating in the formation of a mesosome in a particular region of the peripheral membrane at a particular time is largely a matter of speculation. From studies of Higgins and his associates (120–123), it appears that the formation of septal mesosomes in *Streptococcus faecalis* may be coordinated by division of the bacterial genome and may be related to the growth of a septum and cross wall between the two daughter cells. The septal mesosome represents one type of mesosome which seems fairly universal in the gram-positive bacilli (244). However, individual bacterial cells may contain several mesosomes located at different sites, although considerable controversy exists concerning this point (34, 124, 148, 246). It appears that variation in the numbers and sites of mesosomes, as well as in their ultrastructural appearance (see above), may be related to species, age of the cells, and methods of fixation for electron microscopy (30, 33, 34, 120–122, 124–127, 198–202).

Figure 8 is a diagram indicating one way by which complex lamellar mesosomes or mesosome-like structures (cf. Fig. 5) might be formed from simple invaginations of the plasma membrane. A, B, and C in this sequence are essentially the same as those in Fig. 7 in which the sac formed from the primary invagination of the plasma membrane collapses to form a flattened saccule (cf. Fig. 4) which in certain profiles appears double-lobed or as an inverted "Y" (Fig. 8C). In this scheme as in Fig. 7, further differentiation depends upon the synthesis and "growth" of the membrane. However, Fig. 8C (dotted lines) suggests that an increase in membrane surface occurs not by means of additional, secondary invaginations of the sac as in the model in Fig. 7, but rather via the infolding of the collapsed saccule membrane on itself (Fig. 8D). Although both edges (X and Y) of the collapsed saccule could be extended, for demonstrative purposes here growth is largely limited to one side, $X \rightarrow X'$ (Y to Y' being relatively minor). The formation of new membrane is essential for models shown in both Fig. 7 and Fig. 8; however, in neither case

can it be implied that synthesis of membrane components occurs specifically at the sites of "growth." This idea is not supported by the evidence now available.

The proposed formation of lamellar membrane structures as shown in Fig. 8 differs from that of others in certain respects, e.g., that of Pate and Ordal (227), but fundamentally is similar in that the "sidedness" of the membrane is maintained as in Fig. 7. Figure 8 is unique in that it could explain high local concentrations of external and internal metabolites, cofactors, ions, etc. at specific cellular sites in close contact with membrane surfaces. Compartmentation is achieved merely by the complex folding of the membrane, providing, in a sense, a single closed system formed by one membrane. That complex lamellae actually are formed in this manner is highly speculative at this time; however, the examination of serial sections through "extra membrane" complexes in *E. coli* $O111_a$ suggests that this could be one means by which they are formed, although other possibilities have not been eliminated. As in the model by Pate and Ordal (227) apposition of the invaginating membranes (not shown in Fig. 8) forming the large lamellar structure could provide more complex compartmentation with respect to determining the contents enclosed within the mesosomes.

The degree of internal differentiation of a mesosome, if it is shown that such is not primarily artifactual because of the conversion of one type of membrane to another (244, 313), may be related to the possible multifunctional nature of this organelle. Thus, whereas the septal mesosome may exhibit complex lamellar, tubular, and/or vesicular organization (33, 124–126, 202), the sac-like mesosomes implicated in the excretion of penicillinase in *B. licheniformis* are largely devoid of internal structures (96). Highton (127), however, found no correlation between the presence of mesosomes and the capacity to excrete penicillinase by various strains of *B. licheniformis* and, in general, observed more extensive mesosomal development. In addition, the internal complexity may be related to the number of mesosomes per cell: the more numerous the mesosomes in a cell, the less complex their structural organization seems to be. Vesicles, tubules, and lamellae in some cases all seem to result from an invagination of the mesosomal sac. It is uncertain whether they originate from a single infolding or whether several different regions of the sac may invaginate at the same time. The latter possibility would seem likely in the case of mesosomes with "mixed" internal organization. However, the entire question of the ultrastructure of the mesosomes remains controversial.

ISOLATION AND CHARACTERIZATION OF "MESOSOME-RICH FRACTIONS"

Knowledge of the chemical and biological properties of the bacterial mesosome has been limited largely because of difficulties in finding methods which would cleanly separate these organelles or their membranes from the peripheral (plasma) membrane. Early attempts (259) to separate these two membranes failed to achieve the isolation and separation of the mesosomes from plasma membranes of *M. lysodeikticus*. Meaningful comparisons of the chemical composition and/or enzymatic components of the peripheral versus mesosomal membranes require that each of the fractions be highly enriched if not "pure." The failure to establish specific "mesosomal markers" (257) makes it particularly difficult to separate and to ascertain the homogeneity of a given mesosomal fraction; this problem is magnified by the general tendency of biological membranes to vesiculate upon disruption. An additional problem presents itself in the fact that, at least in certain bacteria, e.g., *B. licheniformis* (34, 124–127, 244, 245), mesosomes themselves appear not to be structurally homogeneous but are composed of both vesicular and tubular elements. For these reasons, it seems prudent at the present time to talk about the isolation of "mesosome-rich" fractions rather than "mesosome" fractions.

More recent attempts at the separation of the mesosomes from peripheral membranes seem to be more successful and reproducible (216, 225, 230), a fact which marks significant progress and improvement in fractionation techniques. At the moment, only knowledge of the sequential structural effects of isolation procedures, e.g., on the release of components, and electron microscopic analysis of fractions can be applied to evaluate the separation of the mesosomes. However, it is important at this stage to begin to apply the criteria of deDuve outlining the use of "marker enzymes" and/or chemical markers in all analyses of subfractions (60). To date, this generally has not been possible, but it should be remembered that the localization of an activity or component in a fraction requires three types of evidence, coupled with ultrastructural analysis: (i) the fraction must have a high *specific activity* of the marker enzyme(s), (ii) a high percentage of the *total activity* must be found in the fraction, and (iii) essentially quantitative *recovery* of the initial (whole cell or protoplast) activity must be accounted for in the

various fractions. One additional point needs to be mentioned. It has not been established in all cases whether investigators are isolating and analyzing *mesosomes* (the organelles) or the mesosomal *membranes*. As has been pointed out earlier, the complex ultrastructure of some mesosomes is indicative of a high degree of compartmentalization within the mesosome itself. Thus, investigators should consciously concern themselves with these questions: Does the isolated mesosome-rich fraction consist of the mesosomal membranes or of the mesosomes? Are other nonmembrane components, albeit functional constituents of the organelles in situ, also present in the fraction, perhaps as soluble proteins enclosed in the tubules or vesicles of the mesosome? Simple mathematical approaches to answering these questions with regard to known enzymatic activities are available (6), but, unfortunately, these have not been applied to any extent in the analysis of microbial systems.

Studies dealing with the isolation of "mesosome-rich" fractions from bacteria have been limited almost entirely to gram-positive species for good reasons. The most promising advances in membrane-mesosome fractionation have been made in studies utilizing *B. subtilis* (72, 73, 225), *B. megaterium* (68, 79, 80), *B. licheniformis* (234, 235, 267), *Listeria monocytogenes* (95), *Lactobacillus casei* (300, 302), *Micrococcus lysodeikticus* (67, 70, 216, 256–262), or *Staphylococcus aureus* (230, 297). These bacteria in general are valid choices for membrane studies (256) since their cell walls virtually can be removed completely by the action of wall-lysing enzymes. Such treatment forms osmotically fragile protoplasts from which membrane ghosts can then be obtained. The complex cell envelope of gram-negative bacteria is more difficult to remove enzymatically. Thus, although the spheroplasts formed are osmotically fragile, they retain components of the cell wall. On the whole, these cells do not lend themselves readily to fractionation of the inner membrane. However, much progress has been made in separating "outer" and "inner" membranes from gram-negative species (215, 272, 332). Also, Greenawalt et al. (103, 328, 329) have succeeded in isolating a fraction highly enriched in the intracytoplasmic membranes of *E. coli* $O111_a$. More sophisticated techniques are required to obtain a cell membrane fraction free from cell-envelope contamination, and a successful separation has been achieved in very few instances (for detailed discussion of the subject, see 257).

Unfortunately, a generalized procedure for the isolation of bacterial mesosomes does not exist at present. A careful survey of the literature indicates that each laboratory has developed and elaborated its own special technique, modifying details to best suit the particular bacterial species being investigated. Such efforts are commendable and probably are essential, particularly when the aim is to obtain membranes in their most "native state" (257), but a critical comparison of the effects of the isolation procedures on the results obtained becomes virtually impossible. Since the isolation of mesosome-rich fractions involves a specific sequence of events, namely the release of mesosomes from the cell and separation of fractions by density-gradient centrifugation followed by recovery of the fractions from the gradients, the major steps in the procedures can be outlined. The fractionation procedures usually are evaluated by means of electron microscopy (most effectively at each step in the procedure), and the distribution of enzymatic activities is determined whenever possible. However, as pointed out above, complete reliance on ultrastructural criteria as a basis for determining the success or failure of a fractionation method is not without hazards.

Release of Mesosomes from the Cell

Fitz-James (79, 80) found that very small amounts of free mesosomal vesicles could be recovered when cells of *B. megaterium* KM were treated with lysozyme, under controlled conditions, and then centrifuged on continuous or discontinuous gradients. Apparently mesosomes were not extruded and released from the protoplasts but remained attached; lysis of protoplasts increased the yield and "relative size" of the mesosomal fraction. Fitz-James subjected the stabilized protoplasts to osmotic shock and then layered the entire protoplast lysate on sucrose gradients made in Tris (10^{-2} M) buffer, pH 7.4, containing KCl (10^{-2} M) and $MgCl_2$ (1.5×10^{-3} M). Two membrane zones were observed: one, at the interface, containing mesosomal vesicles, and the other, at the bottom, composed of protoplast ghosts. Detergents such as Lubrol W in concentrations up to 0.03% and Isodet P-40 (both nonionic surfactants) reduced the recovery of plasma membrane in the pellet and increased the sharpness and density of the interfacial band. The treatment with nonionic detergents, which was presumed to release the mesosomes by breaking the points of their attachment to the peripheral membrane, has not been accepted as a routine in the isolation methods. The damaging effect of detergents even at low concentrations on membranes seemed to be indicated; indeed, Fitz-James himself observed

that the mesosomal vesicles from detergent-treated lysates had a "distorted" appearance in negatively stained preparations.

The separation of a mesosome-rich fraction from a gram-positive bacterium invariably has involved a protoplast stage. The conditions under which protoplast formation takes place seem to play a key role in the effectiveness of release and both quality and quantity of the mesosome-rich fractions obtained. Although this point has been emphasized throughout the literature, few investigators recognized the fact that among the first effects that take place under the conditions used to treat cells with lysozyme is the occurrence of plasmolysis. In 1961 van Iterson (313) showed that mesosomes of *B. subtilis* were extruded into the space (periplasmic?) between the wall and peripheral membrane upon plasmolysis of whole cells; other workers have shown subsequently that this phenomenon occurs with other cells as well. Subsequently, Fitz-James (79) demonstrated that these extruded membranous vesicles could be released into the suspending buffer when cell walls were removed with lysozyme. Despite these reports, it has been acknowledged only recently that the release of mesosomal components is greatly enhanced if cells are plasmolyzed *before* digestion of the wall. Thus, Owen and Freer (216) reported that the amount of membrane released from plasmolyzed cells (in the presence of 10 mM Mg^{2+}) was approximately twice that of control cells. On this basis, the variety of methods which have been employed to form protoplasts can be divided into two general groups (see Fig. 16): (i) "direct" methods in which washed cells suspended in an appropriate hypertonic medium are incubated together with a wall-degrading enzyme (95, 230, 234, 235, 250, 255) and (ii) "indirect" methods in which ample time is allowed for plasmolysis to take place in the hypertonic medium *before* a wall-degrading enzyme is added (66, 67, 216).

Owen and Freer (216) stressed that, if cells are plasmolyzed before wall digestion, a higher Mg^{2+} concentration is required to maintain the stability of protoplasts than when unplasmolyzed cells are used. On the other hand, the release of extruded mesosomes is facilitated by lower Mg^{2+} concentration. For example, Ellar and Freer (67) used ionic shock involving a decrease in Mg^{2+} concentration for detaching the extruded mesosomes of *M. lysodeikticus* from the protoplast membranes.

The choice of ion concentration, e.g., Mg^{2+}, which is used during the protoplast-forming stage may be critical (235, 245) for maximal release of mesosomal components. The optimal Mg^{2+} concentration differs for different microorganisms: Owen and Freer (216) found 10 mM Mg^{2+} optimal for *M. lysodeikticus*, whereas the optimal value was reported to be 20 mM Mg^{2+} for *B. licheniformis* (235) and for *S. aureus* (230). In *B. subtilis* (250) and *Lactobacillus casei* (301), much lower concentrations of Mg^{2+} were used, 0.02 mM and less than 5 mM, respectively.

The exact nature of the effect of magnesium or other divalent cations on the release of mesosomes is not known, although Nachbar and Salton (195, 196) have shown that ionic forces play a role in the association of certain components to the membrane. Both the stability of protoplasts (as indicated by minimal leakage at the optimal cation concentration) and the attachment of extruded mesosomal vesicles to protoplasts are affected. Careful investigation of this effect on protoplasts and mesosomes of *B. licheniformis* by Rogers' group (235, 245) yielded some interesting results. They varied the concentration of Mg^{2+} over the range of 0 to 40 mM. In the absence of Mg^{2+} or in low Mg^{2+} concentration, no stable protoplasts were formed; the membranes appeared damaged and general leakage indicated destruction of some protoplasts. At 20 mM Mg^{2+}, mesosomal vesicles with a normal appearance of strings of beads attached at one point to a stabilized protoplasts were obtained. At Mg^{2+} concentrations higher than 20 mM, mesosomal vesicles tended to adhere or "stick" to the surfaces of protoplasts, and there was a general tendency of protoplasts to "agglutinate." Thus, a skillful manipulation of the ionic environment is a prerequisite for a successful release of mesosomes from bacterial cells. This finding is not unexpected since the effect of divalent cations on the stability of biological membranes has been known and studied extensively in many different systems. In addition, Weibull in his early report describing the stabilization of bacterial protoplasts, found Mg^{2+} to be essential to maintain structural integrity of the protoplasts (325).

Once freed from the surface of protoplasts, mesosomal vesicles appear to retain their stability even in an ion-depleted environment. Owen and Freer (216) reported that mesosomal vesicles from *M. lysodeikticus* appeared unchanged after being washed in buffers free from Mg^{2+} They suggested that the presence of Mg^{2+} in the protoplasting medium may be sufficient to stabilize mesosomal vesicles so that they are not adversely affected during the subsequent washing procedures and gradient centrifugation.

This suggestion is supported by the studies of Lastras and Mũnoz (163), who showed that magnesium ions remain associated with membranes of *M. lysodeikticus* even after numerous washings of these membranes with cation-free buffers. On the other hand, those mesosomal vesicles that are prepared in hypertonic buffers devoid of magnesium or other divalent cations are unstable, as reported in studies of *S. aureus* by Popkin et al. (230).

Another parameter which plays an important role in the release of mesosomes is the nature of hypertonic buffer used for protoplast formation. The advantages of one stabilizing medium over another still must be determined by each investigator at a variety of concentrations (from 0.3 to 2.0 M) for different bacterial species. Although sucrose appears to be the most commonly used stabilizing medium, hypertonic NaCl solutions (230, 297), polyethylene glycol at the minimal concentration of 20% (wt/vol) (235), and Ficoll (95) have been employed for the isolation of protoplasts of different bacteria.

Other parameters tested for their effectiveness in the release of mesosomal membranes from protoplasts were time, mild shearing, and temperature (216). Of these, only temperature (in addition to ionic shock) affected greatly the amount of recovered mesosomal membranes; 30 to 35 C proved optimal for yielding mesosomes from *M. lysodeikticus*.

The means by which extruded mesosomes are detached from the surface of protoplasts is also crucial to the results obtained. The method used by Fitz-James (80), which involves the preparation of total protoplast lysates by means of osmotic shock, is relatively drastic. The problem of fragmentation, vesiculation, and cofractionation of membranes and mesosomes arises during the uncontrolled osmotic shock of protoplasts as a result of undesirable disorganization of membranes. However, under controlled conditions (e.g., 95, 225), undue fragmentation of membranes apparently can be avoided, and the separation of mesosomal vesicles from peripheral membranes can be achieved. Mild osmotic shock (involving 1.5-fold lowering of osmotic strength of the medium), reducing lysis of protoplasts, was skillfully used by Owen and Freer (216) in the preparation of mesosomes from *M. lysodeikticus*. The role of ions in the ionic shock procedure used by Ellar and Freer (67) has been mentioned above. Finally, a number of investigators have found it sufficient merely to centrifuge the stabilized protoplasts under controlled conditions, such that the protoplasts sediment leaving the detached mesosomes suspended in the supernatant fluid (230, 234, 235, 297). Figure 16 summarizes the major steps used in the various methods that have been successful in obtaining a significant release of mesosomal vesicles from bacterial cells.

Separation and Recovery of Mesosomes

After mesosomal vesicles are released from the cells and separated grossly from peripheral membranes by differential centrifugation, the next step in their isolation usually involves density gradient centrifugation, the object being to separate those peripheral membranes or their fragments that remain in the suspending medium after differential centrifugation from mesosomal vesicles. This can be done in a variety of ways and, again, no single fractionation procedure can be singled out as yet as being generally more efficient than another. However, before an analytical fractionation is undertaken, criteria for satisfactory separation and purification of a mesosome-rich fraction must be established. There are several ways of monitoring for "the purity" of gradient fractions. These include electron microscopy of both negatively stained and embedded, thin-sectioned material, and possibly freeze-fractured preparations. In addition, whenever possible, chemical analysis of different gradient cuts and enzymatic assays of gradient fractions should be performed. In fact, every criterion available should be used to judge "the purity" of an isolated mesosome-rich fraction; in several recent studies (216, 225, 297), most of these have been applied.

The uniformity in morphology, e.g., size or degree of fragmentation of membranous vesicles, can be determined qualitatively by skillful electron microscopy. In general, characteristic differences can be observed between the electron microscopic appearance of isolated mesosomal vesicles and that of fragments of the peripheral membrane. In negatively stained preparations, mesosome-rich fractions are characterized by small, uniform, smooth-surfaced vesicles and/or tubules (34, 73, 80, 95, 216, 225, 230, 248). In contrast, fragments of the peripheral membrane appeared as flat, particle-studded sheets of variable sizes and shapes (194, 216, 257). The precise reasons for this difference is not known, but one must ask whether the presence or absence of internal contents accounts for this marked contrast in appearance. (See section on morphology and ultrastructure.) The peripheral membrane fractions are often contaminated by occasional mesosomal vesicles (216) and vice versa. It should be stressed that the extent to which the latter situation occurs

depends largely upon the conditions of forming protoplasts and of releasing the mesosomes. It is observed particularly when fractionation conditions do not favor the stability of the membrane. The chances are that such occasional contamination of mesosome-rich fractions with peripheral membrane fragments may not be detected readily by electron microscopy. Morphological techniques are limited in establishing purity of a given subcellular fraction, as pointed out by deDuve (60). It should be emphasized that the valid application of electron microscopic analysis as a basis for evaluating the separation of these two membranous elements demands *extensive* examination of many samples or sections. Cursory examination will surely be misleading.

Specific enzyme analysis does not suffer from these limitations, provided the detailed study of the distribution of enzyme activities among all the fractions is done, and the sum of activities in all of them corresponds to the activity of the starting material. In addition, enzymatic assays ought to be combined with chemical analyses of all fractions, and the activities should be expressed as relative specific activities. In terms of the fractionation of mesosomal membranes, this means that all recoveries are to be expressed in terms of total membrane content since one is then dealing with membrane-bound enzymes. Thus, it is extremely important to establish whether or not the "mesosome-rich" fraction contains essentially membrane components only. The overall distribution of relative activities is an important indication of purity, particularly since there appear to be few qualitative differences between peripheral and mesosomal membranes and no specific mesosomal enzymes so far have been reported. Certainly, major emphasis should be given to "true" membrane components, as opposed to those of cytoplasmic origin, in comparing the functions of the two membranes. In bacterial membranes biological "markers" are available in the form of phospholipids, carotenoids, menaquinones, cytochromes, and other respiratory enzymes or enzyme complexes (see 257). The problem of determining optimal conditions for the isolation of membranes and membranous structures is of utmost importance in this context, as stressed by Salton (256, 257). Thus, a compromise must usually be made since loss of "membrane markers" into soluble fractions probably indicates breakdown of the structure, yet maximal removal of nonmembrane enzymes and other cytoplasmic macromolecules is desirable.

The problem of structural integrity becomes a critical one when the separation of mesosomal from peripheral membranes is attempted. It must be remembered that conditions found optimal for the stability of peripheral membranes might not be optimal for the stability of extruded internal membranes and vice versa. Will one of the two systems be adversely affected by conditions of density gradient centrifugation? This point may be illustrated by experiments of Fitz-James (79), who did a careful evaluation of the effect of the presence of divalent cations (Mg^{2+}) in gradients on the integrity of membranous structures of *B. megaterium* KM. Mg^{2+}-free sucrose gradients gave a very poor resolution, resulting in a large degree of contamination of mesosomal fraction with fragments of the peripheral membrane. Inclusion of Mg^{2+} at a concentration of 10^{-4} M tended to correct this problem, and the presence of Mg^{2+} (1.5×19^{-3} M) yielded mesosome-rich fractions of the highest quality. Fitz-James worked with total protoplast lysates where the chance of cofractionation and contamination with peripheral membrane fragments is much greater than in methods employing differential centrifugation to separate "intact" protoplasts from released mesosomes. Nevertheless, his observations remain valid. The inclusion of Mg^{2+} ions in gradients may be essential to preserve the peripheral membranes in a physical state such that they can be sedimented reasonably intact. Thus, the possibility of contamination of the "lighter," mesosome-rich band is greatly reduced or avoided altogether. There appears to be a general agreement that released mesosomes are more stable than peripheral membranes; hence, the likelihood of contamination of mesosome-rich fractions with membrane fragments is greater than the reverse situation.

It is apparent that other experimental conditions (e.g., composition of gradients; composition, molarity, and pH of buffers; temperature, length, and speed of centrifugation), in addition to the presence of cations, may influence the stability of membranous structures. In Tables 1 and 2 are listed a number of references to studies in which various conditions were used in different laboratories to separate mesosomes. Ghosh and Murray (95) found that sucrose at concentrations in excess of 0.25 M caused a fragmentation of released mesosomes. Consequently, they included Ficoll in a medium for density gradient fractionation of mesosomes from *L. monocytogenes*. Nevertheless, sucrose seems to be the medium most frequently used, and both continuous and discontinuous sucrose gradients (225, 297) have been employed in different laboratories. Reaveley (234) and Reave-

TABLE 1. *Chemical composition of mesosomal (M) and peripheral (P) membranes of selected gram-positive bacteria*[a]

Microorganism	Membrane	Chemical composition (%)				Reference
		Protein	Lipid	Carbohydrate	RNA	
B. licheniformis	M	44	17	ND	2–10	Reaveley (234)
	P	43–49	18–25	ND	13–15	
S. aureus	M	41	34	4	8	Theodore et al. (297)
	P	56	25	4	15	
M. lysodeikticus	M	30–40	26.1	20[b]	ND	Owen and Freer (216)
	P	42–50	26.4	4.7[b]	ND	

[a] Results expressed as percentages of the total dry weight of membranes. ND, not determined.
[b] Measured as total hexose content.

TABLE 2. *Protein to lipid ratios of mesosomal and peripheral membranes*[a]

Microorganism	Mesosomal membranes	Peripheral membranes	Reference
B. licheniformis	2.6[b]	1.9–2.7	Reaveley (234)
S. aureus	1.2	2.2	Theodore et al. (297), Theodore and Panos (296)
M. lysodeikticus	1.0–1.4	1.6–2.9	Owen and Freer (216)
B. subtilis[c]	0.75	1.85	Fitz-James (79)
L. monocytogenes[c]	2.8[d]	2.94–4.6	Ghosh and Murray (94)

[a] Calculated from results given as percentages of the total dry weight of membranes.
[b] Calculated from a single determination.
[c] Ratios of protein to phospholipid.
[d] This fraction may be partially "contaminated."

ley and Rogers (235) found cesium chloride gradients (32 to 34%, wt/vol) satisfactory for the isolation of mesosome-rich fractions of *B. licheniformis*. On the other hand, Theodore et al. (297) called attention to the fact that CsCl might have dehydration effects and might cause protein denaturation and alterations in structure. They found that the isolation of mesosomes from *S. aureus* was better on sucrose gradients and resulted in more consistent separations and in more uniform (almost entirely vesicular) preparations. Finally, Owen and Freer (216) did not use density gradients at all and obtained a mesosome-rich fraction of *M. lysodeikticus* by the high-speed centrifugation of the supernatant fluid containing released mesosomes. This latter finding serves as an indication that, if conditions for plasmolysis and protoplasting are carefully controlled, the time needed for the purification of mesosome-rich fractions may be significantly shortened by removing the need for time-consuming density gradient centrifugations. Fractionation was also improved by removing this additional experimental variable.

Although the detailed description of various conditions used in gradient centrifugations of mesosomes is beyond the scope of this review, a few procedural points deserve some attention. One is the treatment of crude mesosomal preparations with nucleases, both ribonuclease and deoxyribonuclease, prior to gradient centrifugation (297). Presumably, the purpose of such treatment is to remove "nuclear" or cytoplasmic nucleic acids and/or ribosomes contaminating mesosomal membranes. However, mesosome-associated ribosomes and even mesosome-associated deoxyribonucleic acid (DNA) may represent a true functional state in a cell; there is ample evidence that in cells mesosomes are in contact with nuclear material (248) and also that some ribosomes are associated with the plasma membrane (269). In fact, evidence indicating that ribosomes are associated with the mesosomes has been presented recently (181, 182). Thus, the removal of these components very likely creates an artifactual, if "purified," preparation. The second point deals with the yield of purified mesosomal membranes. The yield of material is important in a practical sense in that it may determine how many and what kind of experiments are to be undertaken. Furthermore, the yield of mesosomal material can be taken as an indicator of irregularities in

the isolation procedure; to a considerable degree, the yield may well reflect the purity of a given fraction and, thus, the specific activity of a marker. An unusually large yield would tend to indicate, for example, the presence of contamination. Available data from a few more recent studies (216, 225, 234) are in fairly good agreement in this regard: the average yeld of mesosomes seems to vary somewhere between 10 and 20% of the total membrane fraction, as determined on the basis of dry weight.

Another important parameter which is experimentally difficult to control completely is the potentially degrading effects of endogenous or excreted proteolytic or lipolytic enzymes on the membranes being isolated (48). To what extent are these hydrolytic enzymes activated during the various stages of treatment and incubation during membrane isolation? Konings et al. (155), studying the transport of L-glutamate by membrane vesicles of *B. subtilis,* minimized the action of proteolytic enzymes found to be excreted by the cells during protoplast formation. By treating the cells with lysozyme in *hypo*tonic medium, the incubation period required to form protoplasts was avoided. The membrane vesicles prepared in this manner retained their transport function for prolonged periods of time, in contrast to vesicles derived from protoplasts. It should be pointed out that in this study no attempt was made to distinguish or to separate mesosomal and peripheral membrane vesicles. Nevertheless, increased concern for such potentially misleading effects seems appropriate now that fundamental methods for the isolation of mesosomal and peripheral membranes seem well established, if not completely perfected. Certainly it is possible by adding known ^{14}C-labeled proteins, for example, to incubation systems to monitor the presence or abence of proteolytic activities during various treatments prior to isolation of the membranes (104, 118a). Also, Santo and Doi (264) recently provided ultrastructural evidence which indicated that proteolytic enzymes were activated during germination of *B. subtilis* spores; localized degradation of the inner and outer spore coats ensued. Mesosomal structures of various configurations became prominent at this time up through the time of net DNA synthesis. Frehel et al. (85) have reported that mesosomes of *B. subtilis* probably do not play a role in the excretion of protease activity, especially esterase activity, since the activity associated with mesosomes remains high during sporulation but activity associated with the plasma membranes decreases. As pointed out by Reusch and Burger (241), however, esterase activity, as well as acid phosphatase activity, is not restricted to membrane fractions; these workers also have attempted to employ a rapid isolation procedure to avoid prolonged density gradient centrifugation and possible degradation of components.

Some mention also needs to be made of the frequent observation of *two* mesosome-rich bands after density gradient centrifugation (225, 234, 235, 297). These are usually referred to as the "upper" and "lower" mesosome bands. Electron microscopy has revealed that the upper band consists exclusively of "purified" mesosomes, and the lower band contains mainly mesosomes plus contaminating fragments and/or particles. Ferrandes et al. (73) noted that mesosomes of *B. subtilis* sediment to a sucrose density of 1.10 to 1.15, whereas the peripheral membrane band is always found at 1.20. They suggested that the difference in density between these two membrane fractions is due to the higher lipid/protein ratio in mesosomal membranes. This difference in chemical composition could account for the appearance of two mesosomal bands if, as suggested by electron microscopy, one contained only mesosomal membranes and the other was contaminated with tightly associated peripheral membrane fragments. Alternately, the upper mesosome band might contain vesicles with a higher lipid content than those in the lower band. However, such fragmentary data must be interpreted with caution: does a lighter buoyant density reflect a higher lipid content or a lower percentage of protein in the membranes? Data should also be expressed as a percentage of dry weight. Even here difficulties may be encountered if dense macromolecules (e.g., RNA) are present as contaminants. In linear CsCl gradients (235), the upper band (density of 1.25) consisted of uniform, small mesosomal vesicles and the lower one (density, 1.28) consisted of larger, mesosome-like vesicles and flagella. It is the upper band that is generally recovered as the "purified" mesosome-rich fraction. Patch and Landman (225) reported that the two mesosome-rich bands behaved differently upon a second density gradient, the upper representing slowly sedimenting mesosomes (SM) and the lower representing rapidly sedimenting mesosomes (RM). Otherwise, the two fractions were similar in all respects. Therefore, at present it is not possible to determine whether the appearance of two bands, instead of a single one, is due to contamination, to imperfect methodology, or to the instability of mesosomes during isolation, or is the result of the separation of different components which together constitute

a functional mesosome. In the light of these findings, omission of density gradient centrifugation as in the procedure of Owen and Freer (216) seems even more attractive.

A final point in regard to separation of mesosomes brings attention to the fact that present isolation methods are still imperfect: only two membrane fractions are obtained (peripheral and mesosomal) instead of three (peripheral, mesosomal, and mesosomal sac). To our knowledge, the separation of mesosomal sacs has not been reported. There is no evidence at present which indicates whether or not the two mesosomal bands represent mesosomal vesicles and separated mesosomal sacs. Rather, the mesosome sacs, which appear to reassociate as part of the peripheral membrane during plasmolysis and protoplasting, may tend to "incorporate" themselves into peripheral membrane and fractionate with it. Until the fractionation and clear-cut separation of all three components of the bacterial membrane system can be achieved, our knowledge about functional characteristics of the mesosome will remain incomplete.

Chemical Composition of Isolated Mesosomal Membranes

Membranes of gram-positive bacteria have a gross chemical composition quite similar to that of membranes from mammalian or plant cells (for review, see 256). They contain approximately 50 to 70% protein and 15 to 30% lipid, and they have a generally low carbohydrate content. Variable but small amounts of RNA are associated with them. Mesosomal membranes do not seem to differ markedly in overall chemical composition from peripheral membranes in those instances where clean separations have been achieved (Table 1). A more thorough examination of available data reveals, however, that there is not clear-cut agreement about the composition of mesosomal membranes. There appear to be two views: one, based on results of Ellar et al. (66, 67, 69, 70), and Landman (225), and Reaveley (234), holds that there are no differences in protein content of the two types of membranes; the other, based on data reported by Theodore et al. (297), Ferrandes et al. (73), and Owen and Freer (216), indicates that mesosomal membranes compared with peripheral membranes are depleted in protein. Ferrandes and collaborators reported that isolated mesosomal membranes of *B. subtilis* contain about one-half of the amount of protein estimated to be present in the peripheral membranes. In studies by Owen and Freer (216), the protein content of mesosomal membranes was consistently lower when determined by two different methods (biuret and Folin-Ciocalteau methods). The protein/lipid ratios for peripheral membranes appear to be higher than those for mesosomal membranes in most organisms listed in Table 2. From the sample of data listed in Table 1, it would seem that the low protein/lipid ratios reflect low protein rather than increased lipid content of mesosomal membranes. Whether this feature indeed represents a unique property of mesosomal membranes in general cannot be ascertained in view of the conflicting experimental evidence. Much more analytical data must be obtained from a greater variety of organisms studied under similar conditions. Improvements in fractionation procedures, as well as better understanding of the need for careful control of growth, isolation, and purification conditions, promise that more definite answers should not be long in coming. In regard to this latter point, van Iterson and Op den Kamp (318) clearly showed that growth of *B. subtilis* under conditions which lowered the pH resulted in an increased amount of lysophosphatidyl glycerol relative to phosphatidyl glycerol in the isolated membranes. This change in composition appeared to be accompanied by increased rigidity of the membrane.

Analyses of total phospholipid (PL) contents of the two types of membranes from gram-positive organisms have been reported. In the case of *B. subtilis*, there is essentially no difference in the amounts of PL in mesosomal and peripheral membranes; the PL content of the peripheral membrane of *M. lysodeikticus*, however, may be significantly higher than the PL in the mesosomal membrane. Ellar et al. (70) found only half the amount of phospholipid (by weight) in mesosomal membranes of *M. lysodeikticus*, compared with peripheral membranes, a finding which suggests that this interpretation may be applicable to the data of Owen and Freer (216). No differences were reported in the phospholipid composition of the two membranes of *M. lysodeikticus* (70). Cardiolipin accounted for 67%, phosphatidyl glycerol, 27%, and phosphatidyl inositol, 6%, on a dry weight basis of the total PL. Neither was there any difference in fatty acid composition, with a C_{15} branched-chain fatty acid constituting more than 90% of the total.

Even more recently, Theodore and Panos (296) showed that the compositions of peripheral membrane and mesosomal vesicles of *S. aureus* are identical, qualitatively, in proteins and fatty acids. However, the fatty acid *content*

of the mesosomal vesicles is 48% greater than that of the plasma membrane. At least 85% of the total fatty acids extracted from both membranes were shown to be the iso- and anteiso-, branched methyl C_{15}, C_{17}, and C_{19} fatty acids. The significance of these quantitative differences is not at all clear, and, although there is no reason to question the validity of the analytical data, one must ask, in view of the possibilities of degradative activities occurring during preparative procedures (see above), if there is any chance that these data reflect such degradation.

Mesosomal membranes of *M. lysodeikticus* contain a significantly higher amount (Table 1) of carbohydrate than peripheral membranes (216). This finding is of interest, particularly in view of the fact that the major hexose component of the mesosomal membranes is mannose. These data suggest the attractive hypothesis that the mesosomes preferentially might be involved in the biosynthesis of envelope components of *M. lysodeikticus*. The studies of Lennarz and Scher (167) showed that bactoprenol (300), a C_{55} isoprenoid alcohol, is involved in polymannan biosynthesis. Other workers have shown this isoprenoid to function as a lipid intermediate and/or carrier for cell wall components (peptidylglycan) across the plasma membrane. However, Thorne and Barker (301, 302) reported that bactoprenol in *L. casei* and *L. plantarum* is in equal concentrations (per milligram of protein) in the peripheral and mesosome membranes. An important observation was made in that initial analysis showed the bactoprenol/protein ratio to be much lower in the plasma membrane than in the mesosomal membrane. However, this difference was found to be due to the presence of lysozyme bound to the peripheral membrane fraction. Upon removal of the lysozyme, the ratios for the two fractions became the same. One-third of the total isoprenoid is found in the mesosomes. Furthermore, these latter workers, by pulse-chase experiments, provided evidence which indicates that the mesosomal bactoprenol is not a precursor to bactoprenol in the plasma membrane. The reported enrichment of mannose in mesosomal membranes may be used to develop new methods for the isolation of highly purified mesosomes by taking advantage of preferential binding of this mannose-rich fraction to lectins, e.g., concanavalin A or other agglutinins. On the other hand, the low carbohydrate content in peripheral membranes of *M. lysodeikticus* reported by Salton and Freer (260) and Owen and Freer (216) contrasts with data of Gilby et al. (100), who found about 20%, and those of Mūnoz and co-workers (personal communication), who found 12% carbohydrate in these membranes. It is possible, of course, that the elevated carbohydrate content of peripheral membranes reported in the latter case was due to the presence of mesosomal membranes in the total membrane fraction assayed. Thorne and Barker (300) have shown that bactoprenol synthesized from [^{14}C]mevalonic acid by *Lactobacillus casei* is released into the medium upon treatment of the cells with trypsin followed by lysozyme and ethylenediaminetetraacetate (EDTA). The labeled lipid was in protein-containing vesicles, possibly mesosomes, having a particle size of about 35 million daltons. Adenosine triphosphatase (ATPase), acetokinase, and phosphotransacetylase activities also were released but could be removed by gel filtration.

Of considerable significance is the very recent finding of Huff et al. (139) that, although no membrane or "intracellular" lipoteichoic acid (245) was detected in the plasma membrane fraction of *S. aureus*, 18% of the dry weight of the mesosomal vesicles was characterized as lipoteichoic acid. Furthermore, comparative analyses of fractions of plasma membranes and mesosomal vesicles isolated from six additional strains of gram-positive bacteria revealed that levels of lipoteichoic acid were from 3 to 30 times greater in the latter fractions than in the former.

Acrylamide disc-gel electrophoresis has been used widely for the characterization of protein patterns in bacterial membranes (see, e.g., 195, 262). It has been recently used in comparative studies of polypeptide composition in peripheral and mesosomal membranes (216, 225, 234, 241). Not surprisingly, the results of comparative disc-gel electrophoresis of the two membrane types from different bacteria are contradictory. In all cases, membranes were dissolved in sodium dodecyl sulfate (SDS) and run in polyacrylamide gels containing SDS. Comparison of the protein profiles of peripheral membranes and mesosomes from *B. licheniformis* (234) showed differences in only three components which appeared to be present in greater amounts in mesosomal membranes. No qualitative differences were detected. Patch and Landman (225) were able to identify approximately 14 polypeptides upon electrophoresis of both membrane types from *B. subtilis*. They also observed great similarity of the two protein patterns. Again, it appeared that if any differences exist, they are only quantitative. In contrast, Owen and Freer (216) found that the polypeptide profile of mesosomal membranes of *M. lysodeikticus* differed from those found with

peripheral membranes. This observation of distinct qualitative differences is in accord with the demonstrated compositional differences between mesosomal and peripheral membranes of *M. lysodeikticus*. It might be argued that vesicular mesosomes could contain soluble proteins trapped inside, which contribute to qualitative and/or quantitative differences observed (see above). However, in both Reaveley's (234) and Owen and Freer's (216) studies, mesosomal vesicles were sonically disrupted and only the insoluble sediments were used for disc-gel electrophoresis. The protein patterns of the insoluble residue were very similar to that of untreated mesosomes.

In their analysis of mesosome and plasma membranes of *S. aureus* ATCC 6538P, Theodore and Panos (296) also found that the protein patterns of the two membranes obtained upon urea-acetic acid and upon neutral SDS disc-gel electrophoresis were different only quantitatively. On the former gels the plasma membrane showed a higher concentration of "slower migrating" proteins, whereas the mesosomal vesicles contained predominantly "faster migrating" proteins. On SDS gels the mesosomal vesicles exhibited a major protein band, with a molecular weight of about 35,000, which is four times greater than the corresponding band observed with the plasma membrane. Reusch and Burger (241) reported also that on SDS gel electrophoresis mesosomal membranes of *B. licheniformis* showed a very prominent band at a molecular weight of 33,000. However, these latter workers found qualitative as well as quantitative differences in the protein patterns of mesosomal versus protoplast (plasma) membranes of both *B. subtilis* and *B. licheniformis* (ATCC 9945). Mesosomes from cells grown in minimal or in rich medium yielded fewer bands than plasma membranes. Also, in contrast to previous findings, these workers reported that esterase and acid phosphatase were not suitable marker activities for the mesosomal fractions. Reusch and Burger (241) concluded also that RNA (ribosomes) associated with the isolated mesosomal fraction was present as contamination; this contrasts with the recent interpretations (above) that ribosomes may be associated with mesosomes in vivo. They also reported preliminary evidence that a material with properties of teichoic acid was at a two- to threefold higher concentration in the mesosomal fractions than in the plasma membrane. This finding is supportive of the data of Huff et al. (139), which indicate that lipoteichoic acid may be localized in the mesosomes of *S. aureus*. Finally, Reusch and Burger (241) found that, in addition to the oxidative enzymes now commonly accepted as being associated with the protoplast membrane, phosphoMurNAc-pentapeptide translocase, teichoic acid synthetase, and UDP-glucose:teichoic acid glucosyl transferase with high specific activities were found in protoplast membrane fractions. These data, together with those of Huff et al. (139), make it necessary to consider further the possible role of mesosomes in the assembly or translocation of envelope (cell wall) components.

Interest has also involved the analysis of the enzymatic composition of peripheral and mesosomal membranes, especially of gram-positive bacteria. Most enzymatic activities considered to be membrane-associated (256, 257) have been found in both peripheral and mesosomal membranes; thus, to date no given enzyme has been reported to be specifically mesosomal. Generally speaking, however, respiratory-associated dehydrogenases have been consistently lower in clean mesosomal fractions. Although differences in specific activities of various enzymes in the two membrane types have been found in almost all cases, the data present a confused picture. Whereas relative specific activities of succinic dehydrogenase in the two membranes obtained in different laboratories for the same bacterial species correspond fairly closely, those for different gram-positive species vary greatly. Whether this variability is a real phenomenon or only a reflection of differences in growth conditions, membrane fractionation, or enzyme assay procedures cannot be determined. Ghosh and Murray (95), for example, studying *L. monocytogenes*, reported very high specific activities of mesosomal succinic and reduced nicotinamide adenine dinucleotide ($NADH_2$) dehydrogenases, relative to values found for other species. The fact that both peripheral and mesosomal membrane fractions were present and assayed does not explain these elevated values. The assay system, based on reduction of nitroblue tetrazolium, was different from that used by most other authors. Thus, lower values have been obtained for succinic dehydrogenase when assayed spectrophotometrically under carefully controlled conditions with 2,6-dichlorophenolindophenol in the presence of phenazine methosulfate (see, e.g., 216). $NADH_2$ dehydrogenase can be measured also spectrophotometrically by the method of Gel'man et al. (93). $NADH_2$ dehydrogenase of *B. subtilis* was reported by Patch and Landman (225), who determined this activity in polyacrylamide gels by measuring the density of formazan deposits after staining the gels in the presence of the substrate and tetranitroblue tetrazolium in Beckman Analytrol. The similarly high activity obtained in this study as in that of Ghosh and Murray for *L. monocyto-*

genes (95) suggests that part of the variability encountered does reflect the methods of assay. This view is supported by observations of Reaveley and Rogers (235) in regard to NADH oxidase activities, which were found to vary widely from one preparation of membranes to another; the variations were attributed to the instability of the enzyme system under the assay conditions used. Another factor may be involved. It is clear that proteins only loosely associated with the membranes may either adhere to or be removed from the membranes depending upon the method of isolation. Weigand and Greenawalt (unpublished data) found that the distribution of NADH oxidase activity of *E. coli* $O111_a$ between the soluble and particulate fractions varied widely depending upon the pressure exerted during disruption of the cells in a French pressure cell. At 10,000 lb/in^2, 70% of the total activity was recovered in the nonsedimentable fraction, whereas at 1,500 lb/in^2 over 90% of the activity was associated with the sedimentable membranes. In both cases cell breakage was greater than 90%, and no evidence was found to indicate that "soluble" NADH oxidase was trapped within membrane vesicles.

A few general, summary comments justifiably can be made at this time concerning the distribution of enzymatic activities between mesosomal and peripheral membranes. These are summarized as follows:

1. Succinate dehydrogenase, $NADH_2$ dehydrogenase, and NADH oxidase appear to be depleted or very low in mesosomal membranes (data of Ferrandes et al. [73] are particularly convincing).

2. Ca^{2+}- or Mg^{2+}-stimulated ATPase appears not to be present and certainly not concentrated in mesosomal membranes of *M. lysodeikticus* (194, 214, 333).

3. A very high autolytic enzyme activity was reported by Ellar and Postgate (69) and Owen and Freer (216) to be present in mesosomes of *M. lysodeikticus*. This activity appears to be a peptidoglycan-hydrolyzing enzyme system (69) acting on isolated cell walls of *M. lysodeikticus* and is 15- to 30-fold more active in the mesosome-rich fraction than in the peripheral membrane fraction. The possibility that contamination with residual lysozyme, used for membrane preparation, accounted for this activity was eliminated by control experiments in which ^{125}I-labeled lysozyme and lysozyme-specific antisera were used to determine the absence of this protein in the fraction.

4. No esterase, acid phosphatase, or glucose 6-phosphate dehydrogenase activities appear to be associated with only mesosomes (85, 240, 241; Reusch and Burger, Fed. Proc. 31:1098, 1972).

5. No single enzyme has been found to be preferentially or exclusively associated with mesosomal membranes of gram-positive bacteria. The mesosome-associated autolytic enzyme system mentioned earlier has been reported for one bacterium only, and further study is needed to determine whether it is present in mesosomes of other species as well. Similarly, the high malate dehydrogenase specific activity found in mesosomes of *B. subtilis* (225) needs further investigation. No clear-cut mesosomal "marker" is available at the present time.

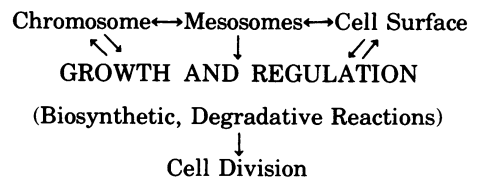
The distribution of cytochromes between mesosomal and peripheral membranes has been a subject of continuing interest and controversy for several years. In 1966, Ferrandes et al. (72) reported that cytochromes were concentrated in the mesosomal fraction of *B. subtilis*. As pointed out by Ryter (249), this observation was supported by circumstantial evidence such as the absence of cytochromes in membranes of L-forms of gram-positive bacteria which do not have mesosomes (327). However, as the methods for fractionation of mesosomal membranes improved, the original observation of Ferrandes et al. (72) could not be reproduced. Reaveley and Rogers (235) found that oxidized-reduced spectra for peripheral and mesosomal membranes of *B. licheniformis* were basically similar, although small differences were noted in the spectrum of the mesosomal membranes, e.g., a small peak at about 500 nm and a shift of the cytochrome *a* peak from 605 nm to 615 nm. More recently, Patch and Landman (225) reported no qualitative differences in spectra for mesosomal and peripheral membranes of *B. subtilis*. One quantitative difference observed was a less shallow trough at 420 nm in the peripheral membrane spectrum, which suggests that peripheral membrane may be richer in cytochrome *c*. A difference in the cytochrome content (for cytochromes *a*, *b*, and *c*) of the two membrane preparations in *M. lysodeikticus* was reported by Ellar et al. (70), but no experimental data were presented. Owen and Freer (216) provided convincing evidence that cytochromes a_{601}, b_{560}, and c_{550} clearly are present in peripheral membranes but are not detectable in mesosome fractions of *M. lysodeikticus*. Cytochrome b_{556} only was present in mesosome membranes. This seeming "partitioning" of cytochromes between the two types of membranes is curious, especially in view of the concept of the necessity for molecular interaction of the electron carriers of the electron transport chain (165). Furthermore, all four cytochromes have been reported to be in peripheral membranes

(93). Owen and Freer (216) pointed out that the absorption of cytochrome b_{556} in peripheral membranes tends to be masked by the absorption peaks of cytochromes b_{560} and c_{550}. Nevertheless, the distribution of cytochromes between the two membrane types must be studied more carefully, perhaps by means of more sensitive low-temperature spectroscopy. In addition, the possibility that cytochromes may be detached during fractionation has been raised (249; see also 92); this demands further investigation. The conditions under which protoplasts are formed may influence the degree to which cytochromes remain attached or are detached from membrane fractions.

It is quite obvious that much needs to be done to clarify and establish the compositional and particularly the enzymatic similarities and/or differences between mesosomal and peripheral membranes. Membranes from more bacterial species need to be prepared under standardized experimental conditions. Close attention will have to be paid to conditions of growth, to age, and to the physiological state of cells used as a source of membranes. The search for a functional mesosomal "marker" is of primary importance and must be continued. With the improved methods for the isolation of purified mesosomal membranes in greater amounts, different experimental approaches may and probably will be undertaken. Immunological comparison and immunochemical analysis of mesosomal and peripheral membrane antigens may yield valuable information. In particular, as additional functional components of membranes become isolated and purified (see 256, 257), specific antisera may be prepared (333) and applied in comparative immunological studies of the two membrane types. Also, physical studies, e.g., infrared spectroscopy, circular dichroism, or optical rotatory dispersion, widely used in membrane studies (166, 324), have not been used with mesosomal membranes. Unquestionably, the scarcity of purified membranous material is the main deterrent to these approaches, but it appears that this will soon be overcome. Infrared spectra of the "inner" and "outer" membranes of *E. coli* have recently been reported (197). Also, it is time that additional reagents such as chaotropic agents (118) be applied in fractionating membranous fractions from microbial cells.

BIOLOGICAL ACTIVITIES OF MESOSOMES: THE QUEST FOR MESOSOMAL FUNCTION

Functional roles postulated for mesosomes have been discussed in comprehensive reviews by Ryter (249) and more recently by Reusch and Burger (240) and by Burdett (30). Views vary from those stating that mesosomes are of no importance for cell survival and, therefore, cannot perform any vital functions (230, 249, 255) to those that attribute a variety of specific and essential biochemical activities to mesosomes. These conflicts have resulted, in part, because workers have attempted to assign specific biochemical functions to these organelles. Of considerable interest is the proposed role of mesosomes as organelles of electron transport, phosphorylation, and oxidation-reduction reactions. Are mesosomes mitochondrial equivalents? As will be discussed below, the data relative to this question are controversial. Much of the vast body of accumulated data supports the position that mesosomes may participate, through a variety of complex interactions, in the overall process of cell division. Suggested roles include synthesis of cell wall and membrane constituents, septum initiation and ingrowth, and partitioning of cell walls. Current concepts would allow further that degradative reactions, compartmented by mesosomal structures, might well be activated at certain stages of the division process to provide turnover of membrane and/or wall constitutents during the remodeling of cell surfaces. Evidence for the role of mesosomes in these reactions will be considered together. In addition, numerous studies suggest that mesosomes may serve as sites of chromosome replication and as a means for the apportionment of DNA. Because of the vast literature supporting this functional role for mesosomes, this aspect will be discussed as a separate topic. However, this is arbitrary and these functions well might be considered part of the same overall process, i.e., cell division. Certainly all these functions are important facets of cell division, and circumstantially mesosomes would appear to provide a physical as well as biochemical link between the complex interactions involved. This concept can be conveyed diagrammatically as follows:



The quest for mesosomal functions will continue unless it is shown beyond doubt that these structures are strictly artifacts (202) or until biochemical functions are clearly established. It is difficult to accept the conclusion that a membranous entity which appears to

form in response to physiological changes, which has been shown to increase in size and complexity, and which seems to divide does not serve essential functions. The challenge to determine the functional roles of mesosomes still beckons. Although final answers have remained elusive, recent advances give indications promising that new insights will be forthcoming in the near future (31–33, 57, 120, 200, 284, 285).

Electron Transport, Phosphorylation, and Oxidation-Reduction Reactions

The excellent review by Harold (116) discusses in considerable detail the mechanisms of energy conservation and transformation in bacteria and mitochondria as we now know them, so little space will be devoted to these considerations here. A few points do warrant comment with regard to these membrane functions and mesosomes as bacterial organelles. There is every reason to believe that the fundamental interrelationships of metabolic energy conservation and energy utilization are the same in bacteria and in mitochondria, especially with regard to oxidative phosphorylation (29, 93). However, the concept of mesosomes being the functional equivalent of mitochondria, at least in toto, has steadily lost ground as more and more data have accumulated concerning the biochemical activities of bacterial subfractions. In no case has evidence been presented which shows mesosomes to play an essential role in the oxidative, coupling, or adenosine triphosphate (ATP)-synthesizing (ATPase) reactions which appear to be common to all oxidative phosphorylation mechanisms. As recently pointed out (227a), characterization of isolated ATP synthetase complexes reveals a multicomponent entity much more complicated than first seemed likely when the soluble ATPase (F_1) moiety was isolated. The picture is complicated also by the diversity of the molecular components comprising the respiratory chains of various bacteria and by the requirement for "soluble coupling factors" to demonstrate phosphorylation by membrane particles prepared from bacteria (29, 116). However, conclusions based on the *absence* of an activity from a subcellular particle must always be viewed with some degree of reservation. The question of membrane composition assumes increased significance when the fluidity of biological membranes is considered (286; cf. mesosomal ultrastructure, above) in the light of the "classical" topology of peripheral, mesosomal sac, and mesosomal membranes, and in view of recent evidence suggesting that lipoteichoic acid may be a specific constituent of mesosomes (139) and that wall-synthesizing enzymes are associated with the peripheral membranes (341).

One point, alluded to above, needs to be emphasized: our present knowledge of the structure-functional relationships in mitochondria, although considerable, is still fragmentary. Recent experiments suggest, for example, that the inner membranes of rat liver mitochondria may be heterogeneous and may be disassembled into constituent structures, lamellae and tubules (101, 263), which appear to differ in ultrastructure (only the latter have projecting particles) and in chemical composition. One might ask what relationship, if any, mesosomes have to the lamellar subfraction of the mitochondrial inner membrane. Also, it must be kept in mind that mitochondria from different species and cell types are not identical, either in ultrastructure or in all details of metabolic reactions, and only a few types of mitochondria have been isolated and studied in great depth. Finally, few studies directed toward the understanding of oxidative phosphorylation in bacterial membranes have been concerned also with the potential contribution of mesosomal membranes as well as the plasma membrane to these activities. Are the "soluble coupling factors" mentioned above soluble in situ or are they derived from membranes during subfractionation? If they are membrane-derived, from which membranes are they removed—the plasma membrane, the mesosomal membrane, or both? Answers to these questions await the further sophistication of fractionation procedures and their application to both mitochondrial and bacterial membranes.

The evidence for involvement of mesosomes in electron transport and oxidation-reduction reactions up to now has been based on three kinds of observations: (i) presence of dehydrogenase activities in mesosome fractions, (ii) localization of cytochromes in mesosomes, and (iii) cytochemical studies. Most recent studies indicate, however, that in all three respects bacterial mesosomes are not exclusive or even major sites of oxidative metabolism. As pointed out in the preceding section, isolated mesosomes seem to be deficient in succinic dehydrogenase, NADH oxidase, $NADH_2$ dehydrogenase, ATPase (ATP synthetase), and cytochromes. ATPase particles, invariably seen in negatively stained preparations of *M. lysodeikticus* membranes and recently shown to react specifically with ferritin-tagged anti-ATPase globulin (214), have not been seen on isolated mesosomes (Salton, personal communication). It should be pointed out that even very recent studies (84, 298) utilizing what appear to be

well-separated and highly enriched fractions do *not* show a *complete absence* of respiratory activities in mesosomal fractions but that specific activities are significantly lower than in the plasma (protoplast or peripheral) membrane fractions. It should be borne in mind that the localized concentration of membranes due to compartmentalization in mesosomal structures could in effect produce a highly active enzyme concentration in a microenvironment within the cell. It would be of interest to calculate, on the basis of specific activities, the relative "oxidative enzyme *densities*" of the "cell" versus the "mesosome" per unit of membrane area and volume.

Cytochemical techniques have been used successfully to demonstrate oxidative reactions; in fact, the formation of potassium tellurite deposits in association with mitochondrial cristae provided some of the earliest evidence for the specialized role of these membranes in oxidative phosphorylation (16). The cytochemical studies of bacterial membranes distinctly fall into two categories:

1. Pre-1970. Rather inconclusive results, which have been summarized by Ryter (249), showed that different cytochemical reagents are not reduced by, or at least reaction products are not deposited on, the same membranous structures. Thus, triphenyl tetrazolium was reduced only in mesosomes (312), tetranitroblue tetrazolium was reduced in both peripheral membrane and mesosomes (164, 277), and potassium tellurite was reduced only in peripheral membranes (86). No conclusion as to the precise location of components of the respiratory chain can be reached on the basis of these reports. Ryter thinks these results may not be contradictory since the reduction of different cytochemical reagents depends on their redox potentials and, therefore, they may show the presence of different enzymes of the respiratory chain. Other factors must be considered in these studies as well. Are the deposits (reaction products) really insoluble in the milieu where the enzymatic activity takes place so that they truly reflect the localized site(s) of the enzyme? In our hands (R. A. Weigand and J. W. Greenawalt, unpublished data), the formazan of tetranitroblue tetrazolium formed upon succinate oxidation by *E. coli* O111$_a$ was readily and visibly solubilized during dehydration of the samples for embedding. Reduced tellurite as a reaction product has distinct advantages in this regard; however, caution must be exerted in its use because of its toxic properties at levels often used for cytochemical studies.
2. Most recently, observations, notably those of Frehel et al. (84, 86), appear to have been made with proper controls and full understanding of difficulties and pitfalls of cytochemical methodology. Frehel's results clearly show that the deposition of potassium tellurite under a variety of conditions is limited to peripheral membrane and does not occur inside mesosomes. The authors' evidence that the formation of tellurite needles truly reflects the respiratory activities of *B. subtilis* is convincing, more so because the number and density of needles were shown to be proportional to the O_2 consumption of the cells. The membrane localization of tellurite needles remained the same during protoplast formation and mesosome uncoiling, which indicates that the methods used for protoplast formation and separation of peripheral and mesosomal membranes did not produce changes in the distribution of respiratory enzymes.

In the light of this new evidence together with previously accumulated data, mesosomes cannot be considered preferential sites of oxidation-reduction reactions; furthermore, major respiratory activities appear limited to the peripheral membrane (see also section on chemical composition). This finding is in itself intriguing; does this absence of respiratory activity mean that the mesosome(s) has different, specialized functions operating, perhaps in the cellular compartmentalization of different biological activities? One may only speculate about the biological significance of such "exclusiveness." Do mesosomes reflect sites of newly synthesized membranous components to which enzymatically active proteins will be added at an appropriate time? The vast majority of the biosynthetic evidence accumulated to date (183–185) strongly indicates that this is not so. Thus, no specific, known membrane function can be assigned to the mesosome at this time.

Replication and Apportionment of DNA

Hypotheses involving mesosomes in DNA replication and cell division are supported by three lines of evidence: (i) morphological, (ii) genetic, and (iii) chemical, the latter being based on the fact that DNA has been found associated with membranous material isolated from several bacterial genera. The replicon model, proposed by Jacob et al. (142), pointed to the existence of a relationship between bacterial DNA and peripheral membrane. The participation of mesosomes in this interaction is not unequivocally established at this time. As a result, a controversy still exists about a role of the mesosome as a "mediator" between DNA and peripheral membrane.

The morphological evidence for this role has been covered in some depth in two reviews by Ryter (248, 249. The main thrust of evidence comes from ultrastructural studies showing the presence of a definite connection between peripheral membrane and nuclear material in sections of both gram-positive and gram-negative bacteria, with mesosomes often, but not always, serving as a connecting link. It has been reported that the number of mesosomes per cell is either one or two, apparently depending closely on the stage of the cell division cycle. However, as noted above (section on ultrastructure), some bacteria contain numerous invaginations ultrastructurally resembling mesosomes, while on the other hand Highton (124–126) reported only one lamellar mesosome per cell. More recently, Garland (91) has found that *B. cereus* consistently contains more than a single mesosome, one of which seems closely associated with septum formation, especially during stationary phase. In exponentially growing cells of *S. faecalis,* apparently a single asymmetrically located mesosome is present (120). Serial thin sections of about 20 cells of *B. subtilis* analyzed by Ryter (248, 249) revealed that either one or two mesosomes remain in contact with nuclear material throughout the entire division cycle. During sporulation in bacilli, one of the two mesosomes remains in contact with the sporangial nucleoid, while the other stays associated with the spore nucleoid and ultimately becomes enclosed in the prespore (78, 80, 248).

The existence of a linkage between DNA and mesosomes can be seen in *B. subtilis* especially well when cells are grown on plates containing hypertonic medium and the mesosomes are in the process of extrusion, pulling nuclear material toward the membrane (249). However, under certain cultural conditions, the attachment of a nucleoid to peripheral membrane is direct, particularly in reverting protoplasts of *B. subtilis* and during germination (255) or when mesosomes are destroyed by incubation for 2 h under anaerobic conditions (248). It should be mentioned that all mesosomes in all cells do not seem to be destroyed or to disappear under anaerobic conditions (54, 64). Ryter concluded that nucleoid material comes into direct constant with the peripheral membrane only when mesosomes disappear and that the point of attachment, ordinarily located on mesosomes, is only transposed onto the membrane after or during their disappearance. Ryter strongly argues that a mesosome is a normal intermediary between the nucleoid and peripheral membrane, except in those cases when for some reason, presumably unfavorable growth conditions, the presence of this intermediary is curtailed. On the basis of such evidence, it appears that the genome is attached to the mesosomal sac and not to inner mesosomal membranes (244). Since the sac may constitute in essence a continuity of the peripheral membrane, it becomes almost impossible to assess the importance of the differential attachment of the genome to mesosomal versus peripheral membrane.

Another question concerns the number of points of contact between the nucleoid and mesosomes at any given period during the cell cycle. Ryter's morphological studies (248, 249), based on random sections of dividing cells as well as germinating spores of *B. subtilis*, led her to propose that two points of attachment are present. At the initial stage of the cycle, each nucleoid is attached to a single mesosome. As a cell continues to grow and approaches the division time, each mesosome divides and then each part moves apart pulling behind it the daughter nucleoid. It is not clear at present what constitutes the driving force moving the two divided mesosomes apart, but it has been suggested that the synthesis and incorporation of new peripheral membrane play that role (80, 139, 248). Ellar et al. (68), working with synchronously dividing cells of *B. megaterium*, reported that throughout the division process the nucleoid is associated at one end with the mesosome at or near the pole of the cell and at the other end with the mesosome close to the septum. The evidence described would indicate that two different attachment points exist; unfortunately, no clue is provided to identify the sites either on the DNA strand or on the mesosome.

The association of the nucleoid and mesosomes has been seen in different gram-positive bacteria (248). Thus, for example, in *S. faecalis* (122) the septal mesosome, often particularly extensive and complex in valine-deprived cells, remains in contact with the centralized nucleoid even after amino acid starvation, the mesosome-nucleoid association being apparently undisturbed in this case. Combining ultrastructural observations with the use of antibiotic inhibitors, to inhibit macromolecular synthesis, Higgins and Daneo-Moore (120) provided evidence for the hypothesis that DNA replication and mesosome growth in *S. faecalis* are connected in that an increase in mesosome area is related to continued DNA synthesis. Thus, it appears that DNA synthesis, rather than cellular events involving protein and RNA synthesis, is a necessary prerequisite for

mesosomal *growth*. Fitz-James (80) observed that, during germination of spores of bacilli, massive mesosomal development could be seen at the time spores began to synthesize DNA. The recent ultrastructural observations of Santo and Doi (264) are in agreement with this finding. Taken together with Ryter's evidence (248, 249), the conclusion that undisturbed and continuous DNA synthesis is required for the increased formation and normal functioning of mesosomes gains in both strength and credibility. However, few details are known concerning the regulation of synthesis of biological membranes, possibly because few, if any, natural systems lend themselves readily to analysis of distinct and separate, newly formed membranes. However, Daniels (58, 59) concluded from pulse-labeling studies of the incorporation of radioactive glycerol into the membranes of *B. megaterium* KM and *E. coli* 3/62 during synchronized and unsynchronized cell cycles that the DNA-membrane complex portion of the membrane was synthesized during cell division. Also, the amount of label per unit weight was greatest in the largest and smallest cells (sized on sucrose gradients), that is, in those cells dividing during the time of labeling. Garland (91) has recently shown that "extra membranes" are formed and that [^{3}H]glycerol continues to be incorporated into cellular phospholipids even after net growth and protein synthesis have been inhibited by actinomycin D. No clear relationship between the synthesis of extra membranes and the formation of mesosomes could be established, but neither has such a possibility been clearly eliminated. This finding is reminiscent of the "extra membrane" massively accumulating in the temperature-sensitive *E. coli* O111$_a$ described by Greenawalt and colleagues (103, 273, 328, 329). These membranes are observed to accumulate maximally after the stationary phase of growth is reached (328) and have been shown to be associated with sites of DNA replication (3, 4). The recent ultrastructural studies of van Iterson and colleagues (315) strongly support the view that DNA fibrils are attached at numerous sites to mesosomes in situ and perhaps are incorporated into the mesosomal structure itself.

The apparent association of DNA with mesosomes has also been reported to occur during the incorporation of transforming DNA into bacterial cells (303, 319, 320, 338). It has been postulated that this association of donor DNA with specific "nuclear" mesosomes (in contrast to "plasma membrane mesosomes") is involved in the processing of the donor DNA into the bacterial genome (338). Recent studies (320) utilizing autoradiographic techniques show that [^{3}H]thymidine-labeled, transforming DNA, incorporated into competent cells of *B. subtilis*, becomes associated with nuclear mesosomes and migrates toward the nucleoid in association with the mesosomes.

Associations of mesosomes with DNA have also been observed in several gram-negative species, notably in *E. coli* (228, 248) and *Pseudomonas aeruginosa* (133). Recently, the existence of a contact point between nucleoid and membrane in *E. coli* had been confirmed by Woldringh and van Iterson (337) in plasmolysis experiments involving a brief exposure of protoplasts to SDS. Their results deserve special attention in view of the fact that, like so many others, they were unable to demonstrate that nucleoplasm is attached to mesosomes by conventional ultrastructural examination of cells prefixed directly in growth medium or in cells plasmolyzed in buffer. Only during "reoccupation" by the protoplast of the plasmolyzed spaces after SDS treatment was a nucleoplasm found in broad contact with peripheral membrane, a situation analogous to that described by Ryter (248) for *B. subtilis* after extrusion of mesosomes in a hypertonic medium.

As mentioned earlier, most gram-negative bacteria do not possess extensively developed mesosomes (56). However, certain strains of *E. coli* (129, 130, 169, 208, 209, 273, 328, 329) form elaborate intracytoplasmic membranes under relatively normal conditions of growth and would seem ideally suited for studies of membrane-DNA associations. Indeed, Altenburg and Suit (3) and Altenburg et al. (4) correlated the appearance of the intracytoplasmic membranes in *E. coli* O111$_a$ with the increase in the DNA content of these cells and confirmed that DNA is firmly attached to these membranes. By examination of serial sections, they estimated that DNA-membrane associations occurred in about 60% of cells containing the extensive membrane systems. Usually only one contact area per nucleoid was seen. Their electron microscopic autoradiography data indicated that the areas of firm contact of DNA and intracytoplasmic membranes might contain the DNA replication forks.

Morphological studies on DNA-membrane associations in bacteria are supported by genetic evidence which strongly indicates that mesosome- and/or membrane-DNA attachment sites may be functional in chromosome replication or segregation, or both. There is extensive literature available concerning all aspects of the replicon model (43), and its current status was discussed by Hirota et al. (129). Only a few

comments directly related to DNA-membrane associations will be made here. It has been argued that if DNA is attached to membrane either via mesosome or directly, and if membrane plays a role in DNA sythesis, then any mutant defective in DNA synthesis (or a part thereof that is membrane-dependent) ought to show an alteration in its membrane. Such membrane alterations in mutants were indeed found in several laboratories (e.g., 141, 279). These findings constitute more direct evidence that the synthesis of DNA takes place in the replicon machinery which is an integral part of a membrane. Furthermore, existence of such a connection with the membrane would provide a convenient way for segregation of daughter chromosomes simply as a result of membrane growth between the point of attachment of DNA to membrane. To the best of our knowledge, this segregation hypothesis is still awaiting solid experimental evidence, for the manner in which a membrane "grows" is still largely unknown. Lin et al. (169) analyzed the segregation of DNA and certain membrane components in *E. coli* by autoradiographic methods. They found that [^{3}H]thymidine-labeled DNA did not segregate preferentially among daughter cells and that membrane phospholipids labeled with either [^{3}H]oleate or [^{3}H]glycerol were evenly distributed among cell progeny. These results seem to indicate, first, that there is no asymmetry in the replicon system and, second, that the insertion of new phospholipid molecules into the membrane occurs at random all over the membrane surface ("dispersed growth").

Since the suggestion made by Jacob and his colleagues (142) that the replicating complex might be attached to the cell membrane, the isolation of membrane-containing DNA complex in various bacteria has been described by Ganesan and Lederberg (90), Tremblay et al. (306), Fuchs and Hanawalt (88), Fielding and Fox (74), and others (242, 293). In *B. subtilis* the newly replicated DNA was resolved from the uncomplexed bulk DNA fragments by zone sedimentation in sucrose gradients (90). However, the possibility exists that, under the experimental conditions used, the DNA may become adventitiously attached to membrane material. Tremblay et al. (306) used a new cell fractionation procedure which depended on the ability of only one of the two components of the complex, namely the membrane, to adhere to crystals of detergent (Sarkosyl). The complex isolated by this method from cells of *B. megaterium*, and named the M-band by the authors, consisted of a portion of the cell membrane, almost all of the cell's DNA, a fraction of ribosomes, and most of the RNA in the process of synthesis. This biochemical evidence was supported by ultrastructural studies of the M-band. Firshein (75) found that the DNA-membrane complex extracted from *Diplococcus pneumoniae* with Sarkosyl (sodium lauroylsarcosinate) plus Mg^{2+} could be subfractionated by electrophoresis on SDS acrylamide gels. SDS tended to inactivate DNA polymerase activity of the DNA-membrane fraction (40 min, 37 C), but at a concentration of 0.16% a fraction of the DNA-membrane complex formed in the gel upon electrophoresis retained about 40% of the activity initially present in the fraction untreated with SDS. Analysis showed the treated fraction to contain 7.5, 9.4, and 24% of the DNA, phospholipid, and protein, respectively, compared with that found in the DNA-membrane complex prior to SDS treatment and electrophoresis. The treated fraction was shown also to have associated with it several nucleases, deoxyribonucleotide kinase, and DNA ligase. These findings were interpreted to mean that a complex of enzymes involved in DNA replication is tightly bound to DNA-membrane complex in pneumococci. To characterize the DNA-membrane complex both functionally and with respect to detailed molecular and enzymatic composition, new isolation methods not involving detergents or proteolytic digestion, which yielded material with low enzymatic activities, were devised. Fuchs and Hanawalt (88) have been successful in releasing a functional replicating complex from *E. coli* lysates by the use of a nonionic detergent (Brij 58), controlled sonic treatment, and sucrose gradient centrifugation. This isolation procedure did not affect the enzymatic activities of polymerases and nucleases located in the complex. However, no specific evidence for the presence of phospholipids in the complex was found, although it did contain a protein moiety with hydrophobic regions that could bind to membranes. Fielding and Fox (74) studied the formation of pulse-labeled DNA, which remains attached to the membrane after further growth in unlabeled medium, at different periods of the cell cycle of synchronized cultures of *E. coli*. The maximal labeling of this species of membrane-bound DNA occurred near division time, providing evidence for the association of DNA at the replication origin with the cell membrane. This DNA remained firmly bound to the membrane even after vigorous sonic treatment. Taken together with Fuchs and Hanawalt's results (88), this could indicate that separate points or structures for replication and segregation of daughter chromosomes exist in *E. coli*.

There seems little doubt that cell membranes of bacteria are involved in replication and segregation of DNA. To what extent these processes are mediated by mesosomes is not at all clear. The only direct evidence linking mesosomes with replication and apportionment of DNA comes from ultrastructural studies. Neither biochemical nor genetic evidence give clues as to the role of mesosomes in these processes. It seems premature at this time to consider mesosomes solely as those membrane regions that control either replication or segregation of DNA, or both, particularly since conditions under which direct association of nucleoid material with the peripheral membrane occur have not been clearly defined.

Cell Surface Remodeling: Biosynthesis, Septum Formation, and Partitioning of Cells

Chapman and Hillier (46) were the first to suggest that "peripheral bodies" (mesosomes), which are often seen in the proximity of a cross-wall septum, might be involved in wall synthesis. The fact that mesosomes are commonly found in the equatorial region, where wall-synthesizing activity is supposed to take place, does not by itself indicate that they play a key role in wall formation. The cell wall-synthesizing activity of this region may simply reflect greater concentration of membranes there, a possibility suggested by Rogers (244). However, not only morphological but also newer biochemical evidence is consistent with the concept that the mesosomes may be a preferential site of wall biosynthesis.

Studies of Higgins and Shockman (122) serve as particularly elegant examples of morphological analysis. Using serial and random sections of *S. faecalis*, these authors reconstructed a model of wall growth for streptococci. The model proposes that in *S. faecalis* peripheral wall elongation is produced by the separation or splitting of the cross wall at its junction with peripheral wall. This results in pushing of the newly synthesized wall bands to subequatorial positions. The appearance of mesosome usually precedes cross-wall initiation. While the linear wall extension is limited to one site, near the edge of centripetally growing septum, wall thickening takes place over the entire bacterial surface. The model predicts that an autolysin must be involved in initiation of new sites of wall synthesis in the old wall, and that the sites of peripheral elongation should show a particularly high autolytic activity. Indeed, Shockman et al. (282) succeeded in isolating and purifying an autolysin, endo-*N*-acetyl-muramidase, from *S. faecalis* and showed that it is localized at the sites of a new wall synthesis.

The participation of a potentially lethal autolysin in biosynthesis of bacterial cell wall would require a careful coordination of its action with the biosynthetic processes. It is possible that mesosomes, almost invariably present at or near the site of new wall formation, perform such a coordinating role, particularly since they are also in contact with nuclear material. Recently, Ellar and Postgate (69) described a peptidoglycan-hydrolyzing enzyme system associated with isolated mesosomal membranes of *M. lysodeikticus*. The presence of such enzyme activity in mesosomes constitutes a strong argument in favor of their involvement in wall synthesis and remodeling. However, Forsberg and Ward (82) investigated the subcellular localization of a lytic enzyme, *N*-acetyl muramyl-L-alanine amidase, in *B. licheniformis* and in its L-form. In the latter cell, this activity was found entirely in the cytoplasmic membrane fraction, whereas in the parent cell it was associated with both protoplast and mesosome membranes. When intact protoplasts or L-forms were mixed with isolated cell walls, it was found that much of the activity was transferred to the walls. These workers found also that the specific activity of the amidase and also D-alanine carboxypeptidase in the mesosomes was only 65% of that in the membranes. They interpreted this reduced lytic activity in mesosomes to be consistent with the idea suggested by Rogers (244) that mesosomes might be areas where membrane growth is faster than wall extension.

From their recent finding that lipoteichoic acid is localized in mesosomal vesicles of *S. aureus*, Huff et al. (139) proposed that cell division involves a sequence of steps initiated by the binding of Mg^{2+} to DNA associated with the plasma membrane, which, in turn, activates the synthesis of lipoteichoic acid by enzymes in the DNA-associated membranes. The new membrane, rich in phospholipids and lipoteichoic acid, forms mesosomal vesicles. The mesosome then becomes actively engaged in the synthesis of plasma membrane and cell wall, producing cross wall and septum. In this model mesosomes perform biosynthetic functions which, in general, have not been found to be localized in these organelles. However, the authors point out that data implying specific localization of biosynthetic enzymes in other cellular constituents are not unambiguous and that lack of activity in subcellular fractions may not reflect accurately the in vivo situation. Also, we suggest that it is not an essential prerequisite that

these enzymatic reactions be restricted exclusively to the mesosomes in order that these organelles function in a special way in the overall process of cell division, but it would be interesting to localize intracellularly the membrane-bound D-alanine carboxypeptidase which is inhibited by penicillin and related derivatives and which is involved in cell wall cross-linking reactions (291).

Thorne and Barker (300–302) found that bactoprenol, the C_{55} isoprenoid alcohol involved in peptidoglycan synthesis, was equally distributed between the mesosome and peripheral-membrane fractions of *Lactobacillus casei* and *L. plantarum*: the concentration of bactoprenol per milligram of protein was the same in mesosomes and in plasma membrane. In addition, pulse labeling with [2-^{14}C]mevalonic acid and chasing with unlabeled precursor showed that bactoprenol is synthesized in both the plasma and mesosome membranes. Thus, mesosomes appear not to be the favored sites in the biosynthesis of this cell wall intermediate. This finding does not exclude the possibility that mesosomes may be preferentially involved in biosynthesis of other peptidoglycan and/or cell wall components. The final judgement has to be reserved until further evidence is available.

Ryter's interpretation of her observations (249) of reverting protoplasts in *B. subtilis* represent a contrast to those linking wall formation with the presence of mesosomes. Mesosomes are observed infrequently under these circumstances and only in protoplasts already engaged in the reversion process. This implies that mesosomes cannot exist in the absence of wall. Indeed, they appear only after the first layer of wall is put down. Ryter interprets this as evidence that mesosomes do not play an essential role in wall synthesis. Such a conclusion may be unwarranted, for clearly reverting protoplasts represent a special case, their behavior being more restricted than that of a healthy bacterial culture in the log phase. For example, the absence of old walls obviates the necessity for an autolytic system. It is likely that the entire membrane of a reverting protoplast is rapidly synthesizing materials necessary for the reversion process and that no functional or morphological distinction between different regions of the membrane exists at this stage.

The idea of mesosomes as active centers of membrane biosynthesis and turnover was advanced by Fitz-James (79, 80) several years ago. He suggested that mesosomes were the chief sites of membrane lipid synthesis on the basis of the higher specific labeling of mesosomal lipid with $^{32}P_i$ and [^{14}C]acetate. His results seemed to point to the mesosome as a primary synthesizing region of the membrane, whose products would then be incorporated into the peripheral membrane. However, subsequent pulse-and-chase experiments from several different laboratories did not support this view. Ellar et al. (70) reported that the specific radioactivities of total lipid and of individual phospholipids in both plasma and mesosomal membrane fractions of *M. lysodeikticus* and *B. megaterium* KM were quite similar. Independent experiments in Salton's laboratory (257) yielded the same results. Thorne and Barker (302) found that bactoprenol is not incorporated preferentially into the mesosomes and then later moved to the peripheral membrane of lactobacilli. Patch and Landman (225) compared the incorporation of [^{3}H]acetate into lipids and [^{35}S]-sulfate into proteins of mesosomal and peripheral membrane fractions of *B. subtilis*. Their pulse-chase experiments also showed no difference in the rate of label incorporation into the two fractions, indicating that the mesosome is not a precursor or a special growing point of peripheral membrane. Bacon and White (11) have recently investigated PL metabolism in *B. megaterium*. Membrane synthesis in bacteria has been studied in detail by Mindich (183, 184) and Mindich and Dales (185). These results also, based on several lines of evidence, including the use of glycerol auxotrophs, indicated that membrane growth in *B. subtilis* does not take place at one or a small number of discrete zones. No large regions of membrane conservation were found in density shift experiments in which the changes in buoyant density of membranes were studied after growth in deuterated media. Mesosomes were ruled out as the precursors of peripheral membrane lipids on the basis of kinetic labeling experiments. Radioautography of thin sections of cells pulse-labeled with tritiated glycerol showed no indication of specific zones of lipid synthesis. Thus, it appears that the bacterial membrane grows by a uniform expansion, and that mesosomes are not the biosynthetic centers for the rest of the membrane. Mindich (184) also reported that regulation of the synthesis of membrane lipids and proteins was not under stringent control. Protein was incorporated into membranes of glycerol auxotrophs of *B. subtilis* in the absence of net lipid synthesis. He did not determine whether the incorporated membranes were functional. He suggested that composition and synthesis of membrane components are primarily regulated by membrane protein synthesis. On the other hand, Wilson and Fox (336)

showed that newly synthesized functional transport proteins in *E. coli* K-12 were preferentially associated with newly synthesized lipids.

Since the membrane apparently does not grow only between the two points of DNA attachment, the question of how the genome is apportioned to daughter cells during cell division remains unanswered. If DNA is attached to the membrane, whose growth is uniform along the entire surface, then this growth cannot be used as a vector for separation of chromosomes. Nevertheless, the elongation of the membrane in the region between mesosomes connected to dividing nuclear bodies has been observed by Ryter (248) in *B. subtilis*. The subject is a controversial one at present, and some authors resort to theorizing that an internal structure analogous to a eukaryotic mitotic apparatus might be involved in the apportionment of DNA in bacteria (e.g., 185). Interestingly, an analogy between bacterial mesosomes and eukaryotic mitotic spindles had been made by some authors (248). Whether such an analogy is valid remains to be seen. It has been suggested that in *S. faecalis* the wall together with the underlying membrane may function in nuclear segregation (123). Upon completion of nuclear replication, these areas may become activated as new points of mesosome and nucleoid synthesis as well as points of new cross-wall formation. There is evidence that in some gram-positive bacteria the wall is conserved during growth (26). On the other hand, there are also data indicating that peptidoglycan and/or surface components are not conserved (e.g., 169). No definitive insights into these important regulatory functions can be given at present, and the role of mesosomes in wall and membrane biosynthesis as well as in nucleoid segregation must remain undecided for the time being.

Morphological evidence for the association of mesosomes with the newly forming septum is firmly established for at least two gram-positive genera, namely *Bacillus* (46, 68, 142) and *Streptococcus* (123). In addition, numerous reports have mentioned the presence of mesosomes near the septum in different bacterial genera, both gram-positive and gram-negative (e.g., 18, 136, 228, 230, 335). This implicates the mesosome in the process of septum formation and controlled perpetuation of cellular shape in bacteria. The subject has been dealt with in great detail by Higgins and Shockman (123), who assign to the mesosome a major role in the cell-division cycle of *S. faecalis*.

As Higgins and Shockman's model indicates, synthesis of the new cross wall is primed by the invagination of peripheral membrane to yield a bag-shaped mesosome, which remains connected to the septum via a narrow stalk throughout most of the cell cycle. The bag portion of the mesosome is in contact with nuclear material. Formation of the mesosome directly precedes cross-wall synthesis, and the localization of the mesosome designates the point at which the new cross wall will start to grow. The septum is "an annual double membrane invagination that encircles the inner side of the cell surface." The cross wall is formed between the two membranes of the septum, and the two events, i.e., septum and cross-wall synthesis, are not separated temporally, occurring as they do at the same time. Initiation of the nascent cross wall establishes its edges as the growing point to which wall precursors may now be added.

The model, reconstructed from serial sections of dividing *S. faecalis*, concludes that mesosomes are involved in cross-wall initiation on one hand and in DNA replication on the other. This conclusion is based on the existence of connections between the newly formed mesosome and nucleoid as well as on the behavior of the mesosome during cell division. When the nucleoid divides, the central mesosome connected to the growing septum is replaced by two mesosomes located below the wall bands in daughter cells. Thus, the disappearance and appearance of mesosomes in the course of cell division correlates rather well with the nucleoid replication. The loss of the central mesosome occurs while cross-wall synthesis is still in progress, indicating that the mesosome is required for cross-wall initiation rather than cross-wall completion.

Additional evidence for involvement of mesosomes in septum formation is provided by Higgins and Daneo-Moore (120). Their analysis of mesosome frequencies in normal and antibiotic-treated cells of *S. faecalis* indicates that in both cases the number of mesosomes per cell is one and that the mesosome is produced every time a cross wall is initiated. Rogers (244) reported that "rod" mutants of *B. subtilis* and *B. licheniformis*, whose morphology and division process are disturbed, do not possess the usual, well-developed mesosomes. Instead, residual structures which may contain electron-dense material are seen (52, 236). Mesosomes are either not present or greatly modified in L-forms (254), whose division and morphology are also abnormal. Although L-forms are able to multiply, the cell division is defective, resulting in bizzare-shaped bodies with a small amount of nuclear material.

It can be concluded that the evidence for

mesosomes as septum initiators during cell division is considerable and fairly convincing but remains, to some degree, circumstantial. There seems to be little disagreement in the literature about assigning this role to the mesosome, although neither the precise mode nor the regulatory mechanisms involved are understood at present. The molecular mechanisms which integrate and regulate the macromolecular synthesis of envelope and cellular constituents are undoubtedly complex and even may vary from organism to organism. However, it seems from recent data and accumulated evidence that mesosomes in gram-negative, as well as in gram-positive, bacteria in some way are intimately involved in the processes leading to the supramolecular events which have been recorded by the ultrastructural analyses.

Important new ultrastructural studies which integrate DNA replication and chromosome separation with the sequence of events during septum formation in synchronously grown *E. coli* B and B/r have just been reported by Burdett and Murray (32). This detailed investigation presents evidence which indicates that septa are formed in these cells at a discrete time of the cell cycle. It has been shown that mesosomes are involved with ingrowth of the septum during the early stages of septum formation after DNA replication has occurred. It has not yet been determined whether mesosomes are present at earlier stages (initiation) of septum formation as occurs in *S. faecalis* (122, 123), and, although the final fate of the mesosomes is not clear, they do not appear to remain associated with the septum during later stages of invagination, thickening, and cleavage as in *B. megaterium* (68). The septum is formed by the plasma membrane and mucopeptide layer of the wall; the outer membrane enters the septum only at the final stages (separation; 32). The authors emphasize the need of using acrolein/glutaraldehyde fixation to stabilize the septum, although they acknowledge the possibility that treatment may contribute to the myelinic appearance of the mesosomes. They suggest that probably only a few (one or two) attachments exist between mesosome and septum.

Possible Role in Secretory Processes

Several years ago, a suggestion was made by Lampen (161) that mesosomes may play a role in the production and release of exoenzymes in bacteria. Subsequently, this possibility has been extensively explored by Lampen and his associates with particular emphasis on the production and secretion (excretion) of penicillinase by constitutive and inducible strains of *B. licheniformis*. Ghosh et al. (96) studied morphology of both these strains and found that, whereas cells of uninduced *B. licheniformis* were characterized by the presence of a single, large septal mesosome, those induced to produce penicillinase by means of cephalosporin C contained a multiplicity of small, tubular and/or vesicular mesosome-like elements on the cell periphery. These structures were formed in response to the inducer. A magnoconstitutive penicillinase producer (749/C) contained similar structures. These were interpreted to represent a penicillinase secretory apparatus, and their resemblance to, but not identity with, mesosomes was noted. Their presence and characteristic morphology were also demonstrated in freeze-fractured and negatively stained cells (97). The structures were composed of tubules and vesicles of variable size surrounded by a unit membrane and situated in an invagination of the peripheral membrane. Upon protoplasting, the tubules and vesicles were released into the external environment, indicating further the mesosomal nature of these structures. Sargent et al. (267) quantitatively correlated such release with the secretion of penicillinase into the surrounding medium where the enzyme activity was associated with tubular and vesicular elements comparable to those seen in intact penicillinase-forming cells. The specific activity of penicillinase in the released structures was six times greater than in the peripheral membrane, and, in contrast to the membrane, they did not contain NADH oxidase.

Beaton (18) noted that when a penicillinase-producing strain of *S. aureus* was grown under conditions in which 98% of the enzyme remained in the bound form, numerous mesosomes of various sizes and shapes were present in the cells. These cells, when transferred and incubated in phosphate-buffered glucose medium, actively released penicillinase into the environment. The release was accompanied by loss of mesosomes and the appearance of membrane invaginations or pockets, some of which contained remnants of smaller vesicles. This observation suggests that penicillinase is accessible for release only when eversion of the vesicles into the periplasmic space occurs. Although no direct causal relationship was demonstrated between vesicle eversion and penicillinase release, Beaton's results and those from Lampen's laboratory indicate that eversion may be an early step in the secretion of penicillinase. Penicillinase is bound to the membrane immediately after synthesis and remains there until membrane growth or remodeling moves it to the outer surface of the membrane and out into

the periplasmic space. The small vesicles filling the membrane pockets seen in *S. aureus* and *B. licheniformis* would represent penicillinase packaged into the membrane and ready to be released, a process somewhat analogous to exocytosis in higher organisms (62, 207).

Caution must be exercised in the interpretation of these results from a number of standpoints. First, only superficial morphological similarity suggests that penicillinase-bearing vesicles are related to mesosomes. The former, as described by Lampen's group, are more variable in size, shape, and number than mesosomes; the contrasting results of Highton (127) mentioned above raise some question of the significance of these differences. In view of their specialized function in penicillinase-producing cells, it is possible to assume that they are membrane invaginations adapted to perform one specific function; in that case, should they be referred to as mesosomes? Second, only the secretion of penicillinase has been correlated with the appearance of these mesosome-like structures. Other exoenzymes, e.g., alkaline phosphatase or nuclease of *B. licheniformis*, do not fit into the model for penicillinase secretion (47). No other evidence relating mesosomes to exocellular secretion is available to the best of our knowledge. It thus appears unlikely that mesosomes, presumed to act as mediators in secretory processes, would be restricted to only one exoenzyme. Instead of assigning a generalized secretory role to mesosomes solely on the basis of one known secretory mechanism, it might be advisable to think of the structures involved in penicillinase liberation, if this picture continues to hold up, as specialized regions of the peripheral membrane.

Other Functions Attributed to Mesosomes

In an effort to find a defined role for the mesosome in a bacterial cell, numerous proposals relating this structure with a variety of functions have been advanced. A few of those that attracted wider attention will be mentioned here.

The mesosome has been labeled a "degradative subcellular organelle" by Reusch and Burger (Fed. Proc. 31:1098, 1972), who measured the distribution of enzymatic activities between purified mesosomal and peripheral membrane fractions of bacilli. Their results indicated that enzymes involved in peptidoglycan and teichoic acid synthesis (i.e., UDP-MurNAc pentapeptide translocase, polyglycerol-phosphate synthetase, and teichoic acid synthetase) were found only in the peripheral membrane, whereas degradative enzymes, acid phosphatase and esterase (tosylarginine methylester esterase), were present exclusively in the mesosomal fraction (cf. 240, 241). A functional analogy of mesosomes to eukaryotic lysosomes was suggested on the basis of these results. The suggestion is an intriguing one and deserves some consideration. It appears that lysosomes are ubiquitous in animal and plant cells, and, thus, bacteria may very well be expected to contain lysosomes or some functionally equivalent structures. According to Novikoff and Holtzman (207), a cytoplasmic particle is a lysosome if electron microscopy proves it to be membrane delimited and if cytochemistry shows it to contain one or more hydrolase activities found associated with lysosomes in biochemical studies. In our minds, the isolation of such bodies and the direct enzymatic assay for these activities would be equally, if not more, convincing. A classical bacterial mesosome representing an elaborate membranous structure usually filled with numerous tubules, lamellae, or vesicles is not morphologically comparable to the classical picture of lysosomes. On the other hand, the vesicles seen inside a mesosomal sac in certain bacteria are more structurally comparable to lysosomes. However, they are not in cytoplasm, or in contact with it, being surrounded by an invagination of the peripheral membrane. There is no cytochemical evidence available for the presence of acid phosphatase, an almost classical lysosomal "marker," in mesosomes. In our view, such evidence would be a prerequisite for any claim concerning lysosomal activities of the mesosome. More important than these considerations is the fact that no concrete evidence has been produced to show that mesosomes engulf cellular components. It is worth reflecting upon the fact that lipid and polysaccharide "storage diseases" in higher organisms are due to genetic lesions leading to deficiencies in lysosomal enzymes. However, in general, bacterial cells tend not to store lipids and polysaccharides so that lysosomal functions, if such exist in bacteria, may be somewhat restricted or different in nature. It is significant, in the present context, that Reusch and Burger (241) more recently reported that degradative enzymes do not constitute unambiguous markers for mesosomes since these activities are not restricted to membrane localization.

One of the most important functions of the cytoplasmic membrane in any cell is its capacity to serve as a permeability barrier. Specific mechanisms for the transport of small molecules into the cell are presumed to be localized

in this barrier. Kaback and his group demonstrated the existence of amino acid and sugar transport in isolated membrane vesicles of *E. coli* and *B. subtilis* (145, 156). One of the ways to compare the peripheral and mesosomal membranes functionally is to look at their respective transport activities in isolated-membrane fractions. This approach was undertaken by MacLeod et al. (177) in the study of Na^+-dependent transport of amino acids associated with membrane and mesosomal vesicles of *B. licheniformis*. Although several amino acids were taken up by peripheral membrane vesicles using various substrates including NADH, no reproducible stimulation of active transport of any of the amino acids into mesosomal vesicles could be detected in the presence of NADH. The authors suggested that the failure of mesosomes to transport was due possibly either to the absence of carrier proteins in the membrane or to the lack of an appropriate energy-coupling mechanism. The latter possibility certainly could account for the results observed since mesosomal membranes of *B. licheniformis* were shown to contain only about 5% of the succinic dehydrogenase, 30% of the NADH oxidase, and 30% of the NADH cytochrome *c* reductase activities of the peripheral membranes (235). It appears, then, that mesosomes represent membrane regions that are depleted also of the transport activities.

Kawakami and Landman (148) entertained a possibility that mesosomes might be sites of attachment of episomes to the membrane. If such were the case, plasmids would be expelled into the supernatant fluid together with the mesosome during plasmolysis of the cell. Thus, protoplasting would tend to "cure" cells of their episomes. The hypothesis was tested in *B. subtilis* W23 carrying phage SP-10, *B. megaterium* 216 carrying megacinogenic factors A and C, and *B. megaterium* C4 M-carrying megacinogenic factor C. No "curing" as a result of protoplast formation in the presence of lysozyme was observed. The conclusion was made that episomes are not localized in the mesosome but at some other position on the interior side of the peripheral membrane.

The role of mesosomes as specialized membrane regions through which the transforming DNA enters the cell has been studied by Tichy and Landman (303). They found that, after removal of walls with lysozyme, protoplasts of *B. subtilis* could no longer be transformed. On the other hand, quasi protoplasts, i.e., protoplasts with residual cell walls, could be transformed by DNA which adhered to them but only if they were allowed to resume wall synthesis. It appeared that complete protoplast formation resulting in eversion of mesosomes blocked all entry of transforming DNA into the cell interior. When competent cells were plated on hypotonic gelatin medium, mesosomes were released, and at the same time their capacity for transforming was sharply reduced. This observation was interpreted as evidence that mesosomes mediated the ability of a cell to transport DNA. In view of the fact that the authors did not demonstrate the presence of transforming DNA in mesosomes or its movement from the cell surface to mesosomes and then to the interior of a cell, this conclusion may be premature. Radioautography coupled with electron microscopy as well as labeling experiments might be needed to obtain a definite answer. Also, the extent of involvement of cell walls in the transformation processes is not at all clear, and neither is the means by which the transforming DNA associated with the mesosome is transported to the interior of a cell. The studies of Vermeulen and colleagues (319, 320, 338) reported above do shed new light on this question, however. The fate of a competence factor during plasmolysis has not been followed, so it is possible also that the observed loss of ability of protoplasts to be transformed may be due to the loss of this factor rather than extrusion of mesosomes.

Are Mesosomes Essential for Bacterial Survival?

There are three ways of looking at a component of a cell: (i) it is an essential part of the cell machinery and, thus, necessary for survival; (ii) it represents a refinement designed to perform a given function more efficiently under certain growth conditions; or (iii) it serves an essential *structural* function and is a product of cellular metabolism but, as such, remains within the cell with no active participation in cellular processes. Is the mesosome an organelle vital to a living, dynamic bacterial cell, is it an elaborate "spare part" formed only under optimal growth conditions in cells capable of synthesizing useful, but nonessential structures, or is it a structural entity, an inert region, of cytoplasmic membrane? These questions are longstanding ones, for the status of mesosomes in bacterial cells has not been clear since they were first described two decades ago. The very number of different functions attributed to mesosomes show how elusive the answers to these questions are. It could be, of course, that mesosomes represent versatile organelles involved in a multiplicity of cell functions with different morphologies in response to

specific but varying needs. The variation and specialization of plasma membranes of various types of eukaryotic cells are also immense.

There are two quite opposite points of view with respect to the importance of mesosomes in cells. One, based on studies of Ryter, Landman, and Frehel (162, 255) and Patch and Landman (225), holds that mesosomes are *not* essential structures. The evidence rests on the observation that a majority of *B. subtilis* cells grown on gelatin medium do not have mesosomes and yet are fully viable and normal in appearance and in cellular functions as judged by rate of growth, cell wall synthesis, and cell division (255). Recent studies by Patch and Landman (225) of the enzymatic activities and labeling of mesosome lipids and proteins did not provide any clear-cut answers as to the role of mesosomes. This was taken as a further indication that mesosomes do not have a well-defined and consistent function in the cell. The lack of differences in size, number, and structure of mesosomes in aerobically and anaerobically grown cells of *S. epidermidis* and *B. macerans* may also be interpreted as negative evidence for the definite mesosomal function (respiration, in this case). Although the cytochrome content and respiratory capacity of the two facultatively anaerobic bacteria were markedly reduced by growth under anaerobic conditions, mesosomes were formed under both anaerobic and aerobic conditions of growth (54; see also 64). This observation could also be interpreted to mean that mesosomes are multifunctional.

The other, more widely held view, that mesosomes do have a definite and essential function in the cell, is supported by little direct evidence. It is difficult at present to link the mesosome definitely with any one cellular process, as indicated earlier, and, indeed, there is no evidence that they may not perform several different, perhaps unrelated, functions. The argument for this viewpoint is that since mesosomes are almost ubiquitous in bacterial cells, and since evolution has preserved them as distinct cellular components, they probably once, if not at the present time, provided some distinct selective advantage. It is only reasonable to assume, therefore, that they are intimately involved in some vital steps of cellular metabolism such as DNA synthesis and replication, cell division, and/or cell wall synthesis. In some cases, they appear to be in a state of dynamic flux in many cells, exhibiting a plasticity of structure that led Ryter (249) to refer to them as "unstable organelles." Obviously, only further research will disclose the true role and significance of mesosomes.

EVOLUTIONARY CONSIDERATIONS OF BACTERIAL AND MITOCHONDRIAL MEMBRANE SYSTEMS

Within the past few years, a great resurgence of interest in the possible phylogenetic relationships linking prokaryotic and eukaryotic cells has resulted in renewed attempts to reconstruct this bit of evolutionary history. To a large degree, this interest has been regenerated by the fact that it has been firmly established that compartmented within mitochondria (and chloroplasts) are unique self-replicating sequences of DNA together with the entire machinery needed to transcribe and translate the information stored in these genomes (7, 8, 22, 23, 118a, 146, 152, 159, 160, 170, 205, 219, 231, 305, 321). Inevitably, these findings revived earlier speculations which suggested that present-day mitochondria (to which we restrict our discussion) evolved by means of the ingestion of ancestral, aerobic bacteria by primitive, anaerobic protoeukaryotes; endosymbiotic specialization followed with the transfer of a major portion of the original bacterial genome to the nucleus of the protoeukaryote resulting in the intimate interdependence of the two cellular components (104, 114, 118a, 219, 232, 268, 288, 310, 331). According to this "symbiotic" theory, the organellar genome and protein synthetic system were established via the incorporation of the prokaryotic cell into the ancestral eukaryote. Much effort has been directed toward trying to determine the products of mitochondrial protein synthesis, the integration and regulation of the mitochondrial and cytoplasmic synthesis of components of the mitochondrion, and the mechanism of transport and incorporation of cytoplasmically synthesized proteins into the organelle (7, 118a, 268, 292, 310). Bennett and Butow (Fed. Proc. 33:1269, 1974) and Kellems and Butow (149–151) have suggested that a specific population of cytoribosomes localized at special sites on the outer mitochondrial membrane of yeasts may synthesize mitochondrial proteins which are vectorially transported into mitochondrial components. The similarities of mitochondrial and bacterial systems are summarized neatly by Raff and Mahler (233), who rightly point out that these resemblances probably have been overstated. Various aspects of this theory have been discussed extensively in recent reviews by Cohen (50), Flavell (81), Raff and Mahler (233), and Stanier (288).

As might be expected, other theories contrary to this symbiotic theory have been considered also; most recently, Raff and Mahler (233)

have expounded what might be referred to as the "plasmid" theory. In this hypothesis the mitochondrion was derived directly from a large, highly evolved, aerobic protoeukaryote by the "pinching off" of specialized regions of the cell membrane containing localized respiratory assemblies. Components of the protein synthetic system (perhaps even a specific population of membrane-attached ribosomes) were included as part of the primitive organelle when it formed from the plasma membrane and cytoplasm of the protoeukaryote. The genome was added subsequently with the incorporation of a stable plasmid containing a limited amount of information, coding for only a few, specific organellar proteins.

Various facets of these proposals have been emphasized by other workers, and modifications of these basic premises have been made. Olson (210), as well as Stanier (288), has discussed these events with respect to the evolution of photosynthesis and the development of aerobic versus anaerobic metabolism. Uzzell and Spolsky (311), while not favoring the symbiotic theory, disagree from a systematics viewpoint with some of the data selected by Raff and Mahler (233) to support their position. It is worth noting that Raff and Mahler (233) in supporting their nonsymbiotic theory argue that bacteria have "mitochondrial equivalents," the mesosomes; in doing so, they refer to data (78, 250) which, as pointed out at considerable length in the foregoing sections of the present review, are no longer widely accepted. Stanier (288) invokes further the possibility that the progressive structural evolution of the eukaryotic line proceeded initially from the acquisition of the unique capacity by the protoeukaryotic cell to perform endocytosis, a function not possessed by prokaryotes but one which would provide distinct selective advantages. deDuve (61), favoring the symbiotic theory, has suggested that both the endosymbiont and host were aerobic cells but that peroxisomes provided the early mechanism for respiratory metabolism in both cell types. An important aspect of deDuve's proposal related to Stanier's (above) is that *differentiation* of the host-cell membranes led to the acquisition of phagocytic capacity, intracellular digestion, and increased cell size, whereas membranes of the symbiont differentiated to develop a respiratory chain (mitochondrial functions) upon the evolutionary decline of the peroxisome.

It is not appropriate nor the purpose of this review to consider these various theories and their ramifications in detail; these are dealt with in excellent fashion in the reviews on this subject to which we have already referred (50, 81, 233, 288). However, the widespread interest in these theories and the implications inherent in them with regard to membrane structure-functional relationships make some comments pertinent to the present topic (44). The fact that membrane specialization constitutes an important evolutionary step(s) regardless of which theory may be preferred has interest here with regard to the still undetermined nature of mesosomes and with respect to the question of whether or not homologous or even analogous relationships exist between bacteria and mitochondria.

Anatomically, some of the most interesting, but at the same time, speculative, reports are those describing "mesosomes" or mesosome-like structures in mitochondria. Despite earlier data to the contrary, it seems clear from the accumulated evidence now available that bacterial mesosomes are not the functional equivalents of mitochondria. However, the converse question—"Do mitochondria have mesosomes?" —has not been answered nor has it received the serious experimental consideration one might have expected. Uncertainty concerning the nature of bacterial mesosomes undoubtedly has been a major deterrent in this regard and makes it difficult to evaluate critically reports of mitochondrial mesosomes. What then is the evidence for the existence of mesosomes in mitochondria? The evidence for mesosomes in mitochondria is largely cricumstantial, based on ultrastructural studies.

A wide variety of mitochondrial inclusions, the nature of which largely remains unknown, has been described in the literature (14), especially in cells or tissues undergong degenerative changes. Therefore, there has been a general tendency to consider all such structures as the result of degradative process. In a number of studies, however, distinctive mitochondrial inclusions or changes (271) have been recorded in cells in various states of differentiation. These latter are most significant in the context of the present discussion.

Yotsuyanagi (339) observed membrane-like whorls in the mitochondria of yeast cells grown under glucose repression and other conditions which reduced electron transport. Release of the inhibitory condition was accompanied by increased respiratory activity and loss of the large inclusions. Cytochemical techniques showed that mitochondrial DNA was associated with these membranous structures, and it was suggested that these inclusions might play a role in mitochondrial function analogous to that hypothesized for the bacterial mesosome in

the "replicon" theory (142). Swift et al. (294) also have reported that mitochondria in yeast adapting to air after anaerobic growth contained membranous structures which perhaps were associated with the mitochondrial DNA. Marchant and Smith (179) and Smith et al. (287) have reviewed in some detail a number of factors resulting in altered mitochondrial structures in yeasts. In summarizing the cytological events occurring in yeasts during adaptation from anaerobiosis to aerobic metabolism and the concomitant formation (biogenesis) of mitochondria, they consider the presence of intramitochondrial membranous inclusions a primary step in this development. McGill et al. (175) have observed the time-dependent formation of electron-dense deposits in mitochondria of Chinese hamster fibroblasts treated with high levels of ethidium bromide. The authors suggest that the dense material may represent the condensed mitochondrial genome and could thus account for the induction of abnormal mitochondria due to this reagent.

Malhotra and colleagues (109, 153, 154, 178) reported "mesosomes" and mesosome-like structures in mitochondria of a respiratory-deficient mutant of *Neurospora*. This "poky" (mi-1) strain is characterized by a cytoplasmically inherited mutation which has pleiotropic effects on mitochondrial functions. Succinate dehydrogenase activity was shown cytochemically to be extramitochondrially associated with the mesosomes and cytoplasmic membrane. Although the term mesosome was applied as a tentative assignment to these structures in *Neurospora* mitochondria, we consider the application of this nomenclature to these structures premature.

Beck and Greenawalt (19) also have reported intramitochondrial membranous structures in *N. crassa*. These were distinguished from cytoplasmic whorls of membranes formed from invaginations of the plasma membrane on the basis of general ultrastructural properties, time of formation during conidial germination, and dependence on the nature of carbohydrate supplementation added to the growth medium.

It has been postulated that intramitochondrial inclusions, sometimes membranous, also play a role in the biogenesis of mitochondria in mammalian cells undergoing developmental changes. Pannese (221, 222) reported that during differentiation of neuroblasts in the spinal ganglia of chick embryos membranous whorls were present in association with the "envelope" and cristae of the mitochondria. These membranous whorls were not restricted to mitochondria since similar structures were also seen in association with the outer nuclear membrane. Pannese suggested that these structures were related to the formation of new mitochondria from the outer nuclear membrane. Barnard and colleagues (13–15) have proposed that intramitochondrial inclusions participate in the formation of new inner mitochondrial membranes during perinatal development of brown adipose tissue in the rat. It was found that unusually large intramitochondrial granules (observed to be membranous in part) were formed during prenatal development. The interconversion of these large structures and smaller ones was thought to be mediated via cyclic adenosine 3′,5′-monophosphate. Furthermore, correlated biochemical data suggested that the large inclusions represented accumulated, excess phospholipids (with respect to the normal proportion of protein); this was interpreted to be due to slower rates of synthesis of mitochondrial inner membrane proteins (e.g., cytochrome oxidase). The finding regarding the relatively high phospholipid to protein ratio is similar to that of Beck and Greenawalt (19).

Two recent studies of serial sections, one by Hoffman and Avers (132) and the other by Brandt et al. (25), present evidence that large, branched, tubular mitochondria are present in yeast (*Saccharomyces cerevisiae*) and rat liver cells, respectively. Both groups of workers suggest that this may be a mitochondrial feature common to many eukaryotes. In the latter case, some but not all mitochondria had this morphology, whereas in the former study it was determined that only one large mitochondrion was present per cell. Grimes et al. (107) found that the morphology and number of mitochondria in *S. cerevisiae* cells were strain dependent and also varied with cell physiology and ploidy. This conflict is not resolved at present (131), but, if these large complex mitochondria prove to be ubiquitous, it may be necessary to reassess currently popular concepts of mitochondrial function and biogenesis (25).

The answer to the question of whether or not mitochondria have mesosomes awaits developments just now emerging for the isolation and characterization of bacterial mesosomes. Such studies on mitochondrial subfractionation are underway, and Santiago et al. (263) have reported the subfractionation of the *inner* mitochondrial membrane into discrete fractions from observation of negatively stained preparations of samples prefixed with OsO_4. Selective loss of phospholipids (phophatidyl ethanolamine and phosphatidyl choline) was mediated by preincubation of the inner mitochondrial membranes with ascorbate.

It is fair to say that the molecular biology of mitochondria, which once was restricted almost

entirely to studies of oxidative phosphorylation and of membrane structure and function, has entered a new phase of growth. Most dramatic has been the growing interest in what we have chosen to call systems-related functions. We would include in this category the biosynthesis of macromolecules such as DNA, RNA, and proteins, but would also consider the cellular sites of these syntheses, the organelles (e.g., nucleus, ribosomes) involved, the transport or translocation of these macromolecules, and the regulation and integration of these biosynthetic reactions. These correspond largely to those functional processes which Luria (173) has called "macro-regulatory phenomena." Much has already been written about the biogenesis of mitochondria, the products of mitochondrial protein synthesis, the properties of mitoribosomes compared with cytoribosomes and with bacterial ribosomes, and the regulation and cooperativity of mitochondrial and nuclear information which direct the genetic apparatus of mitochondria. Similarities and differences in these systems (187, 232, 233) must still be sorted out, as must any evolutionary significance which such comparisons may have.

It is recognized that mitochondrial DNA, like that of bacteria, is generally circular, and Nass (203–205) has shown that it is associated with the mitochondrial membranes.

Recent progress in mitochondrial genetics by Thomas and Wilkie (299), Coen et al. (49), Avner et al. (10), and Howell et al. (138) seems clearly to indicate that recombination between mitochondrial genomes does occur which now makes it feasible to begin to map mitochondrial genomes. In addition, Horak et al. (137) recently reported that mitochondrial DNA in hybrid somatic cells (human-mouse and human-rat) induced by Sindai virus contains sequences containing the two parental DNAs linked together, probably by phosphodiester bonds.

It seems clear that energy-linked vectorial translocations occur similarly across both mitochondrial and bacterial membranes and that these are mediated by the asymmetric topography of constituents comprising the multienzyme respiratory chain and coupling factors (128). Thus, the unifying chemiosmotic coupling hypothesis of Mitchell (189; see also 116) is currently the favored model by which adenosine triphosphate (ATP) synthesis and other energy-dependent processes are explained. Hughes et al. (140), as well as Harold in his excellent, detailed review of energy conservation (116), summarize some of the similarities and differences of mitochondria and energy-transducing membranes of prokaryotes. Certain principles seem applicable to both: for example, the sensitivity of certain enzymatic reactions to the degree of phosphorylation of adenine nucleotides, i.e., energy charge [(ATP) + $1/2$ (ADP)/(ATP) + (ADP) + (AMP)]. The inhibition of ATP-generating systems and acceleration of ATP-utilizing enzymes by high energy charge and the reversal of this phenomenon at low energy charge tends to maintain the ATP-ADP-AMP levels balanced so that metabolic steady state prevails. This mechanism seems important in balancing energy production and utilization both in eukaryotes and in bacteria. Chapman et al. (45) have shown that the energy charge in intact metabolizing cells of a wide variety of types is stabilized near 0.85.

It seems fairly well established now that major constituents of oxidative phosphorylation are associated with the plasma membrane of bacteria (214) and with the inner membrane of mitochondria (101, 102, 105, 274). Packer (217, 218) has summarized the current view of many workers of the molecular topography of mitochondrial inner membranes with respect to molecular interactions of lipid and protein constituents. This general concept is based largely on the fluid mosaic model of membranes proposed by Singer and Nicolson (286).

Knowledge of the constituents comprising the respiratory chain(s) and the factors coupling phosphorylation in bacterial systems is advancing at a fairly rapid rate largely as a result of the successful dissection and isolation of multimolecular complexes comprising these systems (157). John and Hamilton (144) some years ago were able to demonstrate respiratory control in membrane particles isolated in the presence of 1 mM ATP from lysozyme-treated *M. denitrificans*. The great variation in the constituents of the respiratory chain(s) in diverse bacterial species has been recognized for many years and is summarized by Harold (116). Brodie and Gutnick (29) also review various aspects of electron transport and oxidative phosphorylation in microbial systems.

Cavari et al. (41) reported, for example, evidence consistent with the idea that a heat-labile protein may function to regulate or inhibit oxidative phosphorylation in *M. phlei*. Such an inhibitor has been postulated to function in mitochondria. (See review by Pedersen, 227a.) Studies of whole cells and subcellular fractions have shown also that the electron transport chain in bacteria may be sensitive to relatively mild treatment, even to lysozyme (278). However, constituent parts can be separated after detergent treatment (e.g., deoxycholate), and activities, such as NADH oxidase (65, 340, 341), can be reconstituted. The selective release of Ca^{2+}-dependent ATPase from *M. lysodeikticus*,

as well as other membrane constituents, has been studied by Salton and colleagues in considerable detail (194, 256, 257, 261). By critically applying ferritin conjugated to antibody against highly purified ATPase, Oppenheim and Salton (214) obtained data which show rather conclusively that the Ca^{2+}-dependent ATPase of *M. lysodeikticus* is a constituent of the plama membrane but not of the mesosome fraction. Using fatty acid auxotrophs, Farias et al. (71) showed that the allosteric inhibition of the Ca^{2+}-ATPase of *E. coli* by Na^+ was dependent upon the fatty acid composition of the cell membrane. It is known that numerous membrane-bound enzymes when isolated require added phospholipids to restore activity (42, 143). Capaldi et al. (37) have reported recently the isolation of a major hydrophobic protein (as much as 10% of total inner membrane) from the inner mitochondrial membrane by treatment with lysolecithin. Capaldi and Vanderkooi (38) have categorized a number of membrane proteins according to their hydrophobic, nonpolar properties as a means of surveying their potential interactions with lipid constituents of membranes and, thus, as an index of their topographic relationships. Water-soluble, cold-labile ATPase (F_1) have been isolated and purified from beef heart, rat liver, and yeast mitochondria and from chloroplasts and bacteria (see Pedersen's review, 227a; 24, 39, 40, 186). The remarkable similarities in subunit (4–5 polypeptides) and amino acid composition of several of these soluble ATPases are clearly suggestive of multimolecular homology. The ATPase of *S. faecalis*, however, does not appear to fit the precise pattern of the other isolated molecules (275). Significant differences also exist in the properties of the "oligomycin-sensitive" complexes (Pedersen, 227a) isolated from various sources.

As pointed out by Stanier (288), strict homology between functional constituents of microbial and mitochondrial membranes must be established at the molecular level. However, the allotopic nature of membrane proteins (i.e., the modulation or regulation of activity may be governed by interaction with membrane components in the native state) means that alterations occur in proteins upon isolation and purification away from other membrane components. Allotopy is probably a characteristic of most membrane-bound proteins and has been invoked to account for differences in the properties of membrane-associated and solubilized enzymes of the mitochondrion. The molecular interactions of membrane constituents are complex and involve lipid-lipid, protein-protein, and lipid-protein interactions. Cardiolipin is generally recognized as a constituent of most, if not all, energy-transducing membranes (165, 168, 224). In this regard, what the functional role of this phospholipid is, if in fact a specific function is involved, remains a curious, unresolved question. It has been postulated that the acidic ATPase of *S. faecalis* may be found to polyanionic cardiolipid of the membrane by Mg^{2+} through ionic linkages (see 323). In addition, Lastras and Mũnoz (163) recently suggested that the membrane ATPase of *M. lysodeikticus* shows a latency which is modulated by Mg^{2+} via a Mg^{2+}-ATPase-membrane complex. Pedersen (227a) summarizes some of the effects of phospholipids on soluble mitochondrial F_1 (ATPase) and on the oligomycin-sensitive complex. It is of interest that cardiolipin, as well as lysolecithin, activates lipid-depleted preparations of the complex and also can release F_1 from the mitochondrial membrane. In the presence of cardiolipin, the affinity of lipid-depleted oligomycin-sensitive ATPase preparations for ATP is greatly enhanced. Guanieri et al.(108) concluded that cardiolipin molecules in intact rat liver, beef heart, yeast, and blow fly mitochondria are oriented in the membranes in such a way that the polar head groups (antigenic portions) are inaccessible to anti-cardiolipin antibody and, further, that cardiolipin is not involved in binding ATPase to the inner mitochondrial membrane. The basis for these discrepancies needs to be investigated further.

From our foregoing discussions, it would seem clear that mesosomes are not exclusive or even major sites of oxidative reactions, nor do they appear to contain ATPase coupling factors. However, the possibility that they may be involved indirectly in these fundamental processes must still remain open. In a series of papers, White and co-workers (211, 212, 308, 309) found that logarithmically growing *Haemophilus parainfluenzae* could be induced to release membrane fragments upon treatment with EDTA-Tris without loss of cell viability. Analysis of the fragments showed that those released early contained two- to fivefold higher proportions of cardiolipin and phosphatidylglycerol and less phosphatidylethanolamine than the residual membranes. It was concluded that the cell membrane was heterogeneous with respect to the distribution of phospholipids. In addition, a phospholipase D specific for cardiolipin was detected in soluble fraction of a cell homogenate (of this bacterium); the enzyme activity was Mg^{2+}-dependent, not activated by Ca^{2+}, and inhibited by EDTA. It was shown further that cells transferred to medium containing EDTA slowed in growth rate, that cardi-

olipin accumulated rapidly, and that the proportion of cardiolipin in the membrane increased with a corresponding loss of phosphatidylglycerol. These findings suggested that the cardiolipin-specific lipase was involved in the hydrolysis of this phospholipid in vivo. Inhibitors of oxidative phosphorylation, TCS (3,3',4,5'-tetrachlorosalicylanilide) and *m*-CCCP (carbonyl cyanide *m*-chlorophenyl hydrazone), which discharge the proton gradient across the cell membrane thereby interrupting energy-dependent transport also blocked cardiolipin hydrolysis with concomitant cardiolipin accumulation. It thus was suggested that a highly active portion of cellular cardiolipin is in some way linked to the energy-dependent transport process in this cell. Finally, Beining et al. (20) recently have analyzed in more detail the differences in lipid composition of the plasma membrane and mesosomes of *S. aureus*. Qualitatively the lipid composition was the same, but based on equal dry weights of membranes major phospholipids were quantitatively greater in the mesosomal vesicles than in the plasma membranes. Pertinent to the present discussion, cardiolipin was 3.6 times more concentrated in the mesosomal membrane fraction. These authors reopen the question of whether or not the mesosome may be involved in lipid metabolism as suggested much earlier by Fitz-James (80); meaningful conclusions must await further experimental results.

With regard to molecular homology, we have selected to discuss two proteins or types of proteins which, with more detailed investigation, may provide evidence of possible evolutionary links between bacteria and mitochondria; others undoubtedly exist such as the enzymes comprising the L-tyrosine biosynthetic pathway (290). The proteins grouped as iron-sulfur (Fe-S) proteins include ferrodoxins, rubredoxins, high potential iron proteins, and iron-sulfur flavoproteins. All contain nonheme iron in the active center coordinated to cysteine sulfurs. Functionally, these proteins serve as electron transport proteins and function in such widely diverse processes as mitochondrial respiration, photosynthesis, hydroxylation reactions, carbon metabolism, and nitrogen fixation. These proteins appear to be ubiquitous. Remarkable homology (amino acid sequence) has been found between ferrodoxins from various sources, especially those found in anaerobic bacteria of the genus *Clostridium*. The earliest Fe-S proteins are thought to be ferrodoxins with two 4Fe + 4S clusters. It has been suggested that there is an evolutionary development of ferrodoxins from the obligate anaerobic bacteria through the green and red photosynthetic and sulfate-reducing bacteria (all anaerobic) to the blue-green algae and finally to plants. It is thought that an evolutionary relationship may also exist between the ferrodoxins and *c*-type cytochromes. A complex membrane Fe-S protein isolated from *Rhodospirillum rubrum* can substitute for succinate dehydrogenase of the bovine heart mitochondrial electron transport chain. Thus, Fe-S proteins are found in the most primitive to the most evolved organism. These data are reviewed in great detail by Hall and colleagues (112, 115). With respect to mesosomal function, it would be of great interet to determine the intracellular sites at which Fe-S proteins are localized in aerobic bacterial cells.

Superoxide dismutase, which catalyzes the reaction $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$, appears to be present in all oxygen-metabolizing cells and serves to protect aerobic cells against O_2 toxicity (87, 106, 158, 289). Steinman and Hill (289) found that the NH_2-terminal sequences of the superoxide dismutase of chicken liver mitochondria (a manganous enzyme) are highly homologous to two dismutases of *E. coli* (a manganous and an iron enzyme). No homology was detected between the chicken mitochondrial enzyme and that found in bovine erythrocytes (a copper-zinc enzyme). These workers postulated a common ancestral beginning for chicken liver mitochondria and *E. coli,* since these two organisms are phylogenetically widely separated whereas the taxonomic distance between chicken and cattle is relatively short. Fridovich (87) further postulated that, since both the cytosol and mitochondria of eukaryotes contain dismutases, containing copper and zinc versus manganese, respectively, mitochondria not only evolved via symbiosis but that prototypes of present eukaryotes and prokaryotes had already diverged during the anaerobic phase of life's history. These two types of eukaryotic superoxide dismutases suggested also that the symbiosis occurred only after much O_2 had accumulated in the atmosphere and after both the protomitochondrion and host protoeukaryote had each evolved a distinctive enzyme. In the case of this enzyme, as with the Fe-S proteins, the intracellular and intraorganellar localization of this enzyme might shed additional light on possible relationships between mitochondria and mesosomes.

SUMMARY AND CONCLUSIONS

The biochemical functions and physiological importance of bacterial mesosomes remain a mystery still. It seems clear that mesosomes are not equivalents of mitochondria; rather, the available evidence suggests that mesosomes

most likely are multifunctional or that they are of diverse function and vary from cell type to cell type and possibly vary from one growth phase to another. Accumulated evidence suggests that mesosomes are not strictly artifacts of chemical fixation, but it is not certain in all cases what the ultrastructural organization of these structures is in the native state. Improved methods of isolation promise to clarify the chemical and enzymatic composition of mesosomal membranes and to provide a basis by which these organelles can be isolated from a wider spectrum of bacteria and compared. The possibility that mitochondria contain mesosomes remains an open question, and it is premature at present to conclude from ultrastructural evidence only that membranous inclusions in mitochondria are equivalent to mesosomes. It should be remembered that mitochondria from various sources are not identical in all aspects of either ultrastructure or biochemical function. The idea that mitochondria evolved from bacteria is being subjected to closer scrutiny, and more serious objections are being raised than just a few years ago. Attempts to extrapolate directly from knowledge of mitochondrial to bacterial structure and function, or to extrapolate in the reverse direction, in general have proved futile. Knowledge of each system still remains fragmentary despite marked advances. Nevertheless, these concepts, despite their speculative nature, are of value in stimulating new ideas, and hypotheses, when proposed in terms which can be tested experimentally, provide new avenues by which these challenging problems can be attacked.

ACKNOWLEDGMENTS

We are grateful to many colleagues too numerous to mention who in toto provided stimulating discussions of mesosomes and, in a more concrete way, forwarded reprints, preprints, and micrographs of their completed works or of studies in progress. We are especially grateful to D. P. Beck, M. M. Burger, R. M. Cole, H. Fanzadegon, A. Goffeau, D. O. Hall, P. H. Hardy, Jr., F. M. Harold, P. J. Highton, M. Kennedy, O. E. Landman, W. J. Lennarz, J. D. Oppenheim, P. L. Pedersen, V. M. Reusch, H. J. Rogers, F. Rothwell, M. R. J. Salton, J. M. Shively, and R. A. Weigand. We also greatly appreciate the drawings of Ronald Garrett, the darkroom work of Jeffrey Greenwalt, the aid of Ann Gibson and David Joseph in organizing figures and references, and the patience and skill of Shirley Skiles, Elizabeth Saeger, and Linda Knowles in typing the manuscript through its many revisions. The aid of Karen Allen in the final proofreading is also acknowledged. The patience of the editors in seeing this manuscript through to its completion is deeply appreciated. The excellent electron microscopy of Glenn Decker done in conjunction with the original work in the laboratory of J. W. G. is acknowledged with pleasure. This effort was supported by research grants GB 31098 from the National Science Foundation and NS 12016 from the National Institute of Neurological Diseases and Stroke, Public Health Service, to one of us (J. W. G.).

LITERATURE CITED

1. Alexander, M., and P. W. Wilson. 1956. Intracellular distribution of tricarboxylic acid cycle enzymes in *Azotobacter vinelandii*. J. Bacteriol. 71:252-253.
2. Allen, M. M. 1972. Mesosomes in blue-green algae. Arch. Mikrobiol. 84:199-206.
3. Altenburg, B. C., and J. C. Suit. 1970. Relation between deoxyribonucleic acid and intracytoplasmic membranes in *Escherichia coli* O111a$_1$. J. Bacteriol. 103:227-237.
4. Altenburg, B. C., J. C. Suit, and B. R. Brinkley. 1970. Ultrastructure of deoxyribonucleic acid-membrane associations in *Escherichia coli*. J. Bacteriol. 104:549-555.
5. Altendorf, K. H., and L. A. Staehelin. 1974. Orientation of membrane vesicles from *Escherichia coli* as detected by freeze-cleave electron microscopy. J. Bacteriol. 117:888-899.
6. Archakov, A. I., L. F. Panchenko, A. B. Kapitanov, I. I. Efron, T. I. Knyazeua, and N. S. Z'herebkova. 1973. A quantitative estimation of degree of purity of preparations of subcellular structures. Anal. Biochem. 54:223-244.
7. Ashwell, M., and T. S. Work. 1970. The biogenesis of mitochondria. Annu. Rev. Biochem. 39:251-290.
8. Attardi, G., and D. Ojala. 1971. Mitochondrial ribosomes in HeLa cells. Nature (London) New Biol. 229:133-136.
9. Avakyan, A. A. 1971. Principles of submicroscopic organization of microorganisms and their evolution mechanisms, p. 65-75. *In* A. Perez-Miravete and D. Pelaez (ed.), Recent advances in microbiology, Int. Congr. Microbiol., Mexico, D.F.
10. Avner, P. R., D. Coen, B. Dujon, and P. P. Slonimski. 1973. Mitochondrial genetics. IV. Allelism and mapping studies of oligomycin resistant mutants in *S. cervisiae*. Mol. Gen. Genet. 125:9-52.
11. Bacon, K., and D. White. 1974. Phospholipid metabolism and membrane synthesis in *Bacillus megaterium*. J. Bacteriol. 118:225-230.
12. Barksdale, L. 1970. *Corynebacterium diphtheriae* and its relatives. Bacteriol. Rev. 34:378-422.
13. Barnard, T. 1969. The ultrastructural differentiation of brown adipose tissue in the rat. J. Ultrastruct. Res. 29:311-322.
14. Barnard, T., and B. A. Afzelius. 1972. The matrix granules of mitochondria: a review. Sub-Cell. Biochem. 1:375-389.
15. Barnard, T., and O. Lindberg. 1969. Ultrastructural changes in chondriome during perinatal development in brown adipose tissue of

rats. J. Ultrastruct. Res. 29:293-310.

16. Barrnett, R. J., and G. E. Palade. 1957. Histochemical demonstration of the sites of activity of dehydrogenase systems with the electron microscope. J. Biophys. Biochem. Cytol. 3:577-588.
17. Bayer, M. E. 1968. Areas of adhesion between wall and membrane of *Escherichia coli*. J. Gen. Microbiol. 53:395-404.
18. Beaton, C. B. 1968. An electron microscope study of the mesosomes of a penicillinase-producing staphylococcus. J. Gen. Microbiol. 50:37-42.
19. Beck, D. P., and J. W. Greenawalt. 1968. Factors affecting the formation of membranous structures in the cytoplasm and mitochondria of *Neurospora crassa*. J. Cell Biol. 39:11a.
20. Beining, P. R., E. Huff, B. Prescott, and T. S. Theodore. 1975. Characterization of the lipids of mesosomal vesicles and plasma membranes from *Staphylococcus aureus*. J. Bacteriol. 121:137-143.
21. Bertsch, L. L., P. M. Bonsen, and A. Kornberg. 1969. Biochemical studies of bacterial sporulation and germination. J. Bacteriol. 98:75-81.
22. Bisalputra, T., and H. Burton. 1970. On the choloroplast DNA-membrane complex in *Sphacelaria sp*. J. Microscopie 9:661-666.
23. Borst, P. 1972. Mitochondrial nucleic acids. Annu. Rev. Biochem. 41:333-376.
24. Bragg, P. D., P. L. Davies, and C. Hou. 1973. Effect of removal or modification of subunit polypeptides on the coupling factor and hydrolytic activities of the Ca^{2+} and Mg^{2+}-activated adenosine triphosphatase of *Escherichia coli*. Arch. Biochem. Biophys. 159:664-670.
25. Brandt, J. T., A. P. Martin, F. V. Lucas, and M. L. Vorbeck. 1974. The structure of rat liver mitochondria: a reevaluation. Biochem. Biophys. Res. Commun. 59:1097-1103.
26. Briles, E. B., and A. Tomasz. 1970. Radioautographic evidence for equatorial wall growth in a gram-positive bacterium. J. Cell Biol. 47:786-790.
27. Briles, E., and A. Tomasz. 1973. Pneumonococcal Forssman antigen. A choline-containing lipoteichoic acid. J. Biol. Chem. 248:6394-6397.
28. Brock, T. D. 1962. Effects of magnesium ion deficiency on *Escherichia coli*: a possible relation to the mode of action of novobiocin. J. Bacteriol. 84:679-682.
29. Brodie, A. F., and D. L. Gutnick. 1972. Electron transport and oxidative phosphorylation in microbiol systems, p. 599-681. *In* T. E. King and M. Klingenberg (ed.), Electron and coupled energy transfer in biological systems, vol. 1B. Marcel Dekker, New York.
30. Burdett, I. D. J. 1972. Bacterial mesosomes. Sci. Prog. (Oxford) 60:527-546.
31. Burdett, I. D. J., and R. G. E. Murray. 1974. Electron microscope study of septum formation in *Escherichia coli* strains B and B/r during synchronous growth. J. Bacteriol. 119:1039-1056.
32. Burdett, I. D. S., and R. G. E. Murray. 1974. Septum formation in *Escherichia coli*: characterization of septal structure and the effects of antibiotics on cell division. J. Bacteriol. 119:303-324.
33. Burdett, I. D. J., and H. J. Rogers. 1970. Modification of the appearance of mesosomes in sections of *Bacillus licheniformis* according to the fixation procedures. J. Ultrastruct. Res. 30:354-367.
34. Burdett, I. D. J., and H. J. Rogers. 1972. The structure and development of mesosomes studied in *Bacillus licheniformis* strain 6346. J. Ultrastruct. Res. 38:113-133.
35. Burnham, J. C., T. Hashimoto, and S. F. Conti. 1968. Electron microscopic observations on the penetration of *Bdellovibrio bacteriovorus* into gram-negative bacterial hosts. J. Bacteriol. 96:1366-1381.
36. Burnham, J. C., T. Hashimoto, and S. F. Conti. 1970. Ultrastructure and cell division of a facultatively parasitic strain of *Bdellovibrio bacteriovorus*. J. Bacteriol. 101:997-1004.
37. Capaldi, R. A., H. Komai, and D. Hunter. 1973. Isolation of a major hydrophobic protein of the mitochondrial inner membrane. Biochem. Biophys. Res. Commun. 55:655-659.
38. Capaldi, R. A., and G. Vanderkooi. 1972. The low polarity of many membrane proteins. Proc. Natl. Acad. Sci. U.S.A. 69:930-932.
39. Catterall, W. A., W. A. Coty, and P. L. Pederson. 1973. Adenosine triphosphatase from rat liver mitochondria. III. Subunit composition. J. Biol. Chem. 248:7427-7431.
40. Catterall, W. A., and P. L. Pederson. 1971. Adenosine triphosphatase from rat liver mitochondria. I. Purification, homogeneity, and physical properties. J. Biol. Chem. 246:4987-4994.
41. Cavari, B. Z., V. K. Kalra, and R. F. Brodie. 1971. Oxidative phosphorylation in fractionated bacterial systems: effect of chloramphenicol. J. Bacteriol. 108:1017-1025.
42. Cerletti, P., R. Strom, and M. G. Giordano. 1965. Reactivation of succinic dehydrogenase by phospholipids. Biochem. Biophys. Res. Commun. 18:259-263.
43. Chai, N.-C., and K. G. Lark. 1967. Segregation of deoxyribonucleic acid in bacteria: association of the segregating unit with the cell envelope. J. Bacteriol. 94:415-421.
44. Chang, K.-P., and W. Trager. 1974. Nutritional significance of symbiotic bacteria in two species of hemoflagellates. Science 183:531-532.
45. Chapman, A. G., L. Fall, and D. E. Atkinson. 1971. Adenylate energy charge in *Escherichia coli* during growth and starvation. J. Bacteriol. 108:1072-1086.
46. Chapman, G. B., and J. Hillier. 1953. Electron microscopy of ultrathin sections of bacteria.

I. Cellular division in *Bacillus cereus*. J. Bacteriol. 66:362–373.

47. Chesbro, W. R., and J. O. Lampen. 1968. Characteristics of secretion of penicillinase, alkaline phosphatase, and nuclease from *Bacillus* species. J. Bacteriol. 96:428–437.
48. Choules, G. L., and W. R. Gray. 1971. Peptidase activity in the membranes of *Mycoplasma laidlawii*. Biochem. Biophys. Res. Commun. 45:849–855.
49. Coen, D., J. Deutsch, P. Netter, E. Petrochilo, and P. P. Slonimski. 1970. Mitochondrial genetics, methodology and phenomenology, p. 449–496. *In* P. L. Miller (ed.), Control of organelle development, Symp. 24, Soc. Exp. Biol. Academic Press Inc., New York.
50. Cohen, S. 1970. Are/were mitochondria and chloroplasts microorganisms? Am. Sci. 58:281–289.
51. Cohen-Bazirre, G., R. Kunisawa, and J. S. Poindexter. 1966. The internal membranes of *Caulobacter crescentus*. J. Gen. Microbiol. 42:301–308.
52. Cole, R. M., T. J. Popkin, R. J. Boylan, and N. H. Mendelson. 1970. Ultrastructure of a temperature-sensitive rod mutant of *Bacillus subtilis*. J. Bacteriol. 103:793–810.
53. Conti, S. F., and P. Hirsch. 1965. Biology of budding bacteria. III. Fine structure of *Rhodomicrobium* and *Hyphomicrobium* spp. J. Bacteriol. 89:503–512.
54. Conti, S. F., N. J. Jacobs, and C. T. Gray. 1968. Ultrastructure and respiratory capacity of *Staphylococcus* and *Bacillus* grown under aerobic and anaerobic conditions. J. Bacteriol. 96:544–556.
55. Cota-Robles, E. H. 1963. Electron microscopy of plasmolysis in *Escherichia coli*. J. Bacteriol 85:499–503.
56. Cota-Robles, E. H. 1966. Internal membranes in cells of *Escherichia coli*. J. Ultrastruct. Res. 16:626–639.
57. Daneo-Moore, L., and M. L. Higgins. 1972. Morphokinetic reaction of *Streptococcus faecalis* (ATCC 9790) cells to the specific inhibition of macromolecular synthesis: nucleoid condensation on the inhibition of protein synthesis. J. Bacteriol. 109:1210–1220.
58. Daniels, M. J. 1969. Lipid synthesis in relation to the cell cycle of *Bacillus megaterium* KM and *Escherichia coli*. Biochem. J. 115:697–701.
59. Daniels, M. J. 1969. Aspects of membrane synthesis during the cell cycle of *Bacillus megaterium* and *Escherichia coli*. J. Gen. Microbiol. 58:iv.
60. deDuve, C. 1970. Tissue fractionation, past and prêsent. J. Cell Biol. 50:20–55.
61. deDuve, C. 1973. Origin of mitochondria. Science 182:85.
62. deRobertis, E. D. P., W. W. Nowinski, and F. A. Saez. 1970. Cell biology, 5th ed., p. 199–228. W. B. Saunders Co., Philadelphia.
63. Dworsky, P., and M. Schaechter. 1973. Effect of rifampin on the structure and membrane attachment of the nucleoid of *Escherichia coli*. J. Bacteriol. 116:1364–1374.
64. Edwards, M. R. 1963. Electron microscopic observations of the morphological characteristics of *Listeria monocytogenes*, p. 210–220. *In* M. L. Gray (ed.), Second symposium on listeric infection, Montana State College, Bozeman.
65. Eisenberg, R. C. 1971. Restoration of deoxycholate-disrupted membrane oxidases of *Micrococcus lysodeikticus*. J. Bacteriol. 108:964–972.
66. Ellar, D. J. 1969. Structure of the cytoplasmic membrane and mesosomes. J. Gen. Microbiol. 57:vi–viii.
67. Ellar, D. J., and J. H. Freer. 1969. The isolation and characterization of mesosome material from *Micrococcus lysodeikticus*. J. Gen. Microbiol. 58:vii.
68. Ellar, D. J., D. G. Lundgren, and R. A. Slepecky. 1967. Fine structure of *Bacillus megaterium* during synchronous growth. J. Bacteriol. 94:1189–1205.
69. Ellar, D. J., and J. A. Postgate. 1972. Peptidoglycan lytic system associated in mesosomal membranes from *Micrococcus lysodeikticus*. Biochem. Soc. 52nd Annu. Meet., Cambridge.
70. Ellar, D. J., T. D. Thomas, and J. A. Postgate. 1971. Properties of mesosomal membranes isolated from *Micrococcus lysodeikticus* and *Bacillus megaterium*. Biochem. J. 122:44p–45p.
71. Farias, R. N., L. Londero, and R. E. Trucco. 1972. Effect of membrane lipid composition on the allosteric inhibition by sodium of the (Ca^{2+})-adenosine triphosphatase from *Escherichia coli*. J. Bacteriol. 109:471–473.
72. Ferrandes, B., P. Chaix, and A. Ryter. 1966. Localisation des cytochromes de *B. subtilis* dans les structures mesosomiques. C. R. Acad. Sci. 263:1632–1635.
73. Ferrandes, B., C. Frehel, and P. Chaix. 1970. Fractionnement et purification des systemes membranaires cytoplasmiques et mesosomiques de *B. subtilis*. Biochim. Biophys. Acta 223:292–308.
74. Fielding, P., and C. Fox. 1970. Evidence for stable attachment of DNA to membrane at the replication origin of *Escherichia coli*. Biochem. Biophys. Res. Commun. 41:157–162.
75. Firshein, W. 1970. DNA membrane complex: macromolecular content and stimulation of enzymatic activity by polyadenylic acid. Science 169:66–68.
76. Firshein, W. 1973. In situ activity of enzymes on polyacrylamide gels of a deoxyribonucleic acid-membrane fraction extracted from pneumonococci. J. Bacteriol. 118:1101–1110.
77. Fitz-James, P. C. 1958. Cytological and chemical studies of the growth of protoplasts of *Bacillus megaterium*. J. Biophys. Biochem. Cytol. 4:257–265.
78. Fitz-James, P. C. 1960. Participation of the cytoplasmic membrane in the growth and

spore formation of bacilli. J. Biophys. Biochem. Cytol. 8:507-528.
79. Fitz-James, P. C. 1967. A functional and structural comparison of separated fractions of mesosomes and plasma membrane of bacteria. Protides Biol. Fluids, Proc. Colloq. 15:289-301.
80. Fitz-James, P. C. 1968. The collection of mesosome vesicles extruded during protoplasting, p. 124-143. *In* L. B. Guze (ed.), Microbial protoplasts, spheroplasts, and L-forms. The Williams and Wilkins Co., Baltimore.
81. Flavell, R. 1972. Mitochondria and chloroplasts as descendants of prokaryotes. Biochem. Genet. 6:275-291.
82. Forsberg, C. W., and S. B. Ward. 1972. *N*-acetylmuramyl-L-alanine amidase of *Bacillus licheniformis* and its L-form. J. Bacteriol. 110:878-888.
83. Freer, J. H., K. S. Kim, M. R. Krauss, L. Beaman, and L. Barksdale. 1969. Ultrastructural changes in bacteria isolated from cases of leprosy. J. Bacteriol. 100:1062-1075.
84. Frehel, C. 1974. Succinodehydrogenase activity of cytoplasmic and mesosomal membranes during sporulation of *B. subtilis*. Enzymatic analysis and cytochemical study. Biochimie 56:571-581.
85. Frehel, C., C. Duhray, and A. Wolff. 1974. Esterase activity of cytoplasmic and mesosomal membrane during sporulation of *B. subtilis*. Biochimie 56:583-598.
86. Frehel, C., B. Ferrandes, and A. Ryter. 1971. Reactions d'oxydo-reduction au miveau des membranes cytoplasmiques et mesosomiques de *Bacillus subtilis*. Biochim. Biophys. Acta 234:226-241.
87. Fridovich, I. 1973. Superoxide radical and superoxide dismutase. Biochem. Soc. Trans. 1:48-50.
88. Fuchs, E., and P. Hanawalt. 1970. Isolation and characterization of the DNA replication complex from *Escherichia coli*. J. Mol. Biol. 52:301-322.
89. Gale, E. F. (ed.). 1969. Synthesis and organization in the bacterial cell, p. 104. John Wiley & Sons, Inc., New York.
90. Ganesan, A. T., and J. Lederberg. 1965. A cell membrane-bound fraction of DNA. Biochem. Biophys. Res. Commun. 18:824-835.
91. Garland, J. M. 1974. Mesosomes in *Bacillus cereus* 569 and the production of extra membranes by treatment with actinomycin-D. J. Gen. Microbiol. 80:419-431.
92. Garrard, W. T. 1972. Synthesis, assembly, and localization of periplasmic cytochrome C. J. Biol. Chem. 247:5935-5943.
93. Gel'man, N. S., M. A. Lukoyanova, and D. N. Ostrovskii. 1967. Respiration and phosphorylation of bacteria. Plenum Press, New York.
94. Ghosh, B. K., and R. G. E. Murray. 1967. Fine structure of *Listeria monocytogenes* in relation to protoplast formation. J. Bacteriol. 93:411-426.
95. Ghosh, B. K., and R. G. E. Murray. 1969. Fractionation and characterization of the plasma and mesosome membrane of *Listeria monocytogenes*. J. Bacteriol. 97:426-440.
96. Ghosh, B. K., M. G. Sargent, and J. O. Lampen. 1968. Morphological phenomena associated with penicillinase induction and secretion in *Bacillus licheniformis*. J. Bacteriol. 96:1314-1328.
97. Ghosh, B. K., M. G. Sargent, and J. O. Lampen. 1968. Morphological phenomena associated with penicillinase induction and secretion in *Bacillus licheniformis*. J. Bacteriol. 96:1314-1328.
98. Giesbrecht, P. 1960. Uber Organisierte Mitochondrien und andere Fein-Strukturen von *Bacillus megaterium*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1. Orig. 179:538-581.
99. Giesbrecht, P., and H. Ruska. 1968. Uber Veranderungen der Feinstrukturen vor Bakterien unter der Einwirkung von Chloramphenicol. Klin. Wochenschr. 46:575-582.
100. Gilby, A. R., A. V. Few and K. McQuilen. 1958. The chemical composition of the protoplast membrane of *Micrococcus lysodeikticus*. Biochim. Biophys. Acta 29:21-29.
101. Greenawalt, J. W. 1972. Localization of monoamine oxidase in rat liver mitochondria. Adv. Biochem. Psychopharmacol. 5:207-226.
102. Greenawalt, J. W. 1974. The isolation of the outer and inner mitochondrial membranes, p. 310-323. *In* S. Fleischer and L. Packer (ed.), Methods in enzymology. Biomembranes vol. 31A. Academic Press Inc., New York.
103. Greenawalt, J. W. 1974. Isolation and characterization of extra membranes in E. coli 0111_a, p. 633-642. *In* S. Fleischer and L. Packer (ed.), Methods in enzymology. Biomembranes, vol. 31A. Academic Press Inc., New York.
104. Greenawalt, J. W., D. P. Beck, and E. S. Hawley. 1972. Chemical and biochemical changes in mitochondria during morphogenetic development of *Neurospora crassa*, p. 541-558. *In* G. F. Azzone, E. Carafoli, A. L. Lehninger, E. Quagliariello, and N. Siliprandi (ed.), International symposium on the biochemistry and biophysics of mitochondrial membranes, Adriatica Editrice. Academic Press Inc., New York.
105. Greenawalt, J. W., and C. Schnaitman. 1970. An appraisal of the use of monoamine oxidase as an enzyme marker for the outer membrane of rat liver mitochondria. J. Cell Biol. 46:173-179.
106. Gregory, E. M., S. A. Goscin, and I. Fridovich. 1974. Superoxide dismutase and oxygen toxicity in a eukaryote. J. Bacteriol. 117:456-460.
107. Grimes, G. W., H. R. Mahler, and P. S. Perlman. 1974. Mitochondrial morphology. Science 185:630-631.
108. Guarnieri, M., B. Stechmiller, and A. L. Lehninger. 1971. Use of an antibody to study the location of cardiolipin in mitochondrial

membranes. J. Biol. Chem. 246:7526-7532.
109. Gupta, P. D., J. R. Koke, and S. K. Malhotra. 1971. Remarks on membranes of *Neurospora crassa* after embedding in glycol methacrylate. Cytobios 3:117-128.
110. Hackenbrock, C. R. 1972. States of activity and structure in mitochondrial membranes. Ann. N.Y. Acad. Sci. 195:492-505.
111. Hagen, P. O., H. Goldfine, and P. J. le B. Williams. 1966. Phospholipids of bacteria with extensive intracytoplasmic membranes. Science 151:1543-1544.
112. Hall, D. O., R. Cammack, and K. K. Rao. 1974. The iron-sulphur proteins: evolution of a ubiquitous protein from model systems to higher organisms. Origins Life 5:363-386.
113. Hall, D. O., and J. W. Greenawalt. 1967. The preparation and biochemical properties of mitochondria from *Neurospora crassa*. J. Gen. Microbiol. 48:419-430.
114. Hall, D. O., and J. M. Palmer. 1969. Mitochondrial research today. Nature (London) 221:717-723.
115. Hall, D. O., K. K. Rao, and R. Cammack. 1975. The iron-sulphur proteins: structure, function, and evolution of a ubiquitous group of proteins. Sci. Prog. 62:285-316.
116. Harold, F. M. 1972. Conservation and transformation of energy by bacterial membranes. Bacteriol. Rev. 36:172-230.
117. Harold, F. M., and J. R. Baarda. 1968. Inhibition of membrane transport in *Streptococcus faecalis* by uncouplers of oxidative phosphorylation and its relationship to proton conduction. J. Bacteriol. 96:2025-2034.
118. Hatefi, Y., and W. G. Hanstein. 1969. Solubilization of particulate proteins and nonelectrolytes by chaotropic agents. Proc. Natl. Acad. Sci. U.S.A. 62:1129-1136.
118a. Hawley, E. S., and J. W. Greenawalt. 1975. Biogenesis of mitochondrial membranes in *Neurospora crassa*. Mitochondrial protein synthesis during conidial germination. Eur. J. Biochem. 54:585-601.
119. Higaski, T., E. Bogin, and A. F. Brodie. 1969. Separation of a factor indispensable for coupled phosphorylation from the particulate fraction of *Mycobacterium phlei*. J. Biol. Chem. 244:500-508.
120. Higgins, M. L., and L. Daneo-Moore. 1972. Morphokinetic reaction of cells of *Streptococcus faecalis* (ATCC 9690) to specific inhibition of macromolecular synthesis: dependence of mesosome growth on deoxyribonucleic acid synthesis. J. Bacteriol. 109:1221-1231.
121. Higgins, M. L., and L. Daneo-Moore. 1974. Factors influencing the frequency of mesosomes observed in fixed and unfixed cells of *Streptococcus faecalis*. J. Cell Biol. 61:288-300.
122. Higgins, M. L., and G. D. Shockman. 1970. Early changes in the ultrastructure of *Streptococcus faecalis* after amino acid starvation. J. Bacteriol. 103:244-254.
123. Higgins, M. L., and G. D. Shockman. 1971. Prokaryotic cell division with respect to wall and membranes. Crit. Rev. Microbiol. 1:29-72.
124. Highton, P. J. 1969. An electron microscopic study of cell growth and mesosomal structure of *Bacillus licheniformis*. J. Ultrastruct. Res. 26:130-147.
125. Highton, P. J. 1970. An electron microscopic study of the structure of mesosomal membranes in *Bacillus licheniformis*. J. Ultrastruct. Res. 31:247-259.
126. Highton, P. J. 1970. An electron microscopic study of mesosomes in *Bacillus subtilis*. J. Ultrastruct. Res. 31:260-271.
127. Highton, P. J., and D. G. Hobbs. 1971. Penicillin and cell wall synthesis: a study of *Bacillus licheniformis* by electron microscopy. J. Bacteriol. 106:656-658.
128. Hirota, H., K. Altendorf, and F. M. Harold. 1974. Energy coupling in membrane vesicles of *Escherichia coli*. I. Accumulation of metabolites in response to an electrical potential. J. Biol. Chem. 249:2939-2945.
129. Hirota, Y., J. Mordoh, I. Scheffler, and F. Jacob. 1972. Genetic approach to DNA replication and its control in *Escherichia coli*. Fed. Proc. 31:1422-1427.
130. Hirota, Y., A. Ryter, and F. Jacob. 1968. Thermosensitive mutants of *E. coli* affected in the processes of DNA synthesis and cellular division. Cold Spring Harbor Symp. Quant. Biol. 33:677-693.
131. Hoffman, H. P., and C. J. Avers. 1974. Mitochondrial morphology. Science 185:631.
132. Hoffman, H. P., and C. J. Avers. 1973. Mitochondrion of yeast: ultrastructural evidence for one giant, branched organelle per cell. Science 181:749-751.
133. Hoffman, H. P., S. G. Geftic, and H. Heyman. 1973. Mesosomes in *Pseudomonas aeruginosa*. J. Bacteriol. 114:434-438.
134. Holt, S. C., and E. R. Leadbetter. 1969. Comparative ultrastructure of selected aerobic spore-forming bacteria: a freeze-etching study. Bacteriol. Rev. 33:346-378.
135. Holt, S. C., J. M. Shively and J. W. Greenawalt. 1974. Fine structure of selected species of the genus *Thiobacillus* as revealed by chemical fixation and freeze-etching. Can. J. Microbiol. 20:1347-1351.
136. Hopwood, D. A., and A. M. Glauert. 1960. A fine structure of *Streptomyces coelicolor*. J. Biophys. Biochem. Cytol. 8:267-278.
137. Horak, I., H. G. Coon, and I. B. Dawid. 1974. Interspecific recombination of mitochondrial DNA molecules in hybrid somatic cells. Proc. Natl. Acad. Sci. U.S.A. 71:1828-1832.
138. Howell, N., M. K. Trembath, A. W. Linnane, and H. B. Lukins. 1973. Biogenesis of mitochondria. 30. An analysis of polarity of mitochondrial gene recombination and transmission. Mol. Gen. Genet. 122:37-51.
139. Huff, E., R. M. Cole, and T. S. Theodore. 1974. Lipoteichoic acid localization in mesosomal vesicles of *Staphylococcus aureus*. J. Bacteriol. 120:273-281.

140. Hughes, D. E., D. Lloyd, and R. Brightwell. 1970. Structure, function, and distribution of organelles in prokaryotic and eukaryotic microbes. Symp. Soc. Gen. Microbiol. 20:295–323.
141. Inouye M., and A. B. Pardee. 1970. Changes of membrane proteins and their relation to deoxyribonucleic acid synthesis and cell division of *Escherichia coli*. J. Biol. Chem. 245:5813–5819.
142. Jacob, F., S. Brenner, and F. Cuzin. 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. 28:329–348.
143. Janki, R. M., H. N. Aithal, W. C. McMurray, and E. R. Tustanoff. 1974. The effect of altered membrane-lipid composition on enzyme activities of outer and inner mitochondrial membranes of *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 56:1078–1085.
144. John, P., and W. A. Hamilton. 1970. Respiratory control in membrane particles from *Micrococcus denitrificans*. FEBS Lett. 10:246–248.
145. Kaback, H. R. 1972. Transport across isolated bacterial cytoplasmic membranes. Biochim. Biophys. Acta 265:367–416.
146. Kalf, G. F., and A. S. Faust. 1969. The inner membrane of the rat liver mitochondrion as the site of incorporation of radioactively labeled precursors into nucleic acid and protein in vitro. Arch. Biochem. Biophys. 134:103–112.
147. Katz, L. N., and I. H. Torjan. 1968. Change in the membrane structures of *Staphylococcus* under different conditions of cultivation. Mikrobiologiya 37:890–896.
148. Kawakami, M., and O. E. Landman. 1966. Retention of episomes during protoplasting and during propagation in the L state. J. Bacteriol. 92:398–404.
149. Kellems, R. E., V. F. Allison, and R. A. Butow. 1974. Cytoplasmic type 80S ribosomes associated with yeast mitochondria. II. Evidence for the association of cytoplasmic ribosomes with the outer mitochondrial membrane *in situ*. J. Biol. Chem. 249:3297–3303.
150. Kellems, R. E., and R. A. Butow. 1972. Cytoplasmic type 80S ribosomes associated with yeast mitochondria. I. Evidence for ribosome binding sites on yeast mitochondria. J. Biol. Chem. 247:8043–8050.
151. Kellems, R. E., and R. A. Butow. 1974. Cytoplasmic type 80S ribosomes associated with yeast mitochondria. III. Changes in the amount of bound ribosomes in response to changes in metabolic state. J. Biol. Chem. 249:3304–3310.
152. Koike, K., and D. R. Wolstenholme. 1974. Evidence for discontinuous replication of circular mitochondrial DNA molecules from Novikoff rat ascites hepatoma cells. J. Cell Biol. 61:14–25.
153. Koke, J. R., D. D. Gupta, and S. K. Malhotra. 1971. A succinic dehydrogenase activity in "mesosomes" of *Neurospora crassa*. Biochem. Biophys. Res. Commun. 42:576–582.
154. Koke, J. R., and S. K. Malhotra. 1969. Mesosome-like membranous structures in mitochondria of *Neurospora*. J. Cell Biol. 43:71a.
155. Konings, W. N., A. Bisschop, M. Veenhuis, and C. A. Vermeulen. 1973. New procedure for the isolation of membrane vesicles of *Bacillus subtilis* and an electron microscopy study of their ultrastructure. J. Bacteriol. 116:1456–1465.
156. Konings, W. N., E. M. Barnes, and H. R. Kaback. 1971. Mechanisms of active transport in isolated membrane vesicles. III. The coupling of reduced phenazine methosulfate to the concentrative uptake of β-galactosides and amino acids. J. Biol. Chem. 246:5857–5861.
157. Kornberg, H. L. 1959. Aspects of terminal respiration in microorganisms. Annu. Rev. Microbiol. 13:49–78.
158. Krinsky, N. I. 1974. Singlet oxygen as a mediator of the antibacterial action of leukocytes. Science 186:363–365.
159. Kroon, A. M., and C. Saccone (ed.). 1974. The biogenesis of mitochondria. Academic Press Inc., New York.
160. Kuriyama, Y., and D. J. L. Luck. 1973. Membrane-associated ribosomes in mitochondria of *Neurospora crassa*. J. Cell Biol. 59:776–784.
161. Lampen, J. O. 1967. Release of penicillinase by *Bacillus licheniformis*. J. Gen. Microbiol. 48:261–268.
162. Landman, O. E., A. Ryter, and C. Fréhel. 1968. Gelatin-induced reversion of protoplasts of *Bacillus subtilis* to the bacillary form: electron-microscopic and physical study. J. Bacteriol. 96:2154–2170.
163. Lastras, M., and E. Mũnoz. 1974. Membrane adenosine triphosphatase of *Micrococcus lysodeikticus*: effect of millimolar Mg^{2+} in modulating the properties of membrane-bound enzyme. J. Bacteriol. 119:593–601.
164. Leene, W., and W. van Iterson. 1965. Tetranitro-blue tetrazolium reduction in *Bacillus subtilis*. J. Cell Biol. 27:237–241.
165. Lehninger, A. L. 1964. The mitochondrion. W. A. Benjamin, Inc., New York.
166. Lenard, J., and S. J. Singer. 1967. Protein conformation in cell membrane preparations as studied by optical rotatory dispersion and circular dichroism. Proc. Natl. Acad. Sci. U.S.A. 56:1828–1835.
167. Lennarz, W. J., and M. Scher. 1972. Metabolism and function of polyisoprenol sugar intermediates in membrane-associated reactions. Biochim. Biophys. Acta 265:417–441.
168. Levy, M., and M. Sauner. 1967. Composition en phospholipids des membranes interne et externe des mitochondries. C. R. Seances Soc. Biol. Paris 161:277–279.
169. Lin, E. C. C., Y. Hirota, and F. Jacob. 1971. On the process of cellular division in *Escherichia*

coli. J. Bacteriol. 108:375–385.
170. Linnane, A. W., G. Cobon, and S. Marzuki. 1973. Aspects of the evolution of the mitochondrial protein synthesizing system and the effect of membrane structure on mitochondrial protein synthesis and mt DNA replication. Proc. 3rd Int. Specialized Symp. on Yeasts, p. 349–367.
171. Luft, J. H. 1971. Ruthenium red and violet. I. Chemistry, purification, methods of use for electron microscopy and mechanism of action. Anat. Rec. 171:347–368.
172. Luft, J. H. 1971. Ruthenium red and violet. II. Fine structural localization in animal tissues. Anat. Rec. 171:369–416.
173. Luria, S. E. 1970. Phage, colicins, and macroregulatory phenomena. Science 168:1166–1170.
174. McCoy, E. C., A. E. Girard, and J. M. Kornfeld. 1971. Fine structure of resting and germination of *Penicillium chrysogenum* condidiospores. Protoplasma 73:443–456.
175. McGill, M., T. C. Hsu, and B. R. Binkley. 1973. Electron dense structures in mitochondria induced by short term ethidium bromide treatment. J. Cell Biol. 59:260–265.
176. McIntyre, J. A., N. B. Gilula, and M. J. Karnovsky. 1974. Cryoprotectant-induced redistribution of intramembranous particles in mouse lymphocytes. J. Cell Biol. 60:192–203.
177. MacLeod, R. A., P. Thurman, and H. J. Rogers. 1973. Comparative transport activity of intact cells, membrane vesicles, and mesosomes of *Bacillus licheniformis*. J. Bacteriol. 113:329–340.
178. Malhotra, S. K. 1968. Mesosome-like structures in mitochondria of poky Neurospora. Nature (London) 219:1267–1268.
179. Marchant, R., and D. G. Smith. 1968. Membranous structures in yeast. Biol. Rev. 43:459–480.
180. Marr, A. 1960. Enzyme localization in bacteria. Annu. Rev. Microbiol. 14:241–260.
181. Matheson, A., and M. C. M. Kwong. 1972. Electron-dense particles resembling ribosomes in mesosomes of *Bacillus subtilis*. J. Bacteriol. 110:747–750.
182. Matheson, A., M. Kwong Yang, and R. Smith. 1973. Demonstration of ribosomes in mesosomes associated with *Bacillus subtilis* protoplasts. J. Bacteriol. 115:349–357.
183. Mindich, L. 1970. Membrane synthesis in *Bacillus subtilis*. I. Isolation and properties of strains bearing mutations in glycerol metabolism. J. Mol. Biol. 49:415–432.
184. Mindich, L. 1970. Membrane synthesis in *Bacillus subtilis*. II. Integration of membrane proteins in the absence of lipid synthesis. J. Mol. Biol. 49:433–439.
185. Mindich, L., and S. Dales. 1972. Membrane synthesis in *Bacillus subtilis*. 3. The morphological localization of the sites of membrane synthesis. J. Cell Biol. 55:32–41.
186. Mirsky, R., and V. Barlow. 1973. Molecular weight, amino acid composition, and other properties of membrane-bound ATPase from *Bacillus megaterium* K M. Biochim. Biophys. Acta 291:480–488.
187. Mishra, N. C., and E. L. Tatum. 1973. Non-mendelian inheritance of DNA-induced independence of inositol in *Neurospora*. Proc. Natl. Acad. Sci. U.S.A. 70:3875–3879.
188. Mitchell, P. 1959. Biochemical cytology of microorganisms. Annu. Rev. Microbiol. 13:407–440.
189. Mitchell, P. 1970. Membranes of cells and organelles: morphology, transport and metabolism. Symp. Soc. Gen. Microbiol. 20:121–166.
190. Moore, R. T. 1971. An alternative concept of the fungi based on their ultrastructure, p. 49–64. *In* A. Perez-Miravete and D. Pelaez (ed.), Recent advances in microbiology, Cong. Int. Microbiol., Mexico, D.F.
191. Morgan, C., H. S. Rosenkrantz, B. Chan, and H. M. Rose. 1966. Electron microscopy of magnesium-depleted bacteria. J. Bacteriol. 91:891–895.
192. Mudd, S. 1954. Cytology of bacteria. I. The bacterial cell. Annu. Rev. Microbiol. 8:1–22.
193. Mudd, S., T. Kawata, J. I. Payne, T. Sall, and A. Takagi. 1960. Plasma membranes and mitochondrial equivalents as functionally coordinated structures. Nature (London) 189:79–80.
194. Mūnoz, E., M. S. Nachbar, M. F. Schor, and M. R. J. Salton. 1968. Adenosinetriphosphatase of *Micrococcus lysodeikticus*: selective release and relationship to membrane structure. Biochem. Biophys. Res. Commun. 32:539–546.
195. Nachbar, M. S., and M. R. J. Salton. 1970. Characteristics of a lipid-rich NADH dehydrogenase-containing particulate fraction obtained from *Micrococcus lysodeikticus* membranes. Biochim. Biophys. Acta 223:309–320.
196. Nachbar, M. S., and M. R. J. Salton. 1970. Dissociation of functional markers in bacterial membranes, p. 175–190. *In* M. Blank (ed.), Surface chemistry of biological systems. Plenum Press, New York.
197. Nakamura, K., D. N. Ostrovsky, T. Miyazawa, and S. Mizushima. 1974. Infrared spectra of outer and cytoplasmic membranes of *Escherichia coli*. Biochim. Biophys. Acta 332:329–335.
198. Nanninga, N. 1968. Structural features of mesosomes (chondrioids) of *Bacillus subtilis* after freeze-etching. J. Cell Biol. 39:251–263.
199. Nanninga, N. 1969. Preservation of the ultrastructure of *Bacillus subtilis* by chemical fixation as verified by freeze-etching. J. Cell Biol. 42:733–744.
200. Nanninga, N. 1971. The mesosome of *Bacillus subtilis* as affected by chemical and physical fixation. J. Cell Biol. 48:219–224.
201. Nanninga, N. 1971. Uniqueness and location of the fracture plane in the plasma membrane of *Bacillus subtilis*. J. Cell Biol. 49:564–570.
202. Nanninga, N. 1973. Freeze-fracturing of micro-

organisms, physical and chemical fixation of *Bacillus subtilis*, p. 151-177. *In* E. L. Benedette and P. Favard (ed.), Freeze-etching: techniques and applications. Societe Francaise de Microscopie Electronique, Paris.
203. Nass, M. M. K. 1966. The circularity of mitochondrial DNA. Proc. Natl. Acad. Sci. U.S.A. 56:1215-1222.
204. Nass, M. M. K. 1969. Mitochondrial DNA: advances, problems, and goals. Science 165:25-35.
205. Nass, M. M. K. 1969. Mitochondrial DNA. I. Intramitochondrial distribution and structural relations of single- and double-length circular DNA. J. Mol. Biol. 42:521-528.
206. Nierlich, D. P. 1974. Regulation of bacterial growth. Science 184:1043-1050.
207. Novikoff, A. B., and E. Holzman. 1970. Cells and organelles, p. 39-40. Holt, Rinehart and Winston, Inc., New York.
208. Ohnishi, Y. 1971. Phospholipids of virus-induced membranes in cytoplasm of *Escherichia coli*. J. Bacteriol. 107:918-925.
209. Ohnishi, Y., and M. Kuwano. 1971. Growth inhibition and appearance of the membranous structure in *Escherichia coli* infected with bacteriophage fd. J. Virol. 7:673-678.
210. Olson, J. M. 1970. The evolution of photosynthesis. Science 168:438-446.
211. Ono, Y., and D. White. 1971. Consequences of the inhibition of cardiolipin metabolism in *Haemophilus parainfluenzae*. J. Bacteriol. 108:1065-1071.
212. Ono, Y., and D. C. White. 1970. Cardiolipin-specific phospholipase D activity in *Haemophilus parainfluenzae*. J. Bacteriol. 103:111-115.
213. Op den Kamp, J. A. F., W. van Iterson, and L. L. M van Deenen. 1967. Studies on the phospholipids and morphology of protoplasts of *Bacillus megaterium*. Biochim. Biophys. Acta 135:862-884.
214. Oppenheim, J. D., and M. R. J. Salton. 1973. Localization and distribution of *Micrococcus lysodeikticus* ATPase determined by ferritin labeling. Biochim. Biophys. Acta 298:297-322.
215. Osborn, M. J., and R. Manson. 1974. Separation of the inner (cytoplasmic) and outer membranes of gram-negative bacteria, p. 642-653. *In* S. Fleischer and L. Packer (ed.), Methods in enzymology, vol. 31. Academic Press Inc., New York.
216. Owen, P., and J. H. Freer. 1972. Isolation and properties of mesosomal membrane fractions from *Micrococcus lysodeikticus*. Biochem. J. 129:907-917.
217. Packer, L. 1972. Functional organization of intramembrane particles of mitochondrial inner membrane. J. Bioenerg. 3:115-127.
218. Packer, L. 1974. Membrane structure in relation to function of energy-transducing organelles. Ann. N.Y. Acad. Sci. 227:166-174.
219. Palmer, J. M., and D. O. Hall. 1972. The mitochondrial membrane system. Prog. Biophys. Mol. Biol. 24:125-176.
220. Pangborn, J., A. G. Marr, and S. A. Robrish. 1962. Localization of respiratory enzymes in intracytoplasmic membranes of *Azotobacter agilis*. J. Bacteriol. 84:669-678.
221. Pannese, E. 1966. Expansive growth of the nuclear envelope and formation of mitochondria in ganglionic neuroblasts. Z. Zellforsch. Mikrosk. Anat. 72:295-324.
222. Pannese, E. 1966. Structures possibly related to the formation of new mitochondria in spinal ganglion neuroblasts. J. Ultrastruct. Res. 15:57-65.
223. Parsons, D. F. 1965. Recent advances correlating structure and function in mitochondria. Int. Rev. Exp. Pathol. 4:1-54.
224. Parsons, D. F., and Y. Yano. 1967. The cholesterol content of the outer and inner membranes of guinea-pig liver mitochondria. Biochim. Biophys. Acta 135:362-364.
225. Patch, C. T., and O. E. Landman. 1971. Comparison of the biochemistry and rates of synthesis of mesosomal and peripheral membranes in *Bacillus subtilis*. J. Bacteriol. 107:345-357.
226. Pate, J. L., J. L. Johnson, and E. J. Ordal. 1967. The fine structure of *Chondrococcus columnaris*. II. Structure and formation of rhapidosomes. J. Cell Biol. 35:15-35.
227. Pate, J. L., and E. J. Ordal. 1967. The fine structure of *Chondrococcus columnaris*. I. Structure and formation of mesosomes. J. Cell Biol. 35:1-13.
227a. Pedersen, P. L. Mitochondrial adenosine triphosphatase. J. Bioenerg. 6:243-275.
228. Pontefract, R. D., G. Bergeron, and F. S. Thatcher. 1969. Mesosomes in *Escherichia coli*. J. Bacteriol. 97:367-375.
229. Pontefract, R. D., and F. S. Thatcher. 1970. An electron microscope study of mesosomes in irradiation-resistant mutants of *Escherichia coli*. J. Ultrastruct. Res. 30:78-86.
230. Popkin, T. J., T. S. Theodore, and R. M. Cole. 1971. Electron microscopy during release and purification of mesosomal vesicles and protoplast membranes from *Staphylococcus aureus*. J. Bacteriol. 107:907-917.
231. Rabinowitz, M., and H. Swift. 1970. Mitochondrial nucleic acids and their relation to the biogenesis of mitochondria. Phys. Rev. 50:376-427.
232. Raff, R. A., and H. Mahler. 1972. The non symbiotic origin of mitochondria. Science 177:575-582.
233. Raff, R. A., and H. R. Mahler. 1973. Origin of mitochondria. Science 180:517.
234. Reaveley, D. A. 1968. The isolation and characterization of cytoplasmic membranes and mesosomes of *Bacillus licheniformis* 6346. Biochem. Biophys. Res. Commun. 30:649-655.
235. Reaveley, D. A., and H. J. Rogers. 1969. Some enzymatic activities and chemical properties of the mesosomes and cytoplasmic membranes of *Bacillus licheniformis* 6346. Bio-

chem. J. 113:67-79.

236. Reeve, J. N., and N. Mendelson. 1972. Cell morphology of *Bacillus subtilis*: the effect of genetic background on the expression of a rod⁻ gene. Mol. Gen. Genet. 119:11-26.
237. Reichenbach, H., H. Voelz, and M. Dworkin. 1969. Structural changes in *Stigmatella aurantiaca* during myxospore induction. J. Bacteriol. 97:905-911.
238. Remsen, C. 1968. Fine structure of the mesosome and nucleoid in frozen-etched *Bacillus subtilis*. Arch. Microbiol. 61:40-47.
239. Remsen, C., F. Valois, and S. Watson. 1967. Fine structure of the cytomembranes of *Nitrosocystis oceanus*. J. Bacteriol. 94:422-433.
240. Reusch, V. M., and M. M. Burger. 1973. The bacterial mesosome. Biochim. Biophys. Acta 300:79-104.
241. Reusch, V. M., Jr., and M. M. Burger. 1974. Distribution of marker enzymes between mesosomal and protoplast membranes. J. Biol. Chem. 249:5337-5345.
242. Richmond, M. H. 1970. Plasmids and chromosomes in prokaryotic cells. Symp. Soc. Gen. Microbiol. 20:249-277.
243. Robinson, J. P., R. D. Robinson, and J. H. Hash. 1974. Electron microscopy of *Staphylococcus aureus* cells and cell walls after treatment with lysozyme *Chalaropsis*. J. Bacteriol. 117:900-903.
244. Rogers, H. J. 1970. Bacterial growth and the cell envelope. Bacteriol. Rev. 34:194-214.
245. Rogers, H. J., D. A. Reaveley, and I. D. J. Burdett. 1967. The membrane systems of *Bacillus licheniformis*. Protides Biol. Fluids, Proc. Colloq. 15:303-313.
246. Rucinsky, T. E., and E. H. Cota-Robles. 1974. Mesosome structure in *Chromobacterium violaceum*. J. Bacteriol. 118:717-724.
247. Ryter, A. 1965. Etude morphologique de la sporulation de *Bacillus subtilis*. Ann. Inst. Pasteur Paris 108:40-60.
248. Ryter, A. 1968. Association of the nucleus and the membrane of bacteria: a morphological study. Bacteriol. Rev. 32:39-54.
249. Ryter, A. 1970. Structure and functions of mesosomes of gram-positive bacteria. Curr. Top. Microbiol. Immunol. 49:151-177.
250. Ryter, A., C. Frehel, and M. B. Ferrandes. 1967. Compartement des mesosomes lors de l'altaque de *Bacillus subtilis* par le lysozyme en milieu hyperou hypotonique. C. R. Acad. Sci. 265:1259-1262.
251. Ryter, A., Y. Hirota, and F. Jacob. 1968. DNA-membrane complex and nuclear segregation in bacteria. Cold Spring Harbor Symp. Quant. Biol. 33:669-676.
252. Ryter, A., and F. Jacob. 1966. Etude morphologique de la liaison de Noyau à la membrane chez *Escherichia coli* et chez les proplastes de *Bacillus subtilis*. Ann. Inst. Pastéur Paris 110:801-812.
253. Ryter, A., and F. Jacob. 1967. Membrane et segregation nucleaire chez les bacteries. Protides Biol. Fluids, Proc. Colloq. 15:267-278.
254. Ryter, A., and O. E. Landman. 1964. Electron microscope study of the relationship between mesosome loss and the stable L state (or protoplast state) in *Bacillus subtilus*. J. Bacteriol. 88:457-467.
255. Ryter, A., and O. E. Landman. 1968. Morphological study of the attachment of nucleoid to membrane in bacilli, protoplasts and reverting protoplasts of *Bacillus subtilis*, p. 110-123. *In* L. B. Guze (ed.), Microbial protoplasts, spheroplasts, and L-forms. The Williams & Wilkins Co., Baltimore.
256. Salton, M. R. J. 1971. The bacterial membranes, p. 1-65. *In* L. A. Manson (ed.), Biomembranes, vol. 1. Plenum Press, New York.
257. Salton, M. R. J. 1971. Bacterial membranes. Crit. Rev. Microbiol. 1:161-197.
258. Salton, M. R. J. 1956. Studies of the bacterial cell wall. V. The action of lysozyme on cell walls of some lysozyme-sensitive bacteria. Biochim. Biophys. Acta 22:495-506.
259. Salton, M. R. J., and J. A. Chapman. 1962. Isolation of the membrane-mesosome structures from *Micrococcus lysodeikticus*. J. Ultrastruct. Res. 6:489-498.
260. Salton, M. R. J., and J. H. Freer. 1965. Composition of the membranes isolated from several gram-positive bacteria. Biochim. Biophys. Acta 107:531-538.
261. Salton, M. R. J., J. H. Freer, and D. J. Ellar. 1968. Electron transport components localized in a lipid-depleted sheet isolated from *Micrococcus lysodeikticus* membranes by deoxycholate extraction. Biochem. Biophys. Res. Commun. 33:909-915.
262. Salton, M. R. J., and M. T. Schor. 1972. Subunit structure and properties of two forms of adenosine triphosphatase released from *Micrococcus lysodeikticus* membranes. Biochem. Biophys. Res. Commun. 49:350-357.
263. Santiago, E., J. J. Vazquez, J. Eugui, J. M. Macarulla, and F. Guerra. 1970. Disassembly of the inner mitochondrial membrane into different submitochondrial particles. FEBS Symp. 20:17-32.
264. Santo, L. Y., and R. H. Doi. 1974. Ultrastructural analysis during germination and outgrowth of *Bacillus subtilis* spores. J. Bacteriol. 120:475-481.
265. Sanwal, B. D. 1970. Allosteric controls of amphibolic pathways in bacteria. Bacteriol. Rev. 34:20-39.
266. Sargent, M. G. 1973. Membrane synthesis in synchronous cultures of *Bacillus subtilis* 168. J. Bacteriol. 116:397-409.
267. Sargent, M. G., B. K. Ghosh, and J. O. Lampen. 1968. Localization of cell-bound penicillinase in *Bacillus licheniformis*. J. Bacteriol. 96:1329-1338.
268. Schatz, G., and T. L. Mason. 1974. The biosynthesis of mitochondrial proteins. Annu. Rev. Biochem. 24:51-87.
269. Schlesinger, D., V. T. Marchesi, and B. C. K. Kwan. 1965. Binding of ribosomes to cytoplas-

mic reticulum of *Bacillus megaterium*. J. Bacteriol. 90:456-466.

270. Schmidt, G. 1960. Parallel forms in art and nature, p. 33. *In* G. Schmidt (ed.), Form in art and nature. Basilius Press, Basel.
271. Schmitt, H., H. Grossfeld, and U. Z. Littauer. 1973. Mitochondrial biogenesis during differentiation of *Artemia salina* cysts. J. Cell Biol. 58:643-649.
272. Schnaitman, C. A. 1971. Solubilization of the cytoplasmic membrane of *Escherichia coli* by Triton X-100. J. Bacteriol. 108:545-552.
273. Schnaitman, C. A., and J. W. Greenawalt. 1966. Intracytoplasmic membranes in *Escherichia coli*. J. Bacteriol. 92:780-783.
274. Schnaitman, C. A., and J. W. Greenawalt. 1968. Enzymatic properties of the inner and outer membranes of rat liver mitochondria. J. Cell Biol. 38:158-175.
275. Schnebli, H. P., A. E. Vatter, and A. Abrams. 1970. Membrane adenosine triphosphatase from *Streptococcus faecalis*. J. Biol. Chem. 245:1122-1127.
276. Schotz, F., I. G. Abo-Elnaga, and O. Kandler. 1965. Zur strucktur der mesosomen bei *Lactobacillus corynoides*. Z. Naturforsch. 20:790-794.
277. Sedar, A. W., and R. M. Burde. 1965. The demonstration of the succinic dehydrogenase system in *Bacillus subtilis* using tetranitroblue tetrazolium combined with techniques of electron microscopy. J. Cell Biol. 27:53-66.
278. Shah, S. B., and H. K. King. 1966. The action of lysozyme on bacterial electron transport systems. J. Gen. Microbiol. 44:1-13.
279. Shapiro, B. M., A. G. Siccardi, and Y. Hirota. 1970. On the process of cellular division in *Escherichia coli*. Membrane protein alterations associated with mutations affecting the initiation of DNA synthesis. J. Mol. Biol. 52:75-89.
280. Shinohara, C., K. Fukushi, and I. Suzuki. 1957. Mitochondria-like structures in ultrathin sections of *Mycobacterium avium*. J. Bacteriol. 74:413-415.
281. Shively, J. M., G. L. Decker, and J. W. Greenawalt. 1970. Comparative ultrastructure of the thiobacilli. J. Bacteriol. 101:618-627.
282. Shockman, G. D., H. M. Pooley, and J. S. Thompson. 1967. Autolytic enzyme system of *Streptococcus faecalis*. III. Localization of the autolysin at the sites of cell wall synthesis. J. Bacteriol. 94:1525-1530.
283. Short, S. A., D. C. White, and H. R. Kaback. 1972. Active transport in isolated bacterial membrane vesicles. V. The transport of amino acids by membrane vesicles prepared from *Staphylococcus aureus*. J. Biol. Chem. 247:298-304.
284. Silva, M. T. 1971. Changes induced in the ultrastructure of the cytoplasmic and intracytoplasmic membranes of several gram-positive bacteria by variations in OsO_4 fixation. J. Microsc. (Oxford) 93:227-232.
285. Silva, M. T., and J. C. F. Sousa. 1973. Ultrastructure of the cell wall and cytoplasmic membrane of gram-negative bacteria with different fixation techniques. J. Bacteriol. 113:953-962.
286. Singer, S. J., and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. Science 175:720-731.
287. Smith, D. G., R. Marchant, N. G. Maroudas, and D. Wilkie. 1969. A comparative study of the mitochondrial structure of petite strains of *Saccharomyces cerevisiae*. J. Gen. Microbiol. 56:47-54.
288. Stanier, R. Y. 1970. Some aspects of the biology of cells and their possible evolutionary significance. Symp. Soc. Gen. Microbiol. 20:1-38.
289. Steinman, H. M., and R. L. Hill. 1973. Sequence homologies among bacterial and mitochondrial superoxide dismutases. Proc. Natl. Acad. Sci. U.S.A. 70:3725-3729.
290. Stenmark, S. L., D. L. Pierson, R. A. Jensen, and G. I. Glover. 1974. Blue-green bacteria synthesize L-tyrosine by the pretyrosine pathway. Nature (London) 247:290-292.
291. Storm, D. R., P. M. Blumberg, and J. L. Strominger. 1974. Inhibition of the *Bacillus subtilis* membrane-bound D-alanine carboxypeptidase by 6-aminopenicillanic acid covalently coupled to Sepharose. J. Bacteriol. 117:783-785.
292. Storrie, B., and G. Attardi. 1973. Expression of mitochondrial genome in HeLa cells. XVII. Heterogeneity of isolated HeLa mitochondria as assayed for their enzymatic and *in vivo* biosynthetic activities. J. Biol. Chem. 248:5826-5834.
293. Stratling, W., and R. Knippers. 1971. Deoxyribonucleic acid synthesis in isolated DNA-membrane complexes. J. Mol. Biol. 61:471-487.
294. Swift, H., M. Rabinowitz, and G. Getz. 1967. DNA and mitochondrial morphology in anaerobic and adapting yeast. J. Cell Biol. 35:131-132a.
295. Takazoe, I., and J. Ennever. 1969. Ultrastructure of *Bacterionema matruchotii*. Bull. Tokyo Dent. Coll. 10:45-60.
296. Theodore, T. S., and C. Panos. 1973. Protein and fatty acid composition of mesosomal vesicles and plasma membranes of *Staphylococcus aureus*. J. Bacteriol. 116:571-576.
297. Theodore, T. S., T. J. Popkin, and R. M. Cole. 1971. The separation and isolation of plasma membranes and mesosomal vesicles from *Staphylococcus aureus*. Prep. Biochem. 1:233-248.
298. Theodore, T. S., and E. C. Weinbach. 1974. Respiratory activities associated with mesosomal vesicles and protoplast membranes of *Staphylococcus aureus*. J. Bacteriol. 120:562-564.
299. Thomas, D. Y., and D. Wilkie. 1968. Recombination of mitochondrial drug resistance factors in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 30:368-372.

300. Thorne, K. J. I., and D. C. Barker. 1969. Bactoprenol, ATPase and acetate activating enzymes of a vesicular fraction from *Lactobacillus casei*. Eur. J. Biochem. 11:582-591.
301. Thorne, K. J. I., and D. C. Barker. 1971. The bactoprenol and content of plasma and mesosomal membranes from *Lactobacillus casei*. Biochem. J. 122:45p-46p.
302. Thorne, K. J. I., and D. C. Barker. 1972. The occurrence of bactoprenol in the mesosome and plasma membranes of *Lactobacillus casei* and *Lactobacillus plantarum*. J. Gen. Microbiol. 70:87-98.
303. Tichy, P., and O. E. Landman. 1969. Transformation in quasi-spheroplasts of *Bacillus subtilis*. J. Bacteriol. 97:42-51.
304. Tourtellotte, M. E., and J. S. Zupnik. 1973. Freeze-fractured *Acholeplasma laidlawii* membranes: nature of particles observed. Science 179:84-86.
305. Towers, N. R., H. Dixon, G. M. Kellerman, and A. W. Linnane. 1972. Biogenesis of mitochondria. 22. The sensitivity of rat liver mitochondria to antibiotics; a phylogenetic difference between a mammalian system and yeast. Arch. Biochem. Biophys. 151:361-369.
306. Tremblay, G. Y., M. J. Daniels, and M. Schaechter. 1969. Isolation of a cell membrane-DNA-nascent RNA complex from bacteria. J. Mol. Biol. 40:65-76.
307. Tsien, H. C., and M. L. Higgins. 1974. Effect of temperature on the distribution of membrane particles in *Streptococcus faecalis* as seen by the freeze-fracture technique. J. Bacteriol. 118:725-734.
308. Tucker, A. N., and D. C. White. 1971. Detection of a rapidly metabolizing portion of the membrane cardiolipin in *Haemophilus parainfluenzae*. J. Bacteriol. 108:1058-1064.
309. Tucker, A. N., and D. C. White. 1970. Release of membrane components from viable *Haemophilus parainfluenzae* by ethylenediamine tetraacetic acid-tris(hydroxymethyl)-aminomethane. J. Bacteriol. 102:498-507.
310. Tzagoloff, A., and P. Meagher. 1971. Assembly of the mitochondrial membrane system. V. Properties of a dispersed preparation of the rutamycin-sensitive adenosine triphosphatase of yeast mitochondria. J. Biol. Chem. 246:7328-7336.
311. Uzzell, T., and C. Spolsky. 1973. Origin of mitochondria. Science 180:516-517.
312. Vanderwinkel, E., and R. G. E. Murray. 1962. Organelles intracytoplasmiques bacteriens et site d'activite oxydoreductrice. J. Ultrastruc. Res. 7:185-199.
313. van Iterson, W. 1961. Some features of a remarkable organelle in *Bacillus subtilis*. J. Biophys. Biochem. Cytol. 9:183-192.
314. van Iterson, W. 1965. Bacterial cytoplasm. Bacteriol. Rev. 29:299-325.
315. van Iterson, W., J. B. Groen, C. L. Woldrinch, W. Leene, and T. de Vries. 1971. Membranes and DNA, p. 37-48. *In* A. Perez-Miravete and D. Pelaez (ed.), Recent advances in microbiology. Int. Congr. Microbiol., Mexico, D.F.
316. van Iterson, W., and W. Leene. 1964. A cytochemical localization of reductive sites in a gram-negative bacterium. J. Cell Biol. 20:377-387.
317. van Iterson, W., and W. Leene. 1964. A cytochemical localization of reductive sites in a gram-positive bacterium. J. Cell Biol. 20:361-375.
318. van Iterson, W., and J. A. F. Op den Kamp. 1969. Bacteria-shaped gymnoplasts (protoplasts) of *Bacillus subtilis*. J. Bacteriol. 99:304-315.
319. Vermeulen, C. A., and G. Venema. 1974. Electron microscope and autoradiographic study of ultrastructural aspects of competence and deoxyribonucleic acid absorption in *Bacillus subtilis*: ultrastructure of competent and noncompetent cells and cellular changes during development of competence. J. Bacteriol. 118:334-341.
320. Vermeulen, C. A., and G. Venema. 1974. Electron microscope and autoradiographic study of ultrastructural aspects of competence and deoxyribonucleic acid absorption in *Bacillus subtilis*: localization of uptake and of transport of transforming deoxyribonucleic acid in competent cells. J. Bacteriol. 118:342-350.
321. Vignais, P., B. Stevens, J. Huet, and J. Andre. 1972. Mitoribosomes from *Candida utilis*. J. Cell Biol. 54:468-492.
322. Voelz, H. 1965. Formation and structure of mesosomes in *Myxococcus xanthus*. Arch. Mikrobiol. 51:60-70.
323. Wacker, W. 1969. The biochemistry of magnesium. Ann. N.Y. Acad. Sci. 162:717-726.
324. Wallach, D. F., and P. H. Zahler. 1967. Protein conformations in cellular membranes. Proc. Natl. Acad. Sci. U.S.A. 56:1552-1559.
325. Weibull, C. 1953. Characterization of the protoplasmic constituents of *Bacillus megaterium*. J. Bacteriol. 66:696-702.
326. Weibull, C. 1965. Plasmolysis in *Bacillus megaterium*. J. Bacteriol. 89:1151-1154.
327. Weibull, C., and H. Gyllang. 1965. Metabolic properties of some L forms derived from gram-positive and gram-negative bacteria. J. Bacteriol. 89:1443-1447.
328. Weigand, R. A., J. M. Shively, and J. W. Greenawalt. 1970. Formation and ultrastructure of extra membranes in *Escherichia coli*. J. Bacteriol. 102:240-249.
329. Weigand, R. A., S. C. Holt, J. M. Shively, G. L. Decker, and J. W. Greenawalt. 1973. Ultrastructural properties of the extra membranes of *Escherichia coli* $O111_a$ as revealed by freeze-fracturing and negative-staining techniques. J. Bacteriol. 113:433-444.
330. Weijers, P. J. 1971. The inner and outer profile of the inner membrane in heart mitochondria. Mondeel-offsetdrukkerij, Amsterdam.
331. Wheeldon, L. W. 1973. Products of mitochondrial protein synthesis. Biochimie 55:805-814.

332. White, D., F. R. Albright, W. J. Lennarz, and C. A. Schnaitman. 1971. Distribution of phospholipid-synthesizing enzymes in the wall and membrane subfractions of the envelope of *Escherichia coli*. Biochim. Biophys. Acta 249:636-642.
333. Whiteside, T. L., and M. R. J. Salton. 1970. Antibody to adenosine triphosphatase from membranes of *Micrococcus lysodeikticus*. Biochemistry 9:3034-3040.
334. Wiebe, W. J., and G. B. Chapman. 1968. Variation in the fine structure of a marine achromabacter and a marine pseudomonad grown under selected nutritional and temperature regimes. J. Bacteriol. 95:1874-1886.
335. Wildermuth, H. 1971. The fine structure of mesosomes and plasma membrane in *Streptomyces coelicolor*. J. Gen. Microbiol. 68:53-63.
336. Wilson, G., and C. F. Fox. 1971. Biogenesis of microbial transport systems: evidence for coupled incorporation of newly synthesized lipids and proteins into membrane. J. Mol. Biol. 55:49-60.
337. Woldringh, C. L., and W. van Iterson. 1972. Effects of treatment with SDS on the ultrastructure of *Escherichia coli*. J. Bacteriol. 111:801-813.
338. Wolstenholme, D. R., C. A. Vermeulen, and G. Venema. 1966. Evidence for the involvement of membranous bodies in the process leading to genetic transformation in *Bacillus subtilis*. J. Bacteriol. 92:1111-1121.
339. Yotsuyanagi, Y. 1966. Un mode de differenciation de la membrane mitochondriale, evoquant le mesosome bacterien. C. R. Acad. Sci. 262:1348-1351.
340. Yu, L., and M. J. Wolin. 1972. Chemical and physical characteristics of the deoxycholate-soluble and magnesium-reaggregated membrane nicotinamide adenine dinucleotide (reduced form) oxidase of *Bacillus megaterium*. J. Bacteriol. 109:51-58.
341. Yu, L., and M. J. Wolin. 1972. Separation of the primary dehydrogenase from the cytochromes of the nicotinamide adenine dinucleotide (reduced form) oxidase of *Bacillus megaterium*. J. Bacteriol. 109:59-68.
342. Zwillenberg, L. O. 1964. Electron microscopic features of gram-negative and gram-positive bacteria embedded in phosphotungstate. Antonie van Leeuwenhoek J. Microbiol. Serol. 30:154-162.