University of Glasgow

THE BACTERIAL MESOSOME

by

Peter Owen B.Sc.

Presented for the degree of Doctor of Philosophy in the Faculty of Science

Department of Microbiology

August, 1972

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

The facilities where this work was carried out resulted from the dedication to research of the late Professor Iwo Lominski and the generosity of Alexander Stone to the University of Glasgow. The investigation itself was supported by grants from the Medical Research Council and latterly from the University of Glasgow. I also wish to thank the Royal Society for the use of the Balzer's 360M Freeze Etching Unit and of the Pye-Unicam SP.800 spectrophotometer.

I should also like to ackowledge the co-operation and help given to me during the course of this investigation by members of the teaching, technical and secretarial staffs of the Department of Microbiology and of Chemistry: in particular, to Dr John H. Freer for his willingness to discuss impartially, and in detail, any aspect of microbiology; for his guidance and encouragement during this project and for his inexhaustible supply of good humour.

Many people have contributed to the final appearance of this manuscript. Accordingly, I would like to express my gratitude to Dr John H. Freer and to Professor Alistair C. Wardlaw for their constructive criticisms of the original drafts; to Cyril J. Smyth and to Gill, my fiancee, for help in checking them; to my mother and to Anne Strachan for their careful typing of a seemingly incomprehensible document; to Ian McKie for making excellent prints of the many electron micrographs and to Gill for her patience in the intricate lettering of them.

Finally I wish to thank the many friends I have made during the past four years for their interest, understanding and humour.

To Gill, Jo and John,

for many things.

S U M M A R Y.

The role of the mesosome in M.lysodeikticus has been studied using both biochemical and electron-microscopic techniques. During the developement of membrane fractionation procedures, unexpected variations in the specific activity of succinate dehydrogenase (EC 1.3.99.1) were observed in similar membrane preparations. Since this enzyme is an important membrane "marker", a detailed study of the factors affecting its activity was considered an essential preliminary investigation.

The membrane-bound enzyme was inactivated at temperatures above 10°C and its specific activity found to increase between two- and three-fold in diluted membrane preparations equilibrated at 0°C for 6h. Membranes treated with sodium deoxycholate showed no activation by dilution but displayed maximal activity. The increase in specific activity observed on dilution could be partially inhibited by fixation with glutaraldehyde, or by the presence of bovine serum albumin, whereas divalent cations caused an overall depression of membrane-bound succinate dehydrogenase activity. The significance of these observations are discussed in relation to the developement of a reproducible method of assay.

Two strains of M.lysodeikticus N.C.T.C. 2665 have been detected and partially characterised. A strain which gave no detectable contamination of membrane preparations with residual wall peptidoglycan was chosen for a study of the properties of isolated mesosomal membrane.

By precise manipulation of the conditions prior to and during protoplast formation, it was possible

to obtain highly purified preparations of mesosomal membrane. Plasmolysis of cells, before wall digestion was necessary for effective mesosome release. The effects of mild shearing forces, divalent cation concentration, temperature and time upon the release of mesosomal membrane from protoplasts were also investigsted. The optimal yield of mesosomal membrane from stable protoplasts was achieved at 10mM Mg^{2+}, mesosomal membrane fractions prepared at differing Mg^{2+} above 10mM being similar in chemical composition. There was no evidence from freeze-fracturing and etching that structural rearrangement or autodigestion occurred during the adopted fractionation procedure.

Evidence from freeze-fracturing and etching indicated that the mesosome was a metabolically important organelle, the mesosomal sacculus possibly representing a region of localised insertion of some membrane components. Both biochemical and electron-microscopic studies suggested that the mesosome was a region of localised autolytic activity and may thus play a role in wall morphogenesis and in cell-separation. A four- to five-fold increase in the mannose content of mesosomal membrane compared with peripheral membrane may also suggest a role for the mesosome in polymannan biosynthesis.

Isolated mesosomal membrane contained cytochrome b_{556} only, whereas isolated peripheral membrane was shown to possess a full complement of cytochromes. The complement of succinate, $NADH_2$ and malate dehydrogenases in mesosomal membrane was also much reduced compared with peripheral membrane. These results serve to elimate the

mesosome as the centre of respiratory activity in this organism.

Evidence from freeze-fracturing and etching and also from negative staining have suggested the presence of membranous vesicles within mesosomal tubules. These results are discussed in relation to membrane growth and to transport and secretion of extracellular products.

An additional investigation into the multiple forms displayed by staphylococcal α-toxin was also undertaken, and a report of this study is included.

Table of Contents

	PAGE
Object of the Research	1
Abbreviations, Symbols and References	2
Introduction	3-34
Materials and Methods	35-59
Results	60-100
Succinate Dedydrogenase. Properties of the membrane bound enzyme	61-63
Partial characterisation of two strains of M.lysodeikticus	63-72
Developement of a method for the fractionation of mesosomal and peripheral membrane from M.lysodeikticus	72-79
A comparison of the properties of mesosomal and peripheral membrane preparations	79-88
Comparative study by the freeze-fracture/freeze-etch technique of peripheral and mesosomal membrane from M.lysodeikticus	88-100
Discussion	101-145
Two strains of M.lysodeikticus	102-105
Isolation of mesosomal membrane	105-111
Properties of mesosomal and peripheral membrane	112-129
Freeze-etching	130-141
Conclusions	142-145
References	146-172
Supporting Publication	Facing 173

OBJECT OF THE RESEARCH

The anatomy and distribution of the intracellular membranous organelles known as mesosomes, found in heterotrophic bacteria, have been studied extensively, yet their function is poorly understood. The object of this investigation was to elucidate the role(s) of the mesosome in the bacterium *Micrococcus lysodeikticus*

ABBREVIATIONS, SYMBOLS AND REFERENCES.

The style of the abbreviations, symbols and references adopted in this thesis follows that of the Biochemical Journal.

I N T R O D U C T I O N.

In 1953, Chapman & Hillier reported the presence of electron opaque "peripheral bodies" associated with developing septa in ultrathin sections of Bacillus cereus when examined in the electron microscope. Similar observations were later reported in B. megaterium and B. subtilis (Chapman, 1956). There seems little doubt that these were the first observations of the membranous organelle, now almost universally known as the mesosome (Fitz-James, 1960). Their membranous nature, however, was not demonstrated until subsequent improvements in fixation procedures (Kellenberger, Ryter & Sechaud, 1958) allowed resolution of their anatomical features (Ryter & Kellenberger, 1958).

A variety of different terms have been coined to describe these organelles e.g. "peripheral bodies" (Chapman & Hillier, 1953), "chondroids" (Kellenberger et al, 1958), "intracytoplasmic membrane systems" (Glauert & Hopwood, 1960), "lamellar structures" (Koike & Takeya, 1961), and "onion bodies" (Brieger, 1963). However, the term "mesosome", introduced by Fitz-James (1960), following the suggestion by Robertson (1959), has now received wide acceptance in the literature.

Mesosomes, recognised as distinctive membrane structures within the cell (Fitz-James, 1960), are prominent in Gram-positive bacteria, and have been shown in a wide range of such organisms (See Table 1). Mesosomes in Gram-negative bacteria are, in general, much less pronounced. Indeed, in early investigations, their presence in thin sections of some bacteria was revealed only after prior

incubation of cells with compounds such as potassium tellurite or tetrazolium salts (Van Iterson & Leene, 1964b; Leene & Van Iterson, 1965; Hoeniger, Van Iterson & Van Zanten, 1966; Van Iterson, Hoeniger & Van Zanten, 1966). However, several Gram-negative bacteria have now been shown to possess internal membrane systems or mesosomes (Table 2), as have many species of gliding bacteria (Table 3).

In Gram-negative organisms, extensive internal membranous systems, often occupying a large part of the cytoplasmic space, occur in photosynthetic bacteria (Cohen-Bazire & Sistrom, 1966; Oelze & Drews, 1972), nitrifying bacteria (Watson & Mandel, 1971), and methane oxidising bacteria (Landenberg, Bryant & Wolfe, 1968; Proctor, Norris & Ribbons, 1969; Davis & Whittenbury, 1970 ; Smith & Ribbons, 1970; Smith, Ribbons & Smith, 1970; de Boer & Hazeu, 1972). In the photosynthetic bacteria, there is strong evidence to show that extensive membranous systems represent the cellular locations of the photosynthetic pigments (Cohen-Bazire & Kunisawa, 1963; Gibson, 1965; Holt, Conti & Fuller, 1966a,b; Oelze & Drews, 1972). It seems probable that similar membrane systems observed in the nitrifying and methane oxidising bacteria have also a role to play in the unique metabolism of these organisms. Thus it would seem inappropriate to classify the internal membranous systems of autotrophic and methane oxidising bacteria as mesosomes. This being so, the internal membrane systems of iron oxidising bacteria could not be considered as mesosomes. However, some species e.g. Thiobacillus thioparus (Shively, Decker & Greenawalt, 1970) have been shown to possess

internal membranous systems very similar to those observed in certain species of photosynthetic bacteria (Gibbs, Sistrom & Warden, 1965), whereas others e.g. T. thioxidans (Mahoney & Edwards, 1966) and T.denitrificans (Shively et al., 1970) possessed organelles similar to the mesosomes of some Gram-positive bacteria. Therefore, an attempt to define the mesosome in functional terms seems unwise at present, but may become possible as new information accumulates on the role(s) played by these organelles.

An order of bacteria in which internal membrane systems are notably absent is the Mycoplasmatales (see however Allen, Stevens, Florence & Hampton, 1970). Likewise organisms which share with mycoplasmas the absence of a cell wall (protoplasts, spheroplasts and L-forms) appear to lack mesosomes. Notable exceptions are the protoplasts or "gymnoplasts" (Van Iterson & Op den Kamp, 1969) prepared from B. megaterium (Op den Kamp, Van Iterson & Van Deenan, 1967) and B. subtilis (Van Iterson & Op den Kamp, 1969) grown at low pH. The retention of mesosomes in such wall-less gymnoplasts appears to be due to increased membrane rigidity resulting from a change in the membrane phospholipid composition at low pH.

Mesosomes arise by a progressive invagination of the peripheral membrane. Their appearance, however, as observed in thin sections, can vary considerably. The simplest type of mesosome is the slender direct invagination of the peripheral membrane, seen for example in E. coli (Pontefract et al., 1969) or the "S" membrane described for D. pneumoniae (Tomasz et al., 1964). Coiling

of these simple invaginations may lead to the lamellar type of mesosome found in many organisms (Highton, 1969). The initial membrane invagination may expand (Higgins & Shockman, 1970a,b) to give a membranous bag, or sacculus, usually observed to contain membranous vesicles and/or tubules. It has been suggested that the latter structures, hereinafter referred to as mesosomal vesicles or mesosomal membranes, arise from secondary invaginations of the mesosomal sacculus (Fitz-James, 1960; Tomasz et al., 1964; Pate & Ordal, 1967; Kats & Kharat'yan, 1969). This theory requires that the cytoplasmic surface of the peripheral membrane becomes the inner surface of the mesosomal vesicles, and evidence based on the assymetric staining of the two halves of the peripheral membrane and mesosomal vesicles of Ch. columnaris after fixation has been presented to substantiate it (Pate & Ordal, 1967). The study of a "marker" molecule localised exclusively on one surface of the peripheral membrane e.g. ATP-ase (Salton - personal communication) may serve to test this theory for other micro-organisms.

The contents of the mesosomal sacculus may arise, however, from areas of membrane in which membrane growth occurs faster than wall extension (Rogers 1970), the mesosomal sacculus serving as a "template" for the accumulation and layering of these structures (Higgins & Shockman, 1970b).

The difficulty in interpreting the overall mesosome morphology is, however, compounded by the observation that variation of the conditions in fixation prior to embedding and thin sectioning can have a marked effect on the mesosome morphology (Pate & Ordal, 1967;

Highton, 1969, 1970a,b; Burdett & Rogers, 1970; Silva, 1971). Prefixation of cells of Ch. columnaris (Pate & Ordal, 1967) with glutaraldehyde appeared to stabilise its mesosomal membrane system and to give a lamellar organelle, contrasting with the tubular mesosomes observed after standard fixation procedures using osmium tetroxide alone (Ryter & Kellenberger, 1958). A similar phenomenon was later reported for the mesosomes of B.licheniformis strain 749 & 749/C (Highton, 1969, 1970a) and B.subtilis (Highton, 1970b). The lamellar structure of the mesosomes observed in these organisms after fixation (Ryter & Kellenberger, 1958) at 0°C could be replaced by a vesicular morphology by fixation at room temperature, by the addition of molar sucrose to the fixative, or simply by allowing bacteria to remain without shaking for 30 min prior to fixation (Highton, 1969). It was concluded (Highton, 1969, 1970a,b) that the vesicular structure observed was a result of disruption of the native lamellar structure. This interpretation has, however, been contested by Burdett & Rogers (1970). In a detailed study, these authors noted that the morphology of the mesosome of B. licheniformis 6346 could be changed by alteration of either the ionic strength or calcium content of the fixative, or by the addition of sucrose. They thought that the mesosome in vivo consisted of a sacculus filled with inflated tubules and vesicles, but not with lamellar sheets of membrane. Recently, however, these same authors (Burdett & Rogers, 1972) have reported the occurrence of lamellar membranes in some mesosomes of B.licheniformis and have discussed the possibility of a reversible transition between

convoluted sheets of membrane and tubules or vesicles (see also Frehel & Ryter, 1972). Silva (1971) has been unable to reach such firm conclusions. During a study of the effect of fixation conditions on the mesosome morphology of a number of species of Gram-positive bacteria, it was noted that omission of a prefixation step caused the mesosomes to adopt a very simplified structure (not unlike that observed in many species of Gram-negative bacteria). He noted that "The possibility of these (morphological) changes usually receives little attention as reflected by over simplification frequently found in fixation protocols", and concluded that no confident picture could be deduced of mesosome morphology at present.

The fact that mesosomes basically consist of an invagination of the peripheral membrane makes their contents amenable to study by the negative staining procedure (Brenner & Horne, 1959), and mesosomes in many organisms have been revealed by this technique (Ryter & Pillot, 1963; Bladen, Nylen & Fitzgerald, 1964; Bladen & Mergenhagen, 1964; Kawata & Inoue, 1964; Zwillenberg, 1964; Abram, 1965; Ritchie & Ellinghausen, 1965; Takagi, Abe & Ueda, 1965; Takagi, Nakamura & Ueda, 1965; Abram, Vatter & Koffler, 1966; Cohen-Bazire, Kunisawa & Poindexter, 1966; Ghosh & Murray, 1967; Ghosh, Sargent & Lampen, 1968; Matheson & Donaldson, 1968; Stevenson, 1968; Langenberg et al., 1968; Freer et al., 1969; Ghosh, Lampen & Remsen, 1969; Hurst & Stubbs, 1969; Nauman, Holt & Cox, 1969; Davies & Whittenbury, 1970; Rogers, 1970; Shively et al., 1970; Burdett & Rogers, 1972). In most instances the general morphology of the mesosome visualised by the negative

staining procedure is compatible with that observed after fixation and sectioning. However, interpretation of electron micrographs of negatively stained whole cells is made somewhat hazardous by the occurrence of several artifacts. Not least of these is the retraction of the peripheral membrane away from the cell wall, possibly a plasmolysis effect (Burdett & Rogers, 1972), induced during drying of the stain. Indeed, ammonium molybdate, a commonly used stain, at a concentration of one per cent is known to have a similar tonicity to that of 0.12M sucrose (Muscatello & Horne, 1968). This value inevitably rises as the stain dries. Resultant plasmolysis may well account for the periplasmic mesosomes seen by some authors (Ghosh et al., 1968, 1969). Further distortion may occur due to the enormous forces incurred upon cells during drying of the thin film of stain. After studying evidence of mesosome structure obtained from a variety of techniques, including the morphology of isolated mesosome contents, Burdett & Rogers (1972) have proposed a convincing model for mesosome structure. However, the relevance of this model to cocci and bacilli other than B.licheniformis has yet to be ascertained.

Other parameters appear to affect mesosome morphology and produce mesosomes with differing structures in any one species of bacteria. (e.g. Van Iterson, 1961; Edwards & Stevens, 1963; Chen, 1964; Bladen, et al., 1964; Kawata & Inoue, 1965b; Van Iterson, 1965; Valentine & Chapman, 1966a, ; Granboulen & Leduc, 1967; Hoeniger & Headley, 1968; Wiebe & Chapman, 1968a; Hofstad & Selvig, 1969; Pontefract & Thatcher, 1970; Shively et al., 1970).

Undoubtedly some of the variations noted may result from inadequately controlled fixation procedures. However, there is evidence that heterogeneity in mesosome morphology may represent stages in the maturation of this organelle (Tomasz et al., 1964; Higgins & Shockman, 1970b). Anatomical variations as a response to growth conditions e.g. temperature (Wiebe & Chapman, 1968b; Neale & Chapman, 1970), culture medium (Wiebe & Chapman, 1968b; Thomas et al., 1969; Higgins & Shockman, 1970b), oxygen tension (Voelz, 1965; Cohen-Bazire et al., 1966; Kats & Tordzhyan, 1968; Tordzhyan & Kats, 1968; Kats & Kharat'yan, 1969), and phase of growth (Suganuma, 1963; Beaman & Shankel, 1969; Stevenson, 1968; Sasson & Delaporte, 1969) have also been suggested. However, the possibility that the differing morphologies may be indicative of functionally different organelles cannot be discounted (Glauert & Hopwood, 1960; Lundgren & Remsen, 1966; Ellar, Lundgren & Slepecky, 1967; Beaton, 1968; Ghosh, Sargent & Lampen, 1968; Kats & Moroz, 1968; Ghosh, Lampen & Remsen, 1969; Ishiguro & Wolfe, 1970).

The diversity in appearance of the mesosome is paralleled by an equal diversity in number per cell. Stationary phase cultures of C.crescentus have been reported (Cohen-Bazire et al., 1966) to contain over thirty mesosomes per cell, whereas exponentially growing cultures of B.subtilis (Highton, 1970b), B.licheniformis 749 (Highton, 1969) and B.licheniformis 6346 (Burdett & Rogers, 1972) have respectively one, one and three mesosomes, on average, per cell. It seems that the number of mesosomes per cell is a function of the strain of bacterium concerned and also of the physiological state of the cell (Cohen-Bazire et al., 1966;

Beaton, 1968; Hoeniger et al., 1968; Stevenson, 1968; Thornley & Glauert, 1968; Wiebe & Chapman, 1968b; Ghosh et al., 1969; Berksdale, 1970).

Certain temperature sensitive mutants of E.coli when grown at restrictive temperatures e.g. E.coli K-12 (Kohiyama, Cousin, Ryter & Jacob, 1966; Hirota, Ryter & Jacob, 1968; Allison, 1971) and E.coli $O111a_1$ (Schmaitman & Greenawalt, 1966; Altenberg & Suit, 1970; Altenberg, Suit & Brinkley, 1970; Weigand & Greenawalt, 1971) and also bacteriophage infected cells of the same organism (Bradley & Dewar, 1967; Schwartz & Zinder, 1968; Ohnishi, 1971; Ohnishi & Kuwano, 1971) possess large accumulations of intracytoplasmic membrane. The appearance of these "abnormal" membrane systems appears to be linked in the latter case to 'phage formation (Bradley & Dewar, 1967; Ohnishi & Kuwano, 1971) and in the former to aberrant regulation of membrane synthesis in non-dividing cells (Altenberg & Suit, 1970; Weigand et al., 1970). In irradiated cells of E.coli K-12, grown in the presence of high salt concentrations, these membranous structures appear to bear a striking resemblance to the rough and smooth membranes found in eucaryotic cells (Allison, 1971). As has been recorded, however, (Weigand et al., 1970), it seems inappropriate to regard these structures as mesosomes. Their fractionation (Weigand & Greenawalt, 1971) and analysis will no doubt help to clarify the situation.

Since the first description of the mesosome (Chapman & Hillier, 1953), there have been many speculations about its possible function in the cell. Prior to the development of fractionation techniques for the separation

of mesosomal membrane, numerous suggestions were made regarding its possible role in the cell.

In their initial description of "peripheral bodies" Chapman & Hillier (1953) concluded from their association with the developing cell septum that these organelles functioned in the synthesis of cell wall material. Salton (1956b) later suggested that the inability of protoplasts to regenerate a cell wall was a consequence of expulsion of these wall synthesising organelles during protoplast formation. With improved fixation techniques (Ryter & Kellenberger, 1958) the presence of material morphologically similar to cell wall, was demonstrated at the neck of the mesosome (Glauert, Brieger & Allen, 1961; Van Iterson, 1965) and within the mesosomal vesicles (Imaeda & Ogura, 1963; Ellar et al., 1969). Indeed Fitz-James (1964c) claimed that the mesosomal vesicles of B.megaterium contained material sensitive to lysozyme. Subsequent studies, however, revealed that the loss of wall integrity induced by penicillin preceded disorganisation of the mesosome structure (Fitz-James & Hancock, 1965), and Fitz-James (1965) concluded that the mesosome did not function directly in wall synthesis. Nevertheless the widespread occurrence of mesosomes at the site of imminent cross-wall formation and their close association with the septum during its development has convinced many authors of its involvement in cell-septum synthesis (Fitz-James, 1960; Glauert & Hopwood, 1961; Kawata, 1963; Kakefuda, Holden & Utech, 1967; Beaton, 1968; Burdett & Rogers, 1972) in cell-wall synthesis (Glauert & Hopwood, 1959; Van Iterson, 1961; Edwards & Stevens, 1963; Ellar, Lundgren & Slepecky, 1967; Rogers,1970),

in cell division (Vanderwinkel & Murray, 1962; Cohen-Bazire et al., 1966; Petitprez, Roos & Tacquet, 1967; Abadie, 1968; Stevenson, 1968; Kats & Kharat'yan, 1969; Burham, Hashimoto & Conti, 1970) and in the budding process (Ishiguro & Wolfe, 1970). Salton (1967) concluded "From their (mesosome) distribution in dividing cells, some role in deciding the location of the new septal site appears likely even if it is not actively engaged in forming new cross-linked peptidoglycan." Indeed it seems from the elegant work of Ellar and colleagues (Ellar, Lundgren & Slepecky, 1967) that initiation of the cross wall in B.megaterium is determined by the mesosome (see also Higgins, Pooley & Shockman, 1970). This suggestion has been further substantiated recently by statistical analysis of the position of mesosomes in cells of B.licheniformis (Burdett & Rogers, 1972).

Additional evidence for a role of the mesosome in wall synthesis comes from a correlation of mesosome number with both wall thickening (Higgins & Shockman, 1970b) and with disordered septation (Freer et al., 1969).

It had been suggested (Ryter & Landman, 1963; Landman & Halle, 1963) that the inability of L-forms of B.subtilis to revert (Landman & Halle, 1963) or to undergo ordered division (Ryter & Landman, 1963) was a consequence of the loss of mesosomes containing a primer for cell-wall synthesis. Subsequent experiments showed that if grown on gelatin medium such organisms could divide at normal exponential rates although 75% of the cells were devoid of

mesosomes (Landman, Ryter & Frehel, 1968). Similar observations have been made in cultures of B.megaterium (Kusaka, 1971). These conclusive experiments serve to eliminate the mesosome as the sole site of cell-wall synthesis. A similar conclusion was reached in a study of mesosome development in E.coli following treatment with chloramphenicol (Morgan, Rosenkranz, Carr & Rose, 1967).

A functional analogy between the mesosome and the mitochondrion of eucaryotic cells has been suggested by numerous authors (Imaeda & Convit, 1962; Kawata, 1963; Chen, 1964; Petiprez et al., 1967; Ryter, 1968). The proposal of such a "mitochondrial" function was based upon a variety of circumstantial evidence including the localised increase (Fitz-James, 1965) in membrane area (Glauert & Hopwood, 1959, 1960; Koike & Takeya, 1961), the increased number of mesosomes observed in cells starved of oxygen (Voelz, 1965; Cohen-Bazire et al., 1966), the occurrence of mesosomes in both sporulating (Fitz-James, 1960) and germinating (Wyss, Neuman & Socolofsky, 1961) cells, and their common occurrence in cells undergoing vegetative division (Valentine & Chapman, 1966).

The most compelling evidence to support this theory came from cytochemical staining of bacteria. As early as 1951, granules in mycobacteria, visualised in the light microscope, were shown to be the apparent centres of oxidative-reductive enzyme activity (Mudd, Winterscheid, Delameter & Henderson, 1951). Similar "bacterial mitochondria" were observed in other organisms (Mudd, Brodie, Winterscheid, Hartman, Beutner & McLean, 1951) and defined as

"....cytoplasmic granules probably possessing limiting membranes and possessing co-ordinated systems of oxidative enzymes necessary for vital synthetic processes of the cell" (Mudd, 1956). The validity of these conclusions was questioned by Weibull (1953), but the electron-microscopic evidence of membranous structures within bacteria was deemed to support the contention of Mudd (Shinohara, Fukushi & Suzuki, 1957, 1958; Shinohara, Fukushi, Suzuki, Sato, Suzuki & Motomiya, 1959; Takeya, Koike, Mori, Yuda & Toda, 1959; Giesbrecht, 1960; Chapman, Hanks & Wallace, 1959). There have been many more recent reports on the exclusive or enhanced deposition within the mesosome of cytochemical stains designed to detect sites of oxidative-reductive enzyme activity. Difficulties in interpretation of early micrographs were compounded by the fact that the deposited stains (formazans of 2,3,5-triphenyltetrazolium chloride or 2,2^1-di-p-nitrophenyl-5, 5^1diphenyl-3, 3^1 (3,3^1dimethoxy-4, 4^1biphenylene)) were solubilized by the dehydration procedures prior to embedding and sectioning. This serious drawback in the method was not fully appreciated at the time and results of such cytochemical studies were thought to provide direct evidence that the mesosome was the centre of respiratory activity in B.subtilis, S.serpens (Vanderwinkel & Murray, 1962), F.polymorphum (Takagi et al., 1963), Cl.botulinum and Cl.tetani (Takagi, Abe & Ueda, 1965). The use of stains yielding insoluble formazans upon reduction e.g. tetranitrobluetetrazolium (Leene & Van Iterson, 1965; Sedar & Burde, 1965a,b; Ariji & Brown, 1968; Brown et al., 1968) or of potassium tellurite (Van Iterson & Leene, 1964a,b; Van Iterson,1965; Hoeniger et al.,

1966; Van Iterson et al., 1966) has, in similar cytochemical studies, however, provided evidence for the exclusive or enhanced localisation of reduced products in the mesosomes of B.subtilis (Van Iterson & Leene, 1964a; Sedar & Burde, 1965b) L.casei (Brown et al., 1968) E.coli (Ariji, Fukushi & Oka, 1968) P.mirabilis (Hoeniger et al., 1966) and P.vulgaris (Van Iterson & Leene, 1964b; Leene & Van Iterson, 1965). Conversely cells of B.stearothermophilus (Abram, Vatter & Koffler, 1966) and of B.subtilis (Frehel, Ferrandes & Ryter, 1971) preincubated with potassium tellurite never showed reduced tellurite in the mesosome. These divergencies may in part be a reflection of the limitations of the technique, not least of which is the possible mobility of the reduced product. Indeed such a phenomenon may well account for the observation that, whereas reduced formazan appeared to be localised exclusively in the mesosome of S.aureus, deposits of reduced tellurite were found on all membranous structures (Tordzhyan & Kats, 1968. See also Takagi,Abi & Ueda, 1965 for a similar phenomenon). Considerable caution should therefore be exercised in the interpretation of results of this nature (see also Salton, 1968).

A correlation between the number of mesosomes per cell and their cytochrome content (Cohen-Bazire et al., 1966; Weibull & Gylang, 1965) has also been taken as indicative of a respiratory function for mesosomes. However, the reverse situation appears to apply in other organisms (see Conti et al., 1968).

It is apparent from the foregoing that statements allocating specific or even enhanced respiratory

activity to the mesosome cannot be substantiated from evidence available. In addition, it is not possible to reconcile a general respiratory role for this organelle with the occurrence of well developed mesosomes in strictly anaerobic bacteria (see Table 1 & 2 for examples).

The suggestion that the mesosome represents the site of new membrane formation (North, 1963; Tomasz et al., 1964; Lampen, 1965) was based on little experimental evidence. The mesosome does seem a logical site for new membrane synthesis (Salton, 1968), the membrane vesicles and tubules within the membrane may indeed fuse with the peripheral membrane (Ellar, Lundgren & Slepecky, 1967; Salton, 1968) and the mesosomal sac may act as a template for new membrane formation (Higgins & Shockman, 1970). These proposals appear attractive yet remain to be substantiated. Nevertheless, it does seem likely that the mesosome represents (Fitz-James, 1965; Weibe & Chapman, 1968b; Rogers, 1970) more than just a random accumulation of extra membrane.

That membrane growth occurs within the mesosome is indicated by the close association of this organelle with the developing forespore membrane (Fitz-James, 1960; Ohye & Murrell, 1962; Ellar & Lundgren, 1966) and apparent fusion of mesosomal vesicles into it (Freer & Levinson, 1967). Speculations based on other anatomical evidence have been made regarding a role for the sporal mesosome in spore coat formation (Fitz-James, 1962), cell wall primodium synthesis (Freer & Levinson, 1967) exosporium formation (Sansonoff et al., 1971) and in the production of lytic enzymes (Tchan et al., 1962).

Membrane growth between the points of attachment of freshly replicated DNA is an essential part of the hypothesis for nuclear segregation proposed by Jacob, Brenner & Cuzin (1963). These authors later showed attachment of the chromatin of B.subtilis to the mesosome (Ryter & Jacob, 1963, 1964), and proposed (Jacob, Ryter & Cuzin, 1966) that longitudinal division of the mesosome effected separation of the chromosomes i.e. that membrane growth occurs at the mesosome. Numerous other workers have since shown intimate association of the chromatin with mesosomes, observations which seem hardly surprising in the light of the large size of many of these organelles. Mesosomes do, however, appear capable of division (Ellar et al., 1967, Highton, 1970b; Rogers, 1970) as predicted by this theory, and the chromatin does, under certain conditions, appear firmly attached to the mesosomal sacculus (Ryter & Jacob, 1963; Ryter, 1968). The mesosomal attachment site is not universal, since in some instances, nuclear division can occur in the absence of these organelles (Landman, Ryter & Frehel, 1968). The possibility of several attachment points of the nuclear material to membrane (Rosenberg & Cavalieri, 1968) cannot be discounted. The recent report (Van Iterson & Groen, 1971) of fibrils of DNA apparently connected to extruded mesosomal vesicles of B.subtilis adds a further complication to this already complex area of bacterial cytology.

A somewhat different mechanism of nuclear division in E.coli has been proposed by Pontefract and co-workers (Pontefract et al., 1969). Electron micrographs of dividing cells revealed two polar mesosomes in newly

formed daughter cells. Both mesosomes were proximal to the nucleus and it was proposed that one remained attached to the chromosome received from the parent cell, whereas the other initiated replication of the new chromosome. Separation of the chromosomes was envisaged by wall growth in the central region of the cell, the newly synthesised genome containing the area or gene which induces formation and synthesis of the mesosome from the cell membrane (Pontefract & Thatcher, 1970). The essential differences between this hypothesis and that of Ryter & Jacob (1964) is that, in the former, longitudinal division of the mesosome is not a requirement and membrane growth does not occur at the attachment point. A necessary consequence of both theories, however, is that the region between the two mesosomes is the area of freshly synthesiscd membrane. In this respect, the observations of Jacob et al. (1963) and those of Ryter (1971) using reduced tellurite (Jacob et al., 1963) and flagella (Ryter, 1971; Frehel & Ryter, 1972) as markers for old membrane are compatible with both hypotheses.

The elegant model proposed by Pontefract and his colleagues (Pontefract et al., 1969; Pontefract & Thatcher, 1970) for nuclear division certainly accounts for recorded instances in other bacteria of contact between DNA and two polar mesosomes (Ellar et al., 1967). However, the situation is complicated by instances where cells contain only a single mesosome e.g. B.licheniformis (Highton, 1969, 1970a) and B.subtilis cells with one or two nuclei (Highton, 1970b). Highton (1970b) concludes "However, one mesosome is insufficient to separate even a single replicating nucleus by any single mechanism of pulling apart.

In a dinucleate cell it does not even seem possible that a single mesosome could have contact with DNA in one half of the cell." The possibility that the observed association between mesosomes and nuclear material may represent a fixation artifact cannot, at present, be discounted. Normal fixation conditions may result in condensation of chromatin to more central regions of the cell, and thus not surprisingly it would then appear to be associated only with internal membranous systems of the cell. Better fixation techniques may thus reveal multiple attachments between bacterial DNA and the cell membrane (Drieger, 1970) and lend support to the biophysical evidence to this effect (Rosenberg & Cavalieri, 1968; Ivarie & Pene, 1970; Burrell, Feldschreiber & Dean, 1971; Daniels, 1971). Alternatively nuclear condensation may be a response to the cell's metabolic activity (Daneo-Moore & Higgins, 1972). There is evidence that actively growing cells of S.faecalis possess dispersed nucleoids (Daneo-Moore & Higgins, 1972). The same authors have also indicated that mesosome size in this organism is directly related to DNA synthesis (Higgins & Daneo-Moore, 1972) thus implicating the mesosome as the site of DNA replication. It would appear from all the available evidence, however, that the function of the mesosome in DNA manipulation is far from fully elucidated.

Recognition that the membranous contents of the mesosome were in contact with the environment prompted suggestions that they functioned to aid transport of compounds into and out of the cell (Glauert & Hopwood,1960; Van Iterson, 1965; Valentine & Chapman, 1966). The peripheral nature of many mesosomes in some cells (Kats &

Moore, 1968; Kats & Kharat'yan, 1969) and the observation of a possible pore in the wall at the base of such mesosomes (Bladen et al., 1964) has been taken as evidence for such a function. Perhaps the most convincing evidence for a secretory role has been shown from a study of penicillinase formation in S.aureus (Beaton, 1968) and B.licheniformis 749/C (Lampen, 1965; Lampen, 1967a,b; Sargent, Ghosh & Lampen, 1967a,b; Ghosh et al., 1968, 1969). Beaton (1968) observed the appearance of peripheral and periplasmic mesosomes only after induction of penicillinase secretion in S.aureus. This agreed with the detailed hypothesis of the mechanism of secretion proposed earlier (Lampen, 1965) in which penicillinase was envisaged as being synthesised together with the membrane and inserted into the membrane at the mesosome. Continued membrane synthesis would apparently cause the penicillinase to migrate around the periphery of the mesosomal sac and possibly into the cytoplasmic membrane (Kushner & Pollock, 1961; Lampen, 1967a,b), followed by its transport through the cell wall (Smirnova, Kushnarev & Tshaikovskaja, 1971) and eventual release as exoenzyme. Ultrastructural studies (Ghosh et al., 1968, 1969) have indicated the presence of periplasmic mesosomes in the magnoconstitutive strain (749/C) of B.licheniformis, and also their appearance in the penicillinase inducible strain (749) after induction. These structures appeared important in the retention and storage of penicillinase (Ghosh et al., 1969) but were not a prerequisite for secretion since actively growing protoplasts of this organism were capable of synthesis and secretion of this enzyme (Sargent et al., 1969b).

As well as being implicated in secretion of extracellular products, a plausible role for the mesosome in the uptake of transforming DNA has been proposed (Tomasz et al., 1964; Wolstenholme, Vermeulen & Venema, 1966; Tichy & Landman, 1966). Electron-microscopic autoradiographs of cells of B.subtilis exposed to ^{3}H-thymidine-labelled donor DNA during the phase of maximum competence indicated that donor molecules were closely associated with the cytoplasmic surface of the mesosomal sacculus. These observations led to the suggestion that the mesosome was involved in the production of enzymes essential for incorporation of transforming DNA into the bacterial genome (Wolstenholme et al., 1966). The observed low efficiency of transformation in either protoplasts or cells grown on gelatin medium, both of which lack mesosomes (Tichy & Landman, 1969) suggested a requirement for this structure in DNA entry into the cell.

In addition to this already formidable list of functions proposed for the mesosome is the suggestion that, in B.bacteriovorus, it may possess enzymes lytic to the host cell wall, thus aiding penetration of the parasite (Burnham et al., 1968). The concept that the mesosome may contain muralytic enzymes (Rogers, 1970) is intriguing, and indeed the mesosome does represent a logical location for a cell-separating enzyme. It is interesting that autolytic activity in the vicinity of the mesosome is an integral requirement in the theoretical model proposed by Thompson (1971) for the growth and morphogenesis of bacterial cell walls.

From a consideration of the divergent suggestions about mesosome function, it is apparent that

evidence based upon studies *in situ* have not been instrumental in elucidating a universal role for this organelle, or indeed in providing a convincing role for it within any one species of bacterium. The possibility that a given mesosome in a cell is polyfunctional (Fitz-James, 1967; Kats & Tordzhyan, 1968; Conti *et al.*, 1968) is undoubtedly the most accommodating explanation. Different functions may also be ascribed to different mesosomes within one bacterial cell or to mesosomes of varying species (or strains) of bacteria. However, it became clear that a better understanding of the involvement of the mesosome in cellular functions would, no doubt, arise from its isolation and subsequent characterisation.

Several investigators have reported upon the properties of "mesosome fractions" isolated from a variety of Gram-positive bacteria (see Table 4). The methods of isolation of such fractions involved detergent treatment of total cell lysates (Fitz-James, 1967, 1968) density gradient separation of total cell lysates (Ghosh & Murray, 1969; Patch & Landman, 1971) and a variety of methods involving separation of mesosomes via the protoplast stage (Ferrandes *et al.*, 1966, 1970; Rogers *et al.*, 1967; Reaveley, 1968; Ellar & Freer, 1969; Ellar, 1969; Reaveley & Rogers, 1969; Sargent, *et al.*, 1969a; Sargent & Lampen, 1970; Daniels, 1971; Ellar, Thomas & Postgate, 1971; Popkin, *et al.*, 1971; Thorne & Barker, 1971, 1972) or via the spheroplast stage (Thorne & Barker, 1969).

The rationale for most of the methods involving separation of mesosomal membrane from protoplasts has been based upon the observation by Van Iterson (1961)

that the mesosomal contents of Bacillus subtilis were extruded into the periplasmic space upon plasmolysis of whole cells. Similar observations have been made for many other bacteria either by direct observation of thin sections (Ryter & Jacob, 1963; Fitz-James, 1964a,c; Ryter & Jacob, 1964; Ryter & Landman, 1964; Weibull, 1965; Ferrandes et al., 1966; Ryter & Jacob, 1966; Ghosh & Murray, 1967; Kakefuda et al., 1967; Ghosh et al., 1968; Popkin et al., 1971; Van Iterson & Groen, 1971; Frehel & Ryter, 1972) or negatively stained whole cells (Ryter, Frehel & Ferrandes, 1967; Matheson & Donaldson, 1968). Similar processes of extrusion appear to arise in some bacteria in the early stages of autolysis (Silva, 1967), as a response to temperature shock (Fitz-James, 1965) or pH shock (Op den Kamp et al., 1967) or to oxygenation (Kats & Tordzhyan, 1968; Kats & Kharat'yan, 1969) or treatment with various chemicals (Kats & Tordzhyan, 1968; Ellison, Mattern & Daniel, 1971).

Following the observation of mesosome eversion on plasmolysis, Fitz-James (1964c) showed that removal of the cell wall from plasmolysed cells resulted in the release of mesosomal contents into the suspending buffer. It seems that only the contents are released, since the mesosomal sacculus was reported to be incorporated into the bounding membrane of protoplasts (Ryter, 1968). (The situation may be more complex and, in some instances, a break in the peripheral membrane adjacent to the mesosome may allow the release of the sacculus and its contents together e.g. Fig. 5 of Van Iterson & Groen, 1971). It is apparent, however, that mesosomal vesicles are not

necessarily released from the parent protoplast but may remain attached, as has been shown by metal shadowing (Ryter, 1968) sectioning (Ryter & Jacob, 1966; Ghosh & Murray, 1967; Op den Kamp, Van Iterson & Van Deenan, 1967; Fitz-James, 1968; Ghosh et al., 1968; Ryter, 1968; Sargent et al., 1968; Van Iterson & Op den Kamp, 1969; Popkin et al., 1971; Burdett & Rogers, 1972) and by negative staining (Ghosh & Murray, 1967, 1969; Ryter et al., 1967; Fitz-James, 1968; Ryter, 1968). Thus the crucial step in the fractionation of mesosomal membrane is effecting its efficient release without concomitant disruption of the parent protoplast. Protoplast lysis would necessitate the fractionation of mesosomal membrane from peripheral membrane, a procedure which in many instances would appear very difficult (Salton & Chapman, 1962). Several workers have attempted such fractionation procedures by using the difference in density between the two fractions (Fitz-James, 1967, 1968; Ghosh & Murray, 1969; Patch & Landman, 1971). This method is not totally satisfactory as mesosomal and peripheral membrane may well remain attached or become vesicularised during protoplast disruption (Salton & Chapman, 1962). Fitz-James (1967, 1968) attempted to overcome this difficulty by addition of detergent. However, the resultant disruption of membrane morphology (Fitz-James, 1968) outweighs by far any gain in mesosomal yield, and casts doubts on the validity of his arguments.

Of the reports concerning characterisation of mesosomal membrane, only those of Rogers and his colleagues (Rogers et al., 1967; Reaveley & Rogers, 1969) attempt to define in any detail conditions under which

concomitant mesosomal membrane release and protoplast stability are achieved. This omission on the part of many workers, together with the inherent difficulties in the fractionation of total lysates, may account in part for the relatively wide range of properties attributed to isolated mesosomal membranes fractions. However, it should be recalled that there is sound justification for not comparing the properties of mesosomes isolated from different organisms, or indeed the same organisms by different methods (Salton, 1968).

It is paradoxical that the fractionation techniques developed to clarify the role of the mesosome have served to increase speculation about its function. Fitz-James (1967, 1968) from pulse labelling experiments with 1-^{14}C-acetate, ^{32}P and ^{56}Fe proposed that the mesosome of B.megaterium represented the site of synthesis of membrane lipid, folic acid and cytochrome (but not of membrane protein). However, it can reasonably be argued that these results, based upon the use of such biologically disruptive agents as detergents, bear little relevance to the properties of the intact mesosome. Indeed recent pulse-labelling experiments have failed to indicate the mesosome as the preferential site of membrane lipid synthesis in B.megaterium (Daniels, 1971; Ellar, Thomas & Postgate, 1971) B.subtilis (Daniels, 1971; Patch & Landman, 1971), L.monocytogenes (Ghosh & Murray, 1969) or M.lysodeikticus (Ellar, Thomas & Postgate, 1971), the mesosomal membrane lipid being synthesised independantly of the peripheral membrane lipid in some instances (Ghosh & Murray, 1969).

A plausible role for the mesosome of

L.casei in cell wall synthesis has been suggested from a comparison of its mesosomal and peripheral membranes (Thorne & Barker, 1969,1971, 1972; Barker & Thorne, 1970). This organism is known to metabolise mevalonic acid mainly to a C_{55} isoprenoid alcohol, bactoprenol (Thorne & Kodicek, 1962), a membrane bound carrier lipid involved in peptidoglycan synthesis (Dietrich, Colucci & Strominger, 1967; Higashi, Strominger & Sweeley, 1970). Subsequent analysis of mesosomal membrane fractions from L.casei (Thorne & Barker, 1971, 1972) and from L.plantarum (Thorne & Barker, 1972) indicated that bactoprenol was equally distributed between mesosomal and peripheral membrane, and that its concentration in the mesosomal membrane was approximately equal to that found in peripheral membrane. Evidence from pulse and pulse-chase labelling experiments with (2-^{14}C-)-mevalonate suggested that biosynthesis of bactoprenol occurred simultaneously in mesosomal and peripheral membrane. It would thus appear likely that peptidoglycan synthesis does occur in the mesosome of this organism, the bactoprenol present in the peripheral membrane possibly functioning in the process of wall thickening (Thorne & Barker, 1971,1972).

The postulated role of the mesosome of B.licheniformis 749/C in penicillinase secretion (Lampen,1965; 1967a,b; Sargent et al., 1969; Ghosh et al., 1968, 1969) has been further supported by evidence that isolated mesosomal vesicles of this organism contained six times the concentration of penicillinase found in peripheral membrane (Sargent, et al., 1969a). Penicillinase bound to these vesicles, unlike that bound to peripheral membrane, was

found to be a precursor for part of the excreted exoenzyme (Sargent *et al.*, 1969b), and appeared from its elution pattern on Biogel to be a hydrophobic conformational variant of the hydrophilic exoenzyme (Sargent & Lampen 1970). In contrast penicillinase bound to peripheral membrane appeared to be a hydrophobic polymer of the enzyme. Secretion from whole cells thus involved (Sargent & Lampen, 1970) incorporation of freshly synthesised enzyme in a hydrophobic conformation (Bettinger & Lampen, 1971) into the cell membrane, to be either secreted immediately in a hydrophilic conformation as the exoenzyme, or to be incorporated into the membrane at its growing point. It was suggested that the membrane bound enzyme was either polymerised in the peripheral membrane, or released into mesosomal vesicle, where it became available for secretion as the exoenzyme.

Although there is good evidence to substantiate a role (although not a unique one) for the mesosome in peptidoglycan synthesis in *L.casei* and in secretion of penicillinase in *B.licheniformis* 749/C, fractionation techniques to date have not helped to uncover a convincing role for the mesosome in other species of bacteria*.

---

* It should be noted that the report of exclusive localisation of cytochromes ($a+a_3$), b,c,y and z in the "mesosome fraction" isolated from *B.subtilis* (Ferrandes *et al.*, 1967) has since been retracted (Ferrandes, Frehel & Chaiz, 1970).

The object of this investigation was to elucidate the function of the bacterial mesosome. As is evident from the foregoing discussion an efficient method is first required for the fractionation of mesosomal membrane from intact protoplasts. The successful isolation of pure mesosomal membrane would then provide an opportunity for investigation of the distribution of functions in the bacterial membrane system.

The Gram-positive lysozyme-sensitive organism M.lysodeikticus was chosen for this investigation because of the detailed information already available on the properties of the total (i.e. peripheral plus mesosomal) membrane fractions (For reviews see Salton, 1956a, 1965, 1967a,b,c; Gel'man, Lukoyanova & Ostrovskii, 1967; Munoz, Salton & Ellar 1969; Nachbar & Salton 1970b; Salton & Nachbar, 1970). The growth requirements of this organism (Wolin & Naylor, 1957; Grula, Luk & Chu, 1961; Salton, 1964a; Walsh, O'Dor & Warren, 1971) and the factors involved in the isolation (Salton & Chapman, 1962) of its membrane system, free from cytoplasmic contamination (Salton, 1967c,d), have been reported previously. Gross chemical analysis of such fractions indicated that the membrane is composed mainly of protein (Gilby, Few & McQuillen, 1958; Salton & Freer, 1965),consisting of many individual polypeptides (Salton, Schmitt & Trefts, 1967; Ostrovskii, Tsfasman & Gel'man, 1969; Grula & Savoy, 1971; Sofronova, Ostrovskii & Gel'man, 1971) and of lipid (Gilby et al., 1958; Macfarlane, 1961a,b, 1964; Salton & Freer, 1967),consisting mainly of mono- and di-phosphotidyl glycerol (Macfarlane, 1961a,b,1964;

Butler, Smith & Grula, 1967; Ellar, Thomas & Postgate, 1971; Whiteside, de Siervo & Salton, 1971; Pollock, Linder & Salton, 1971).

The fatty acid composition of the lipid constituents has been determined (Macfarlane, 1961a; Thorne & Kodicek, 1962; Cho & Salton, 1964; Cho, Corpe & Salton, 1964) and several minor lipid components detected (Gilby *et al.*, 1958; Macfarlane, 1961a,b, 1964; Lennarz, 1964; Lennarz & Talamo, 1965, 1966; de Siervo & Salton, 1971; Pollock *et al.*, 1971; Estrugo, Lennarz, Corrales, Dutch & Munoz, 1972). Phospholipids are located almost exclusively in the membrane system of *M.lysodeikticus* (Macfarlane, 1961a) as are the carotenoid pigments (Gilby *et al.*, 1958; Gilby & Few, 1958; Rothblat, Ellis & Kritchevsky, 1964; Salton & Ehtisham-ud-din, 1965; Salton & Schmitt, 1967a) and components of the electron transport system *e.g.* cytochromes (Smith, 1954; Jackson & Lawton, 1959; Gel'man, Lukoyanova & Oparin, 1960; Lukoyanova & Biryuzova, 1965; Salton & Ehtisham-ud-din, 1965; Lukoyanova, Biryuzova, Simakova & Gel'man, 1967; Lukoyanova & Taptykova, 1968; Salton, Freer & Ellar, 1968; Simakova, Lukoyanova, Biryuzova & Gel'man, 1969; Gel'man, Tikhonova, Simakova, Lukoyanova, Taptykova & Mikelsaar, 1970) menaquinones (Bishop & King, 1962; Salton, 1965; Salton & Schmitt, 1967a) and succinate dehydrogenase (Gel'man, Zhukova, Lukoyanova & Oparin, 1959; Lukoyanova, Gel'man & Biryuzova, 1961; Mitchel, 1963; Salton *et al.*, 1968; Nachbar & Salton, 1970b; Pollock *et al.*, 1971). The demonstration of exclusive localisation of these cell components in the membrane makes them useful "membrane markers".

Other enzymes (Mitchel, 1963; Nachbar & Salton, 1970b; de Siervo & Salton, 1971), notably those of electron transport and oxidative phosphorylation (Ostrovskii & Gel'man, 1963, 1965; Biryuzova, Lukoyanova, Gel'man & Oparin, 1964; Ishikawa, 1970; e.g. $NADH_2$dehydrogenase (Gel'man, Zhukova & Oparin, 1963; Zhukova, Ostrovskii, Gel'man & Oparin, 1966; Nachbar & Salton, 1970a) and ATP-ase (Munoz, Freer, Ellar & Salton; Munoz, Nachbar, Schor & Salton, 1968; Simakova, Lukoyanova, Biryuzova & Gel'man, 1968, 1969; Munoz, Salton, Ng & Schor, 1969; Lastras & Munoz, 1971) are localised preferentially in the membrane system of this bacterium. Unlike membrane succinate dehydrogenase, these enzymes may be dissociated from the "basic" membrane structure (Salton et al., 1968) by manipulation of the suspending medium. Thus membrane bound ATP-ase (Munoz, Freer, Ellar & Salton, 1968; Simakova et al., 1968) may be released by osmotic shock treatment (Munoz, Nachbar, Schor & Salton, 1968), a procedure designed to rupture ionic bonding via cations. This has provided a convenient method for the purification of this enzyme (Munoz, Salton, Ng & Schor, 1969) and allowed a study of its biochemical (Munoz, Salton, Ng & Schor,1969; Lastras & Munoz, 1971) and serological (Whiteside & Salton, 1970; Whiteside et al., 1971) properties. Similar fractionation procedures have been instrumental in detecting differential inhibitory effects of chloramphenicol on the synthesis of membrane bound ATP-ase and cytoplasmic enzymes of this organism (Vambutas & Salton, 1970a,b).

Similar treatment of membranes from

M.lysodeikticus with a chelating agent (EDTA) removed a vesicle fraction rich in $NADH_2$dehydrogenase (Gel'man, Zhukova & Oparin, 1963; Nachbar & Salton, 1970a), cardiolipin (Nachbar & Salton, 1970a) and cardiolipin synthetase (de Siervo & Salton, 1971) and suggested regional distribution of specific proteins in the membrane. Treatment with detergent (Salton et al., 1968; Simakova, Lukoyanova, Biryuzova & Gel'man, 1969; Gel'man et al., 1970) urea (Simakova et al., 1968, 1969) proteases (Lukoyanova et al., 1967) lipases (Lukoyanova & Biryuzova, 1965) and glutaraldehyde (Ellar, Munoz & Salton, 1971) has provided further information on the nature of the bonding and inter-relationship of components within the membrane.

Physical techniques such as infra-red spectroscopy (Oparin, Lukoyanova, Shvets, Gel'man & Torkhovskaya, 1965; Green & Salton, 1970; Grula & King, 1971), electron paramagnetic resonance spectroscopy (Gol'dfel'd, Ostrovskii & Rozantsev, 1970) differential scanning calorimetry (Ashe & Steim, 1971), disc gel electrophoresis (Salton & Schmitt, 1967b) ultrasonication (Salton & Netschey, 1965; Salton, 1967d) reaggregation experiments (Butler et al., 1967; Grula, Butler, King & Smith, 1967; Salton, 1967c) and cation binding experiments (Lukoyanova et al., 1961; Cutinelli, Galdiero & Tufano, 1969; Lastras & Munoz, 1971) have also been instrumental in elucidating the molecular architecture of this bacterial membrane system. Recently the immunology (Fukui, Nachbar & Salton, 1971a) and immunochemistry (Fukui, Nachbar & Salton, 1971b) of membranes isolated from M.lysodeikticus have also been studied.

Added to this extensive knowledge on the properties of the membrane M.lysodeikticus is an understanding of the chemical composition and structure of the cell wall of this organism (for reviews see Salton,1964b; Rogers & Perkins, 1968; Ghuysen, 1968; also Balyuzi, Reavely & Barge, 1972; Hoshino, Zehavi, Sinay & Jeanloz, 1972). The involvement of the membrane in the biosynthesis of peptidoglycan (Anderson, Matsuhashi, Haskin & Strominger, 1965, 1967; Kats, Matsuhashi, Deitrich & Strominger, 1967; Deitrich, Collucci & Strominger, 1967; Higashi, Strominger & Sweeley, 1967) polymannan (Scher, Lennarz & Sweeley, 1968; Scher & Lennarz, 1969; Lahav, Chiu & Lennarz, 1969) and cardiolipin (de Siervo & Salton, 1971) has also been studied.

Thus it is apparent that the choice of M.lysodeikticus for a study of the mesosome is a logical one. Indeed preliminary reports on the properties of isolated mesosomal vesicles from this organism have recently been published (Ellar & Freer, 1969; Ellar, 1969; Ellar, Thomas & Postgate, 1971).

MATERIALS AND METHODS.

Chemicals

Egg white lysozyme (E.C.3.2.1.17) and yeast hexokinase (E.C.27.1.1) were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.) and bovine deoxyribonuclease (E.C.3.1.4.6.) from Worthington Biochemical Corp. (Freehold, N.J., U.S.A.). Yeast nicotinamide adenine dinucleotide (reduced) sodium salt ($NADH_2$), horse-heart cytochrome c, sperm-whale myoglobin and ovalbumin were from Koch-Light Laboratories Ltd. (Colnbrook, Bucks., U.K.), bovine serum albumin Fraction V from Armour Pharmaceutical Co. Ltd. (Eastbourne, Sussex, U.K.) and bovine pancreatic chymotrypsinogen from Miles-Seravac Laboratories Inc. (Maidenhead, Berks., U.K.). Decon 90 and Domestos cleaning fluids were products of Decon Lab. Ltd. (Brighton, Sussex, U.K.)and of Lever Bros. Ltd. (London, U.K.) respectively. All other chemicals and reagents were of analytical grade.

Organisms

Micrococcus lysodeikticus (N.C.T.C. 2665) was used throughout the course of these investigations. Two strains of this organism have, however, been detected and distinguished viz. a laboratory strain, designated M.lysodeikticus St.O, and obtained originally from the National Collection of Type Cultures and a second strain, designated M.lysodeikticus St.A, obtained directly from that source. St. O had been maintained by subculture for several years, whereas St.A was studied within 6 months of initial culture.

Cultivation Conditions

Cells of M.lysodeikticus were cultivated under the conditions described by Owen & Freer (1970b. See reprint facing p.61), and maintained on plates containing 1.5%^{W}/v Bacto-Agar, 5%^{W}/v Bacto-Peptone, 0.1% Difco Yeast Extract, 0.5% Nacl, pH7.2 and subcultured once per two weeks.

Harvesting Conditions

After growth for the requisite period of time, cells were harvested at 4^{o}C (2000g for 25min) and washed once with distilled water and once with 50mM-tris-HCl buffer, pH7.5. (This buffer, referred to as "tris" was used throughout this work, unless otherwise stated.

Comparison of St.O and St.A of M.lysodeikticus

Growth Curves. Cells were grown under the conditions described by Owen & Freer (1970b) and 3ml aliquots of the culture removed aseptically at 1h intervals. Cell suspensions were immediately diluted with fresh medium to give an E^{1cm}_{620} of less than 0.40. Their extinction at this wavelength was then recorded in a Pye Unicam SP.600 spectrophotometer. Recorded values were the mean of three such determinations.

Pigment Production. 5ml aliquots of culture fluid were removed aseptically at intervals over the growth cycle. Cells were sedimented by bench centrifugation (2000g for 20min) and the supernatant fluid recentrifuged. The visible spectrum of this second supernatant fluid was then recorded in a Pye Unicam SP800 spectrophotometer against fresh medium. The extinction at 405nm (λmax) was taken

as an estimate of the pigment content of the culture.

Lysozyme Sensitivity. A comparison of the lysozyme sensitivity of the two strains was made in the following way. Aliquots of cells were removed aseptically from the culture medium at various time intervals, harvested and washed as described by Owen & Freer (1970b) and finally suspended in tris buffer at 30°C to an optical density of less than 2.0 at 620nm (620nm was chosen to avoid the absorption due to the carotenoid pigments present in the membrane (Fig.1) and an initial extinction value of approximately 2.0 was necessary to monitor the optical density at the later stages of the reaction). To 2.9ml of cell suspension was added 0.1ml of lysozyme solution to give a final concentration of 100ug/ml. The decrease in E^{1cm}_{620} was then recorded automatically in a Pye Unicam SP800 spectrophotometer fitted with temperature controlled (30°C) cuvette holders and a recorder.

Preparation of "standard" membranes and membrane residues from Strains O and A of M.lysodeikticus

Preparation of "standard" membranes from total lysates. "Standard" membrane preparation was based on the method of Salton & Freer (1965) and is detailed by Owen & Freer (1970b. See reprint facing p.61). A "standard" membrane suspension may be defined as a suspension of washed (Salton, 1967d) total membranes (i.e. peripheral and mesosomal membranes) at a concentration of between 10-20mg dry wt. membrane/ml, prepared as detailed by Owen & Freer (1970b).

Effect of sodium dodecyl sulphate (SDS) on membrane turbidity. "Standard" membrane suspensions were diluted with an equal volume of tris buffer containing SDS at varying concentrations.

Their E^{1cm}_{620} values were recorded in a Pye Unicam SP.600 spectrophotometer after 5min incubation at 30°C.

Extraction of "standard" membranes with SDS. "Standard" membrane suspensions were diluted with an equal volume of tris buffer containing sodium dodecyl sulphate (SDS) at varying concentrations. After 5min incubation at 30°C suspensions were centrifuged at 65 000g for 1h at 4°C and the carotenoid content of the supernatant fluid determined in a Pye Unicam SP.600 spectrophotometer by its extinction at 446nm (See p.43).

Deoxycholate extraction of "standard" membranes. Deoxycholate extraction was performed essentially as reported by Salton et al. (1968). "Standard" membrane suspensions from M.lysodeikticus St.0 were extracted with an equal volume of 2% ($^{w}/v$) sodium deoxycholate+ (DOC) in tris buffer* at 4°C. Centrifugation at 38 000g for 45min at 4°C gave an insoluble residue (DO1) which was extracted a further five times with 1% ($^{w}/v$) sodium deoxycholate+ (DOC) in tris buffer, and then three times with tris buffer alone to remove residual deoxycholate. Supernatant fluids from these extractions were combined and centrifuged at 200 000g for 1h at 4°C to give a second pellet (DO2). Centrifugation of the resultant supernatant fluid for 7h at 200 000g and 4°C gave a third

---

* Solution of deoxycholate in tris buffer were held overnight at 4°C and filtered to remove insoluble precipitate. + Abbreviation: DOC sodium deoxycholate.

pellet (DO3). Pellets DO2 and DO3 were extracted a further twice with 1% DOC in tris buffer, and then washed three times in tris buffer alone to remove residual DOC. Membrane residues DO2 and DO3 were sedimented at 200 000g for 7h at 4°C during extraction and washing (See Flow Diagram 1).

"Standard" membrane suspensions from M.lysodeikticus St.A were extracted with DOC in a similar manner to give pellets DA1 and DA2. No significant pellet corresponding to DO3 was obtained.

For dry weight and chemical analysis the membrane residues were dialysed against three changes of 100 vol. distilled water at 4°C and freeze dried.

Extraction of "standard" membrane with EDTA. "Standard" membrane suspensions (10-15mg dry wt. membrane/ml)were extracted with an equal volume of tris buffer containing 10mM ethylene diamine tetraacetic acid disodium salt (EDTA). Membrane residues were extracted a further once with tris buffer containing 5mM EDTA and then washed three times in tris buffer to remove residual EDTA. Membrane residues were sedimented at 65 000g for 1h at 4°C during extraction and washing.

Ionic shock treatment of "standard" membranes. "Standard" membranes were washed a further three times using either distilled or deionized water, both being equally efficient. Membrane residues, or "shocked" membranes, were sedimented at 65 000g for 1h at 4°C during preparation.

Estimation of membrane yield. Membranes or membrane

residues were made up to a known volume with tris buffer, an aliquot removed, and dialysed exhaustively (at least three changes of x100 vol.) against distilled water at 4°C. Suspensions were frozen at -25°C and freeze dried in an Edwards Freeze Drier (Model 30P2) prior to weighing. These results were compared with those obtained after freeze drying of a known aliquot of washed whole cells.

Preparation of mesosomal and peripheral membrane fractions

Cultivation. M.lysodeikticus St.O was used throughout these mesosome studies. Cells were cultivated as previously described (Owen & Freer, 1970b. See reprint facing p.61.) and harvested (2 000g for 25min at 25°C) after 10h growth at 30°C. Sedimented cells were washed twice in tris buffer at 21°C before further treatment.

Plasmolysis. In procedures involving plasmolysis, washed cells were resuspended to 1/10 of the culture volume in tris buffer which was made 2.0M with respect to sucrose, and contained $MgCl_2$ at the desired concentration (see Results). Cells were allowed to equilibrate in this solution for 1.5h at room temperature (21°C).

Protoplast formation. Washed cells were suspended to 1/10 of the original culture volume in tris buffer, 0.8M with respect to sucrose and containing $MgCl_2$ at the desired concentration. Cells were then equilibrated for 1.5h at 21°C. After equilibration in 0.8M buffered sucrose, or, in the case of plasmolysed cells, in 2.0M buffered sucrose, lysozyme was added to a final concentration of 250ug/ml and suspensions were incubated for 45min at 30°C in a circulating water bath unless otherwise stated. The

resulting protoplast suspension was then diluted with 1.5vol. of tris buffer containing sufficient sucrose to give a final molarity of 0.8M. $MgCl_2$ was incorporated in the diluent at the desired concentration.

Swirling. 60ml of protoplast suspension were subjected to swirling in a 250ml Erlenmeyer flask at 85 rev./min in an orbital shaker-incubator at 30°C for 2h.

Sedimentation of mesosomal and peripheral membrane fractions. Protoplasts were sedimented from the suspending medium by centrifugation at 12 000g for 2h at 15°C. The 12 000g supernatant fluid was recentrifuged at 12 000g for 2h at 15°C to remove any residual protoplasts. This second supernatant fraction, subsequently referred to as the "protoplast supernatant fraction" contained the released mesosomal membranes. These could be sedimented from the protoplast supernatant fraction by centrifugation at 280 000g for 2h and were washed six times in tris buffer, sedimenting under similar conditions.

Pelleted protoplasts were combined and osmotically lysed by the addition of tris buffer (approx. 10 vol.) and of deoxyribonuclease to a final concentration of approx. 0.5ug/ml. Peripheral membranes were prepared from this osmotic lysate by washing six times in tris buffer as previously described (Owen & Freer, 1970b).

Procedures for monitoring protoplast stability and mesosomal membrane release

Protoplast leakage. An estimate of leakage of protoplast content during the release of mesosomal membrane was achieved by monitoring the E_{260}^{1cm} of the protoplast supernatant

fraction made 0.1% with respect to SDS. The extinction value was compared with the E^{1cm}_{260} of an osmotic lysate of the protoplast suspension clarified by the addition of SDS to a final concentration of 0.1%. This method, although not totally satisfactory, offers a rapid and convenient method of assessing the intactness of protoplasts. (Reavely & Rogers, 1969).

Release of mesosomal membrane. Convenient "marker" molecules for membrane fractions of M.lysodeikticus are the carotenoid pigments (see Salton & Ehtisham-ud-din, 1965). The E_{446} values for carotenoid absorption (Fig.1) correlates directly with the dry weight of membrane in suspension (Fig.2) and as such offer a convenient method for assaying the concentration in aqueous suspensions. The carotenoid content per unit of dry weight of membrane does not differ appreciably (see Results and Fig.2) between peripheral and mesosomal membranes. Before extinction values were measured, membrane suspensions were clarified by addition of SDS to a final concentration of 0.1%. The E^{1cm}_{446} values of the protoplast supernatant fractions and those of the total protoplast lysates were compared and expressed as a percentage (e.g. see Table 18).

Electron microscopy. All membrane preparations were examined by the negative staining procedure (Brenner & Horne, 1959) using 2.0% ammonium molybdate. Before examination, mesosomal membrane were washed three times in tris buffer. For further details of procedure see Methods p.56 .

Chemical analysis of membrane fractions

All glassware was either cleaned in chromic

acid or in Decon 90 before use.

Where recorded, ultrasonic clarification of membrane suspensions was performed essentially as described by Salton & Netschey (1965), using an M.S.E. 100W Ultrasonic disintegrator fitted with a 3/8" or 3/4" titanium probe as required.

Carotenoids. Fresh membrane suspensions were clarified with SDS (0.1%) and the extinction value measured at λ max (Fig.1) of 446nm. Values for carotenoid content were obtained assuming a molar extinction coefficient of 3×10^3 (Salton & Schmitt, 1967a). It should be noted that consistent results were only obtained using fresh membrane preparations. It seems that the conditions of lyophilisation cause partial destruction of the pigments. Recorded values are the means of at least two determinations.

Extractable lipid. Extractable lipid was estimated gravimetrically after treatment of lyophilised membrane preparations (50-100mg) with acetone-methanol (7:2 by vol.) as described by Salton & Schmitt (1967a). Recorded values are the means of at least two determinations.

Bound Lipid. The insoluble residues remaining after removal of extractable lipid from the membrane were pooled and subjected to mild acid hydrolysis for 1h (refluxing 5% conc. HCl in methanol $^{v}/v$) as described by Yudkin (1967). Acid methanol was removed under reduced pressure in a rotary evaporator, and the residue extracted three times with ether (approx.10ml) followed by three extractions with 10ml aliquots of acetone-methanol (7:2). Ether and the acetone-methanol were evaporated under vacuum in a rotary evaporator at room temperature and the separate extracts dried over

phosphorous pentoxide and sodium hydroxide pellets prior to gravimetric estimation. Lipid extracts were maintained under an atmosphere of nitrogen where possible. All estimations were performed in duplicate and the meansrecorded.

Sodium dodecyl sulphate. The quantity of residual SDS present in SDS insoluble membrane residues was determined on lyophilised preparations. Hydrated samples (approx.0.8mg dry wt. residue/ml) were extensively sonicated, SDS extracted as the alkyl sulphate-methylene blue complex into chloroform and estimated by reading the optical density of the chloroform solution at 655nm (Ray, Reynolds, Polet & Steinhardt, 1966; Reynolds, Herbert, Polet & Steinhardt, 1967; Reynolds & Tanford, 1970). The previously published procedure was modified by using 10ml chloroform, 5ml HCl (0.03N), 5ml methylene blue (700ug/ml) and 5ml of sample. Calibration curves were run using standard solutions of SDS of the appropriate concentrations.

Phosphorus content. Phosphorus content was determined by the colorimetric method of Allen (1940). The presence of a fine precipitate during the determination of orthophosphate in membrane preparations prevented a quantitative estimation of inorganic phosphate. The contribution of orthophosphate to the total phosphorus content, however, appeared to be negligible from a visual comparison of samples in the two colorimetric assays. Potassium dihydrogen phosphate (dried at 105^{o}C overnight) was used as standard and recorded values for membrane phosphorus content were the mean of three determinations.

Protein estimation. Protein was estimated on lyophilised

membrane following dispersion by ultrasound and solubilisation with 0.1% SDS by the method of Lowry, Rosenbrough, Farr & Randall (1951) and by that of Gornall, Bardawill & David (1949). Bovine serum albumin in the presence of 0.1% SDS was used as a standard in both procedures. In estimations utilising the Biuret reagent (Gornall et al., 1949), the extinction value obtained at 560nm was corrected for absorption at this wave-length by membrane pigments (see Fig.1). All estimations were performed in triplicate, and the means recorded. A separate standard curve was plotted with each set of results.

Polyacrylamide disc gel electrophoresis. The procedure for electrophoresis of insoluble membrane proteins was a modification of that described by Davis (1964). In initial studies the method of Davis (1964) was utilised but with the addition of 0.1% SDS to all buffers and gel systems. Electrophoresis was performed in a Shandon analytical polyacrylamide electrophoresis tank. Gels were fixed and stained overnight as described by de Vito & Santome (1966), followed by electrophoretic destaining.

This method was unsatisfactory for a number of reasons. The major problem was exclusion of samples from the separating gel, as judged by the amount of staining material present in the sample gels and at the interfaces of sample gel and the stacking gel. This in turn caused distortion of the gels during electrophoresis, and resulted in a poorly resolved spectrum of protein bands. Almost total penetration was achieved by subjecting membrane samples to extensive ultrasonication prior to "solubilisation" with SDS, the elimination of a sample gel and the use of a

mechanically strengthened stacking gel. Increased resolution of individual components was obtained by lowering the applied current to 1mA/gel. This procedure, however, resulted in a diffuse band of tracking dye, a phenomenon which could be overcome by raising the applied current to 4mA/gel over the final few mm of the run. Rehydration of fixed and stained gels (de Vito & Santome, 1966) before destaining avoided the problem of mechanical distortion often encountered at this stage.

It should be noted that ageing of the acrylamide solutions affected the pattern of bands obtained after electrophoresis of samples. Thus gels were always prepared from fresh acrylamide solutions.

The protocol finally adopted and detailed below allowed good resolution of discrete membrane polypeptides. Lyophilised membrane preparations were dispersed in tris-glycine buffer (Davis, 1964) by extensive ultrasonication (15min at 0°C) and then made 0.2% with respect to SDS. To 1ml of "solubilised" membrane suspension (1-2mg/ml) was added 3 drops of glycerol. Aliquots of this suspension were then layered on top of the mechanically strengthened stacking gel, which was made 4%^{W}/v with respect to acrylamide (de Vito & Santome, 1966), 1%^{W}/v with respect to NN'-methylenebisacrylamide* (bis), and 0.1% with respect to SDS. Separating gels and buffers were as

---

* Abbreviation bis: NN'methylenebisacrylamide.

described by Davis (1964) but made 0.1% with respect to SDS. Bromophenol blue was used as tracking dye (Davis, 1964) and electrophoresis performed at 1mA/gel during the run, but elevated to 4mA/gel when the tracking dye reached the final few mm. Gels were immediately removed and fixed and stained overnight in a solution containing 1%^{W}/v amido black, 10%^{V}/v acetic acid and 50%^{V}/v methanol. Prior to electrophoretic destaining (at 5mA/gel) gels were rehydrated in 7%^{V}/v acetic acid for 4h.

Although allowing good resolution of several membrane components, the use of 7%^{W}/v acrylamide separating gels (Davis, 1964) did not give adequate resolution of faster migrating components. Indeed approx. 50% of the stainable material entering the separating gel was found to migrate as one band with the tracking dye (Plate 1. See also Munoz, Salton, Ng & Schor, 1969, and Estrugo et al., 1972 for a similar phenomenon).

Resolution of this fast moving complex was achieved by decreasing the pore size of the separating gel. Plates 1-3 show the effect of varying concentrations of acrylamide and bis in the separating gel upon the spectrum of resolved membrane polypeptides. These plates show that optimal resolution of the faster migrating components combined with minimal distortion of bands is achieved in gels having a composition of 11.7% (^{W}/v) acrylamide and 0.153% bis (Plate 2,A_{15}). To obtain maximum resolution of membrane polypeptides it was necessary, however, to utilise separating gels containing both 11.7%^{W}/v and 7%^{W}/v acrylamide. (Recently Grula & Savoy, (1971) have detailed a method for polyacrylamide gel electrophoresis which

appears to give good resolution of a wide range of membrane proteins from M.lysodeikticus on a single gel.

Several authors have reported that the electrophoretic mobilities of proteins were proportional to their molecular weight when dissolved in SDS (Shapiro, Vinuela & Maizel, 1967; Shapiro & Maizel, 1969; Weber & Osborn, 1969), a phenomenon attributed to the loss of charge specificity of the proteins on binding SDS (Reynolds & Tanford, 1970). It is thus possible to estimate the molecular weight of discrete protein components by comparing their Rf value (i.e. the distance migrated by the protein divided by the distance migrated by bromophenol blue) with those of proteins of known molecular weight electrophoresed under similar conditions. A typical plot of the molecular weight against Rf value of several highly purified proteins, electrophoresed in 11.7%^{W}/v acrylamide gels, is illustrated in Fig. 3. Caution, however, must be exercised in the determination of molecular weights based upon this method alone, as some proteins have been shown to migrate anomolously in these systems (Swank & Monkres, 1971; Tung & Knight, 1971; Williams & Gratzer, 1971; Griffith, 1972).

The use of SDS-polyacrylamide gels containing 11.7%^{W}/v acrylamide has also proved invaluable in the study of proteins released by membranes subjected to various washing procedures and also in an investigation of the multiple forms displayed by the α-toxin from Staphylococcus aureas. A full report of this latter investigation has been published (McNiven, Owen &

Arbuthnott, 1972) and is included in this thesis. (See attached reprint facing p.173).

Paper chromatographic analysis of amino acids. Approx.20mg of lyophilised membrane were hydrolysed for 18h at 105°C in evacuated ampoules containing 3ml 6M HCl. Hydrolysates were filtered through Whatman No. 1 paper and evaporated to dryness at 100°C. The residue was resuspended in distilled water and evaporated to dryness again. After repeating this procedure six times, the residue was dissolved in distilled water, filtered, evaporated to dryness and finally dissolved in 0.5ml distilled water.

The resultant amino acids were separated by two dimensional ascending paper chromatography (Whatman No.1) using butanol-acetic acid-water (120:30:50 by vol.) and water saturated phenol (500g phenol + 125ml water) - ammonia (200:1 by vol.) as solvent systems. Chromatograms were washed in two changes of acetone to remove traces of phenol, dried, and developed at 90°C for 5min after spraying with 0.5% ninhydrin in butanol. The resultant patterns of amino acids were preserved by dipping chromatograms in dilute copper nitrate after first marking the position of proline and hydroxyproline. Amino acids were identified by comparison with chromatograms of standard mixture of amino acids run under the same conditions.

Ion-exchange chromatographic analysis of amino acids. Approx. 25mg of lyophilised membrane preparations were hydrolysed under a nitrogen atmosphere for 24h at 100°C in sealed ampoules containing 6M HCl. Individual amino acids were resolved in a Locarte Model 4 automatic amino acid analyser

and estimated by comparison with known standards. The author is indebted to Mr. L. Lobel of Locarte Ltd (London) for running the samples, and also to Dr. G. Leaf of Dept. of Biochemistry (Glasgow) for providing a value for the colour constant of glucosamine.

Cytochromes. Reduced spectra and difference spectra (reduced vs. air oxidised) of membrane preparations and membrane residues were recorded at 21°C in a Pye Unicam SP800 fitted with a slave recorder. The use of freshly prepared membranes was found to be essential since lyophilisation caused partial destruction of cytochromes. Membrane preparations (10-15mg dry wt.membrane/ml) were clarified by ultrasound (5min) at 0°C and any particulate matter removed by bench centrifugation (1 000g) for 5min. Where indicated Triton X-100 (100%^{W}/w) was added to membrane preparations to a final concentration of 1%^{V}/v and Vitamin K_3 (menadione) to the concentration indicated. The test cuvette contained 0.40ml membrane suspension which was reduced by addition of either 5ul of sodium dithionite (80mg/ml), 5ul of $NADH_2$ (0.20M), or 5ul of sodium hydrogen malate (0.40M) to give final concentrations of 1mg/ml, 2.5mM and 5mM respectively (Gel'man et al., 1970). In instances where the kinetics of reduction were studied, the visible spectrum was scanned between 625nm and 530nm at 30s intervals. The content of cytochromes $a_{601}, b_{556} + b_{560}, c_{550}$, was calculated according to the method of Lisenkova & Mokhova (1964). Wave-lengths used for determination of the absorbance (ΔE) of the above cytochromes, as well as the coefficients of millimolar extinction, are as documented by Simokova et al. (1969) and are reproduced in Table 5.

As observed by Gel'man and colleagues (1970) chemical reduction of cytochromes is not instantaneous. Therefore, in all experiments that required complete chemical reduction (e.g. calculation of cytochrome content) the kinetics of reduction were followed. Complete chemical reduction of membrane cytochromes was achieved after approx. 10min (Fig.4) or if performed in the presence of 1.0%^{v}/v Triton X-100, approx. 5min (Fig.4).

Nucleic acid. Nucleic acid was determined by comparison of extinctions at 260nm and 280nm (Warburg & Christian, 1941). The extinction at 260nm and 280nm of fresh membrane preparations clarified by extensive ultrasonication (10min), or by addition of 0.1% SDS were found to be identical, and hence the latter method was used throughout.

The direct determination of DNA in membrane preparations by the method of Burton (1956) was found to be unsatisfactory, negative results being recorded consistently for some preparations.

Total hexose determination. Total hexose content was determined on lyophilised membrane preparations after ultrasonic dispersion for 10min and "solubilisation" with 0.1% SDS, by the anthrone reaction method of Morris (1948). Glucose in the presence of 0.1% SDS was used as standard. Recorded values for hexose content were the mean of at least three determinations and a suitable standard curve was prepared for use with each set of results.

Paper chromatographic analysis of hexoses. Sugars were separated by paper chromatography following acid hydrolysis (Cummings & Harris, 1956). 10-20mg of lyophilised membrane

preparation were hydrolysed at 105°C for 2h in sealed ampoules containing 1.0ml of 2M H_2SO_4. Hydrolysates were neutralised by addition of solid barium carbonate, filtered through Whatman No. 1 paper, evaporated to dryness in vacuo over sodium hydroxide pellets and phosphorus pentoxide and finally dissolved in distilled water (approx. 0.5ml). Sugars were separated by one dimensional descending paper chromatography (Whatman No. 4, 57cm x 23cm) using ethyl acetate-pyridine-water (160:40:20: by vol.) as solvent. Chromatograms were run for 18h and developed by dipping in aniline hydrogen phthalate reagent (Cummings & Harris, 1956) followed by heating at 105°C for 5-10min. Individual sugars were identified by comparison with known standards run in parallel.

Glucose determination. Glucose was determined quantitatively in neutralised acid hydrolysates by the glucose oxidase method (Hugget & Nixon, 1957) using the "Glucostat" enzyme kit marketed by Worthington Biochemical Corp. (Freehold, N.J.).

Differentiation of mannose and fructose. Mannose and fructose have similar Rf values when run in most chromatographic solvent systems (see Partridge, 1948), including the one mentioned above. It thus became necessary to distinguish between these two sugars. Due to the lack of a direct analytical method for distinguishing these two sugars, save by gas liquid chromatography (Holligan, 1971), the identity of the component running in the position of fructose/or mannose was ascertained by testing for the presence of keto sugars (Ikawa & Nieman, 1949). Aliquots (0.3ml) of sugar hydrolysate containing 20ug of total hexose (Morris, 1948) were mixed with 84%$^{w}/_{w}$ sulphuric

acid (2.7ml), cooled, and held at 25°C for 2h prior to recording E^{1cm}_{310} in a Pye Unicam SP500 spectrophotometer. The extinction values were compared with standard curves for fructose, mannose and glucose, recorded under similar conditions (Fig.5). Unlike fructose, sugar hydrolysates of all membrane preparations failed to show a λ max at 310nm after incubation for 2h in 79%^{W}/w sulphuric acid, the E^{1cm}_{310} indicating a fructose content of less than 4% of the total hexose (assuming that the absorption at 310nm in hydrolysates is attributable solely to fructose).

Total pentose determination. Total pentose content was determined in lyophilised membrane preparations, after ultrasonic dispersion and "solubilisation" with 0.1% SDS, by the Bial reaction (Dische & Schwartz, 1937). However, the reaction time of 20min necessary to ensure hydrolysis of nucleotides, produced interference due to hexose in the preparations. This problem was overcome by correcting the observed pentose value for absorption due to hexose. The correction for hexose absorption at the pentose λmax (Dische & Schwartz, 1937) was determined from absorption of mannose equivalent in concentration to the hexose content of the test membrane suspension. The hexose content was determined by the method of Morris (1948). Ribose in the presence of 0.1% SDS was used as standard. Recorded values were the mean of three separate determinations.

Paper chromatographic analysis of individual pentoses. Individual sugars were separated by paper chromatography following acid hydrolysis. The procedure adopted was identical to that described for hexose sugars, except that chromatograms

were run for 6h prior to development.

Enzyme analysis of membrane fractions

All spectrophotometric enzyme assays were carried out on freshly prepared membrane fractions using a Pye Unicam SP800 spectrophotometer fitted with temperature controlled cuvette holders and a slave recorder. Enzyme activities were expressed as ΔE_{600}^{1cm}/min/mg dry wt. of membrane (except for the autolytic enzyme(s)), and recorded values were the mean of at least two separate determinations.

$NADH_2$ dehydrogenase (EC.1.6.99.3) and malate dehydrogenase (EC.1.1.1.37). The activities of both $NADH_2$ dehydrogenase and malate dehydrogenase were determined spectrophotometrically at 25°C as described by Gel'man et al. (1970).

Succinate dehydrogenase (EC 1.3.99.1). Succinate dehydrogenase activity was determined by a modification (Owen & Freer, 1970b. See reprint facing p. 61) of the spectrophotometric method of Ells (1959). Prior to assay, fresh membrane preparations were diluted in tris buffer at 0°C to approx. 250ug dry wt. membrane /ml and held at 0°C for at least 6h (Owen & Freer, 1970). Assay was performed at 15°C except where noted otherwise.

Autolytic enzyme. The lytic activity of membrane preparations against whole cells of M.lysodeikticus St. O was estimated in the following way. Washed exponential phase cells were suspended in tris buffer to an extinction of 0.4 at 450nm. The reaction cuvette contained 2.9ml cell suspension and 0.1ml of freshly prepared membrane suspension. The blank cuvette contained 2.9ml of cell suspension and 0.1ml of tris buffer. The assay was

performed at 30°C and ΔE_{450}^{1cm}/min was determined in a Pye Unicam SR800 spectrophotometer over the linear portion of the plot. One unit of activity was defined as a ΔE_{450}^{1cm} of 0.001 per min in a suspension of M.lysodeikticus of E_{450}^{1cm} equal to 0.4 at 30°C, pH 7.5 and total volume of 3ml. Enzyme activities were compared with that of egg-white lysozyme assayed under similar conditions.

Infrared Spectroscopy

A Hilger-Watts Infrascan spectrophotometer Model H900 was employed throughout. Spectra of membrane preparations were determined on solid films essentially as described by Wallach & Zahler (1966). Thin films were prepared by air drying (21°C) aqueous membrane suspensions spread on silver chloride plates. Lipid fractions, dissolved in chloroform-methanol (2:1 by vol.), were filmed in a similar manner onto sodium chloride plates. All films were dried *in vacuo* over phosphorus pentoxide prior to examination.

Electron Microscopy

All specimens were examined in a Philips EM300 electron microscope using double condenser illumination, a 50um objective aperture and a liquid nitrogen anti-contamination device. The operating potential was 60kV and the magnification was calibrated from diffraction grating replica ruled at 2160 lines/mm. Photographic records were made utilising Ilford N.50 plates developed with Ilford ID-11 developer.

Negative staining. Washed whole cells and membrane preparations were examined by the negative staining method

(Brenner & Horne, 1959) using ammonium molybdate as stain. Samples were diluted with 2%^{W}/v ammonium molybdate pH 5.2 or 2%^{W}/v ammonium molybdate + 2%^{W}/v ammonium acetate pH 6.9 to yield a lightly turbid suspension. With a capillary pipette the suspension was applied to grids covered with carbon-coated formvar and the excess removed with a filter paper point. Grids dried within a few seconds and were examined, immediately after preparation, by electron microscopy.

Thin sectioning. Bacterial cultures at the appropriate stage of growth were made 5%^{V}/v with respect to glutaraldehyde. Cells were then sedimented by centrifugation at 2 000g for 10 min and resuspended in fresh fixative i.e. 5% glutaraldehyde in Kellenberger's veronal-acetate buffer (Kellenberger, Ryter & Sechaud, 1958) pH 6.1 for 2h. After thorough washing in veronal-acetate buffer cells were again fixed in veronal-acetate buffered 1% osmium tetroxide for 3h at room temperature. The twice fixed, washed pellets were stained with 0.5% magnesium uranyl acetate for 1.5h at room temperature, washed once with veronal-acetate buffer, and embedded in 1.5% Bacto agar. Agar blocks (0.5mm^{3}) were next dehydrated in an ethyl alcohol series (25%, 50%, 75%, 95%, 100% ^{V}/v ethanol), infiltrated and embedded in Epon (Luft, 1961) as described for Araldite (Glauert & Glauert, 1958). Sections (silver-pale gold) were cut with glass or diamond knives on the LKB Ultratome Model 8802A, collected on distilled water and heat stretched (Roberts, 1970) with a Polaron Equipment Ltd heat pan. After collection on formvar-covered, carbon-coated grids, sections were either double stained with uranyl acetate and lead citrate by the method of Frasca & Parks (1965), or single stained with

lead citrate by the method of Venable & Coggeshall (1965).

Freeze-etching. Fresh membrane preparations were suspended in either 0.01 M Tris-Hll buffer pH 7.5 or in 20% glycerol in 0.01 M Tris-HCl buffer pH 7.5 before freezing. With whole bacteria, washed cells were impregnated with 20% buffered glycerol for 3h at room temperature. In experiments involving protoplasts (stabilised in 0.8M sucrose/tris buffer containing 10mM Mg^{2+}) fixation was carried out in 1%^{v}/v glutaraldehyde for 2h, followed by washing twice with 0.01 M Tris-HCl buffer pH 7.5 prior to freezing.

Drops of thick sample suspension (approx.10ul) were placed onto gold specimen supports and rapidly frozen by immersion in Freon 22 at liquid nitrogen temperature for 2-3s. Specimens were rapidly transferred into liquid nitrogen and stored until use (always less than 24h). Freeze-fracturing was performed in a Balzer's 360M Freeze etching unit essentially as described by Moor (1964) and Moor & Muhlethaler (1963). Specimen discs were placed on the precooled specimen table at liquid nitrogen temperature and cleaved at -100°C until a large evenly chipped face was obtained. In preparations where etching was desired the final chipped specimen face was held for 1min at -100°C approx. 2mm under the base of the knife holder (Moor, 1969) with a temperature differential between specimen and knife of 97°C. Platinum-carbon replicas were prepared immediately after fracturing or etching, floated onto distilled water and cleaned by transfer into 5%^{w}/v SDS for approx. 18h, followed by transfer into 70%^{v}/v sulphuric acid (approx. 5h) and subsequent transfer into Domestos

(overnight). After washing in distilled water, replicas were collected on formvar-covered, carbon-coated grids and examined in the electron microscope.

R E S U L T S.

Factors Influencing the Activity of Succinic Dehydrogenase in Membrane Preparations from *Micrococcus lysodeikticus*. By P. OWEN and J. H. FREER (*Department of Microbiology, University of Glasgow*)

Differences in the distribution of respiratory chain enzymes, including succinic dehydrogenase (SDH), between cytoplasmic and mesosomal fractions of Gram-positive bacteria have been reported. The SDH activity appeared largely in the mesosome fraction (Ghosh, B. K. & Murray, R. G. E. (1969), *J. Bact.* 97, 426), or solely in the cytoplasmic membrane (Rogers, H. J., Reaveley, D. A. & Burdett, I. D. J. in *Protides of the Biological Fluids*, Vol. 15, p. 303. Amsterdam: Elsevier; Ellar, D. J. 2nd meeting of the North West European Microbiological Group Symposium, 1969; Reaveley, D. A. & Rogers, H. J. (1969), *Biochem. J.* 113, 67). Cytochemical evidence suggested a distribution of activity throughout the cell membranes, but predominating in the internal membranes (Sedar, A. W. & Burde, R. M. (1965), *J. Cell Biol.* 27, 53).

The present work reports several factors which influence the SDH activity in membrane preparations, with some comments on the spectrophotometric assay.

Preparation of membranes: SDH activity in membrane preparations is reduced by exposure to temperatures above 10°. A loss of 25 to 30 % of activity occurs after exposure to 30° for 10 min.

The specific activity of SDH is dependent upon membrane concentration. At 0·3 to 0·4 mg. membrane/ml. the specific activity is 2- to 3-fold greater than that for concentrations of 10 to 15 mg. membrane/ml. Washing with sodium deoxycholate results in a similar increase in activity. Activation by dilution is largely inhibited if the diluent includes bovine serum albumin (1·0 mg./ml.) and totally eliminated in membranes mildy fixed with glutaraldehyde. In all cases activity remains in the sedimentable fraction.

Spectrophotometric assay: The assay method used was based upon the method of Ells, H. A. (1959, *Archs Biochem. Biophys.* 85, 561). Factors influencing the observed SDH activity are temperature, age of membrane and light.

Results suggest that SDH is firmly bound to the membrane, and that its activity is controlled to some extent by the presence of an 'inhibitor', the dissociation of which leads to an increase in activity.

iochem. J. (1970) **120**, 237–243
rinted in Great Britain

Factors Influencing the Activity of Succinate Dehydrogenase in Membrane Preparations from *Micrococcus lysodeikticus*

BY PETER OWEN AND JOHN H. FREER
Department of Microbiology, University of Glasgow, Garscube Estate, Glasgow, U.K.

(*Received* 26 *June* 1970)

1. Some properties of succinate dehydrogenase [succinate–(acceptor) oxidoreductase, EC 1.3.99.1] in membrane preparations from *Micrococcus lysodeikticus* (N.C.T.C. 2665) were investigated. 2. In the spectrophotometric assay system adopted the reaction velocity was shown to be proportional to the amount of membrane added. Dichlorophenol-indophenol, reduced photochemically in the presence of phenazine methosulphate, or enzymically by the membrane-bound enzyme, was shown to undergo reoxidation in the dark. 3. The membrane-bound enzyme was found to be inactivated at temperatures above 10°C. 4. The specific activity of membrane-bound succinate dehydrogenase was found to increase between two- and three-fold in diluted membrane preparations equilibrated at 0°C for 6h. Membranes treated with sodium deoxycholate showed no enzyme activation on dilution but displayed maximal activity, all activity being sedimentable at 103000*g*. The increase in specific activity observed on dilution could be partially inhibited by fixation with glutaraldehyde, or by the presence of bovine serum albumin. 5. The addition of Mg^{2+} or Ca^{2+} ions to membrane suspensions caused an overall depression of enzyme activity. 6. The results suggest the presence of an 'inhibitor' that affects the expression of membrane bound succinate dehydrogenase activity.

Several reports on the fractionation of bacterial nembrane systems have been published (Ferrandes, haix & Ryter, 1966; Fitz-James, 1967; Reaveley, 968; Ghosh & Murray, 1969; Reaveley & Rogers, 969). The fractionation methods usually yield a inor small vesicle fraction [the so-called 'mesoome' fraction (Fitz-James, 1960)] which has been hown to differ from the major membrane fraction n several respects. A differential distribution of omponents of the respiratory chain between major nd minor fractions has been shown in several rganisms (Ferrandes *et al.* 1966; Reaveley, 1968; hosh & Murray, 1969; Reaveley & Rogers, 1969). arious membrane fractions from *Micrococcus ysodeikticus* showed different amounts of succinate ehydrogenase [succinate–(acceptor) oxidoreducase, EC 1.3.99.1] activity. The purpose of this nvestigation was to determine to what extent ifferences in activity could be accounted for by ariations in membrane preparation procedure or onditions of the enzyme assay.

MATERIALS AND METHODS

Chemicals. Lysozyme (EC 3.2.1.17) was obtained from igma Chemical Co. (St Louis, Mo., U.S.A.) and deoxyribonuclease (EC 3.1.4.6) from Worthington Biochemical Corp. (Freehold, N.J., U.S.A.), DCIP,* PMS and sodium dodecyl sulphate were from BDH Chemicals Ltd. (Poole, Dorset, U.K.), tris, disodium succinate (enzyme grade) and sodium deoxycholate were from Koch–Light Laboratories Ltd. (Colnbrook, Bucks., U.K.), glutaraldehyde was from Taab Laboratories (Reading, Berks., U.K.) and bovine serum albumin Fraction V was from Armour Pharmaceutical Co. Ltd. (Eastbourne, Sussex, U.K.). All other chemicals and reagents were of analytical grade.

Preparation of 'standard' membranes. Membrane preparation was based throughout on the method of Salton & Freer (1965). Cells of *Micrococcus lysodeikticus* (N.C.T.C. 2665) were grown from a 10% inoculum in 2 litre conical flasks containing 500ml of a medium containing 5% Bacto-Peptone, 0.1% Difco Yeast Extract, 0.5% NaCl, pH7.2, on an orbital shaker at 30°C (150 rev./min). After 18h of growth cells were harvested at 4°C (2500*g* for 25min) and washed once with distilled water and once with 50mM-tris–HCl buffer, pH7.5. (This buffer, referred to as 'tris', was used throughout this work, unless otherwise stated.)

Cells were resuspended in tris buffer to approx. 50mg dry wt./ml. Cell walls were removed with lysozyme at a concentration of 100μg/ml by incubation at 30°C for 45min. Deoxyribonuclease was added to the lysed protoplast suspension to decrease the viscosity. Membranes were sedimented from the total lysate by centrifugation at 38000*g* for 50min at 0°C and kept at 0°C during all subsequent procedures, unless otherwise stated. Membrane fractions were washed six times with tris buffer by

* Abbreviations: DCIP, 2,6-dichlorophenol-indophenol; PMS, phenazine methosulphate.

resuspension and recentrifugation and finally resuspended in tris buffer to 10–15mg dry wt. of membrane/ml. This suspension is referred to as 'standard' membranes, and was always prepared within 18h of harvesting the cells.

Preparations of 'equilibrated' membranes. 'Standard' membranes were diluted in tris buffer to give 200–500μg dry wt. of membrane/ml and kept at 0°C for at least 6h. This suspension was termed 'equilibrated' membranes.

Glutaraldehyde fixation. 'Standard' membrane suspensions (10–15mg dry wt. of membrane/ml) were diluted with glutaraldehyde in tris buffer to give a final concentration of approx. 5mg dry wt. of membrane/ml of buffered glutaraldehyde (0.5% glutaraldehyde in tris buffer). The 'standard' membrane suspension was rapidly diluted and mixed in the fixative solution to prevent aggregation. The membrane suspension was then immediately centrifuged for 50min at 38000g and the membrane pellets were resuspended and washed twice in tris buffer to remove glutaraldehyde before resuspension in tris buffer to give 10–15mg dry wt./ml. All operations and reagents were at 0°C.

Deoxycholate extraction. Deoxycholate extraction was performed essentially as reported by Salton, Freer & Ellar (1968). 'Standard' membrane suspensions were extracted six times with 1% (w/v) sodium deoxycholate in tris buffer at 0°C and washed three times in tris buffer to remove residual deoxycholate. Membrane residues were sedimented at 38000g for 1h at 0°C during extraction and washing and then resuspended in a volume of tris buffer equivalent to that of the 'standard' membrane suspension before extraction.

Succinate dehydrogenase assay. The procedure for assay of succinate dehydrogenase was based on the spectrophotometric method of Ells (1959) with succinate as substrate, KCN as an inhibitor of the terminal oxidase, PMS as intermediate electron acceptor, and DCIP as terminal electron acceptor. The test cuvette (1cm light-path) contained 0.06ml of 2.5mM-DCIP, 0.30ml of 10 mM-KCN, 0.15ml of PMS (3mg/ml, freshly prepared), 0.60ml of 20mM-disodium succinate and membrane suspension. Tris buffer was added to give a final volume of 3.00ml. All reagents except for PMS were present in the 'blank' cuvette. Membrane suspensions were brought rapidly from 0°C to assay temperature, and the reaction was initiated with succinate. Decrease in E_{600} was recorded in a Pye Unicam SP.800 spectrophotometer fitted with temperature-controlled cuvette holders and a recorder. The reaction velocity, expressed as ΔE_{600}/min was measured over the linear part of the plot, which occupied the first 50% of the reaction. Assay was performed at 15°C unless otherwise stated. To minimize the photoreduction of DCIP found to occur in the presence of PMS the test cuvette was covered with aluminium foil until the reaction had been initiated.

RESULTS

Factors affecting the assay of succinate dehydrogenase in membrane preparations. In the assay system adopted the reaction velocity was found to be proportional to the amount of membrane added, no reduction of DCIP occurring in the absence of succinate. It was found that the reduction of DCIP by membrane preparations in the absence of PMS amounted to approx. 10% of that observed in the complete assay system (considerably higher values were found in assays of total lysate activity).

To measure only the PMS mediated reduction o

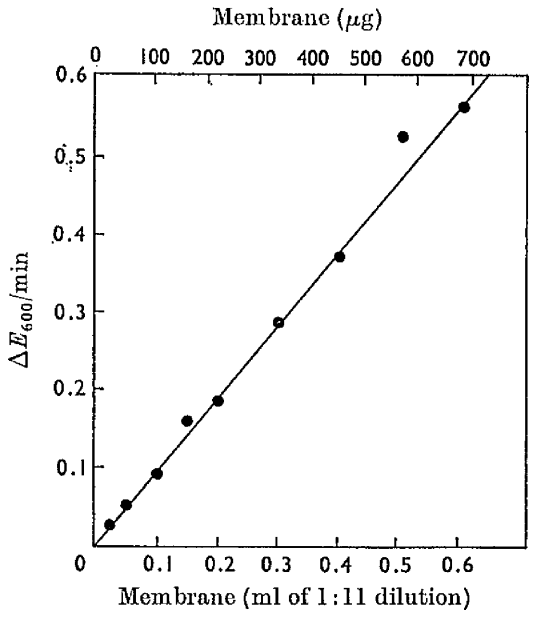


Fig. 1. Relationship of succinate dehydrogenase activity to the amount of membrane present in the assay. 'Standard' membrane suspensions were diluted (1:11) and equilibrated in tris buffer at 0°C for 12h before assay at 30°C.

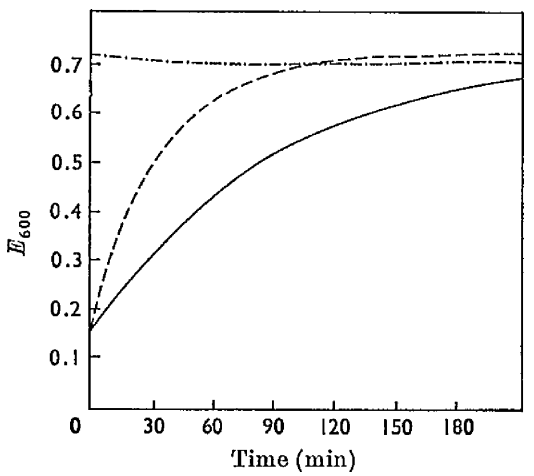


Fig. 2. Reoxidation of DCIP after photoreduction in the presence of PMS. The assay system without enzyme was exposed briefly to sunlight and reoxidation was recorded continuously at 25°C (----); reoxidation after photoreduction in the presence of 0.67% sodium dodecyl sulphate was also recorded (——). ·-·-·-·, Assay system without enzyme and protected from sunlight.

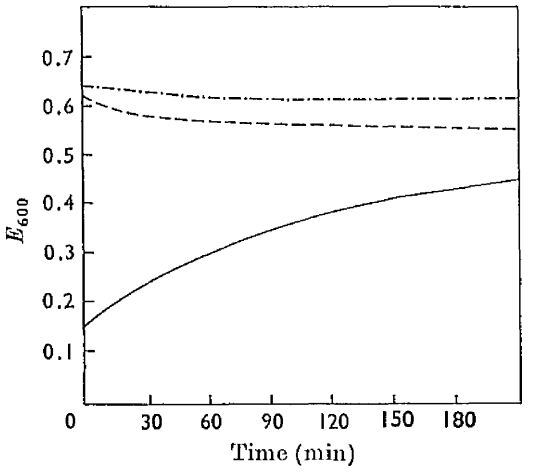


Fig. 3. Reoxidation of DCIP at 25°C after enzymic reduction, in the absence of photoreduction. The enzyme was inactivated by 0.67% sodium dodecyl sulphate after enzymic reduction of DCIP (——) or before addition of enzyme to the assay system (– – – –). ·–·–·–·, Assay system without enzyme and protected from sunlight.

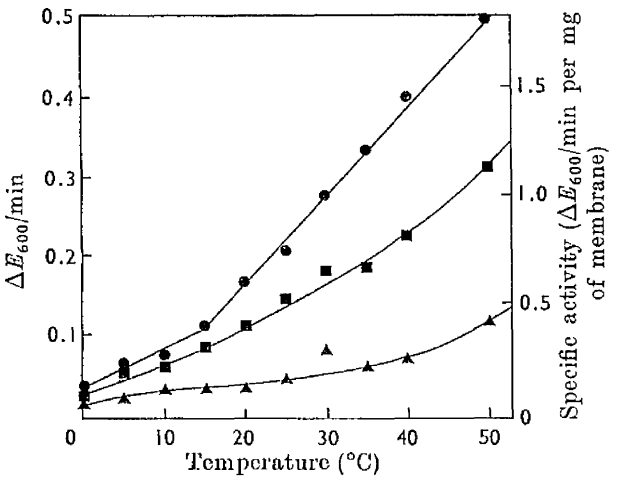


Fig. 4. Effect of assay temperature on the activity of succinate dehydrogenase in membrane suspensions equilibrated for 12h at 0°C in various diluents. ●, Tris buffer; ■, tris buffer containing 10mg of bovine serum albumin/ml; ▲, tris buffer containing 12mM-disodium succinate.

DCIP, it is essential to omit PMS from the 'blank' cuvette. The inclusion of PMS necessitates the elimination of either enzyme, substrate or terminal acceptor from the 'blank'. Under these latter conditions the overall reaction rate is a measure of both PMS-mediated and non-PMS-mediated dye reduction.

The enzyme reaction rate was found to be dependent on the concentration of PMS, and within the range of PMS tested (0.05–0.30mg/ml of assay mixture), the rate of dye reduction was proportional to the amount of membrane added. This result, for PMS at a concentration of 0.15mg/ml (as in the adopted assay system), is illustrated in Fig. 1.

It was found that spontaneous reduction of DCIP in the presence of PMS occurred on exposure to sunlight, a reaction that did not appear to be grossly affected by the other constituents of the assay system. This observation necessitated the protection of the test cuvette from direct light during assay. After photoreduction, the dye is reoxidized in the dark, the rate being proportional to the concentration of PMS. This process can be monitored at 600nm, being unaffected by irradiation at this wave-length. From Fig. 2 it can be deduced that the rate of reoxidation is also proportional to the concentration of reduced DCIP. After enzymic reduction of DCIP under assay conditions, reoxidation was again evident, at a rate similar to that observed for the photoreduced dye (see Fig. 3). In the experiment illustrated in Fig. 3, enzyme was inhibited after dye reduction by addition of sodium dodecyl sulphate to a final concentration of 0.67% (w/v).

Variation of enzyme reaction velocity with temperature is illustrated in Fig. 4. In experiments conducted with membrane preparations equilibrated in tris buffer alone a point of inflexion was consistently observed ata pprox. 15°C. The temperature of assay that was finally adopted was 15°C, and the reasons for this choice are evident from the results presented below. The effect of temperature on enzyme activity is illustrated in Table 1. 'Equilibrated' membrane suspensions were rapidly brought to assay temperature and the change in enzyme activity at the particular temperature was recorded at intervals over the next 1h. At temperatures below 10°C the loss of activity over the first 1h was slight (less than 13%), most of this occurring within the first 20min. However, at temperatures exceeding 15°C, an appreciable and progressive loss of activity was noted over the first 1h (greater than 55% at 25°C).

Preincubation of membrane suspensions with succinate for 1h appeared to increase the initial rate of enzyme inactivation, and at temperatures up to 25°C resulted in an overall depression of activity (see Table 1). Equilibration of membrane suspensions in the presence of succinate also resulted in a depression of specific activity when compared with suspensions equilibrated in either buffered bovine serum albumin solutions or buffer alone. The depression was evident over the range of assay temperatures 0–50°C (Fig. 4). This result contrasts with the reported activation of mitochondrial succinate dehydrogenase by preincubation with substrate (Kearney, 1957).

Enzyme activity and membrane concentration. The specific activity of succinate dehydrogenase in

Table 1. *Time-course of thermal inactivation of 'equilibrated' membrane suspensions at various temperatures*

Membrane suspensions (228 μg dry wt. of membrane/ml) were equilibrated for at least 12 h at 0°C in tris buffer (Expt. *a*) and in tris buffer containing 12 mM-disodium succinate (Expt. *b*). The suspensions were then brought to the desired temperature and succinate dehydrogenase activity was assayed at that temperature over the next 1 h.

Temperature (°C)	Expt. type	100 × Succinate dehydrogenase activity (ΔE_{600}/min) Time ... 0	15 min	30 min	45 min	60 min
5	*a*	6.1	5.4	5.0	5.0	4.9
	b	4.2	4.0	3.7	3.1	2.2
15	*a*	10.1	9.2	8.7	8.4	8.1
	b	7.6	5.3	4.9	4.8	4.8
25	*a*	16.7	14.4	12.0	9.7	7.4
	b	12.3	8.3	7.8	7.2	6.3

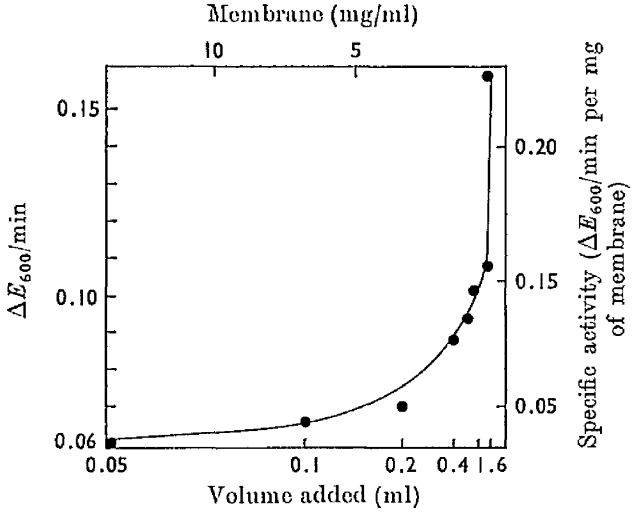


Fig. 5. Effect of membrane concentration on the activity of succinate dehydrogenase. 'Standard' membrane suspensions were diluted in tris buffer to various concentrations and equilibrated for 12 h at 0°C before assay at 0°C. The upper abscissa represents the membrane concentration in the suspensions and the lower abscissa the volume of that suspension present in the assay system. All determinations of enzyme activity were performed on the same dry wt. of membrane.

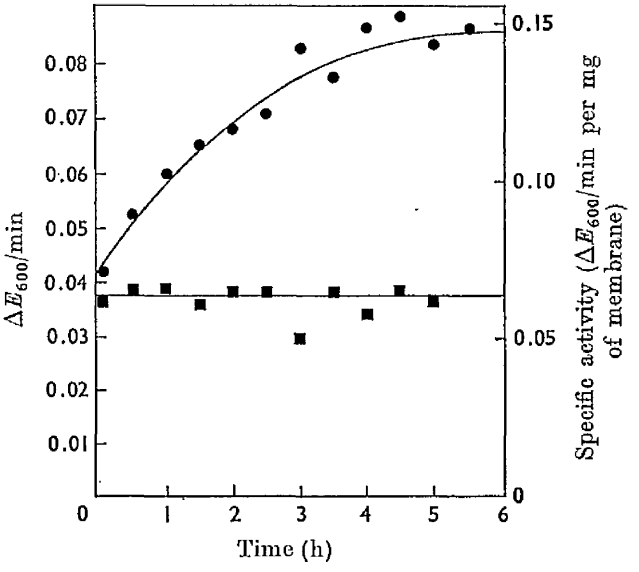
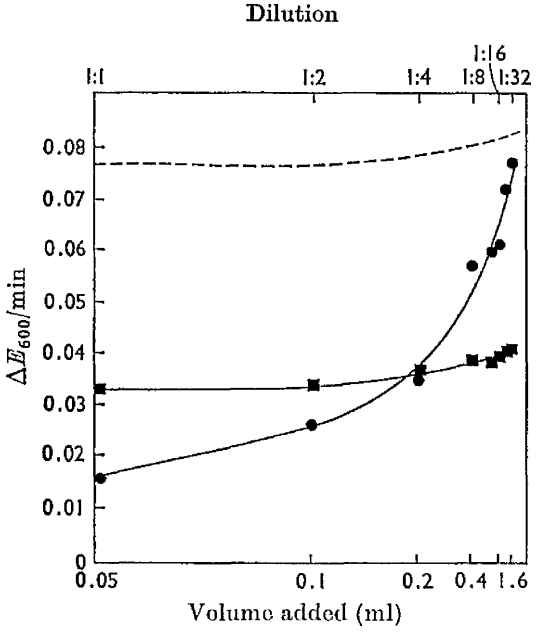


Fig. 6. Time-course of 'activation by dilution'. ●, Change in enzymic activity of a dilute membrane suspension (367 μg dry wt. of membrane/ml) prepared from a 'standard' membrane suspension (11.7 mg dry wt. of membrane/ml) by dilution in tris buffer at 0°C; ■, corresponding plot for the 'standard' membrane suspension All assays were performed at 15°C on the same dry wt. of membrane.

membrane suspensions was found to be dependent on membrane concentration. Over a range of concentration from 15 mg to 200 μg dry wt. of membrane/ml the specific activity was found to increase up to sevenfold although usually a two- to three-fold increase was observed on dilution (see Fig. 5). This 'activation by dilution' appeared to reach an equilibrium approx. 6 h after initiation (see Fig. 6). No change in activity was observed during the 6 h of equilibration in the 'standard' membrane suspensions.

Effect of deoxycholate extraction. Activation of succinate dehydrogenase by dilution (Fig. 5) was not observed in membranes that had previously been extracted with deoxycholate. Nevertheless, the combined activity of both the deoxycholate-extracted residue (38000*g* sediment) and that of the deoxycholate washes (sedimentable at 103000*g*) was equivalent to that observed for 'equilibrated' membrane suspensions (Fig. 7). This effect was observed in deoxycholate residues obtained from both 'standard' membranes and those of membrane suspensions obtained directly from total lysates.

Effect of glutaraldehyde and bovine serum albumin.



g. 7. Effect of dilution on the activity of succinate hydrogenase in 'standard' and deoxycholate-extracted mbrane suspensions. The upper abscissa represents the ution factor of the 'standard' and deoxycholate-tracted membrane suspensions used, and the lower scissa the corresponding volume of that suspension sent in the assay system. The 'standard' (●) and responding deoxycholate-extracted membrane suspen-ns (■) were equilibrated at various dilutions in tris ffer for 12 h at 0°C before assay at 15°C. ----, Total zymic activity present in the deoxycholate-extracted mbrane residues (38000*g* sediment) plus activity in deoxycholate washes (sedimentable at 103000*g*).

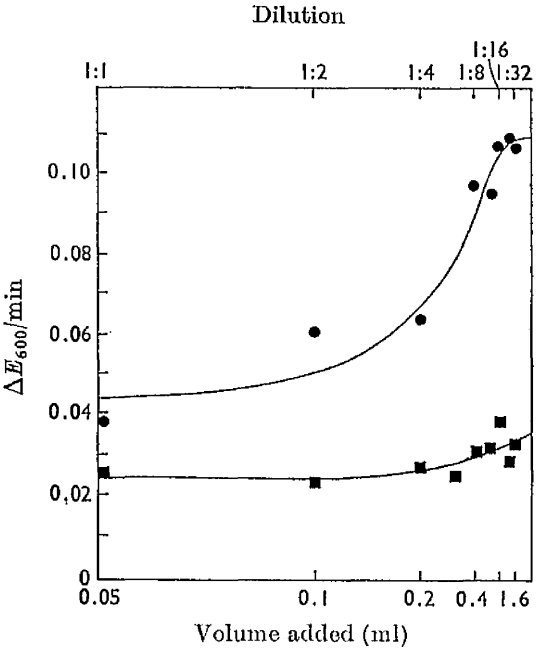


Fig. 8. Effect of dilution on the succinate dehydrogenase activity of 'standard' and glutaraldehyde-fixed membrane suspensions. The upper abscissa represents the dilution factor of the 'standard' and glutaraldehyde-fixed membrane suspensions, and the lower abscissa the corresponding volume of that suspension present in the assay system. The 'standard' (●) and glutaraldehyde-fixed (■) membrane suspensions were equilibrated at various dilutions in tris buffer for 12 h at 0°C before assay at 15°C.

ie loss of 'activation by dilution' after deoxycho-e extraction could also be observed in 'standard' mbranes subjected to mild fixation with glutar-ldehyde (Fig. 8). However, to eliminate virtually the 'activation by dilution', the degree of fixation quired was such that an overall loss of about 30% enzyme activity occurred, when compared with e activity of undiluted (i.e. 'standard') mem-anes.

The addition of bovine serum albumin to the uent buffer also resulted in a depression of the zyme activation normally observed on dilution 'standard' membranes. The extent of depression pended on the concentration of bovine serum oumin and appeared to reach a maximum value of prox. 40% inhibition at a concentration of mg of bovine serum albumin/ml of suspension. ie presence of bovine serum albumin (10 mg/ml) 'equilibrated' membrane suspensions caused an erall depression of enzyme activity when assayed various temperatures between 0 and 50°C (Fig. 4). ie point of inflexion observed to occur at 15°C th 'equilibrated' membranes was no longer ident.

Effect of bivalent cations. The influence of bivalent cations on the specific activity of membrane suspensions was investigated and the results are illustrated in Fig. 9. After dilution of 'standard' membranes in tris buffer containing either Mg^{2+} or Ca^{2+} ions, enzyme activity was observed to increase with time. However, the specific activity in suspensions containing bivalent cations was consistently lower than that of control suspensions. The extent of the cation-mediated depression of activity was dependent on the concentration of added ions and increased as the cation concentration was raised. The results of a similar experiment with deoxycholate-extracted membranes are shown in Fig. 10. Again, the presence of Mg^{2+} and Ca^{2+} ions caused an overall depression of specific activity when compared with control suspensions.

From the results presented in Figs. 9 and 10 it is evident that the presence of Mg^{2+} and Ca^{2+} ions does not influence 'activation by dilution', but does cause an immediate depression of enzyme activity.

DISCUSSION

Our results for *M. lysodeikticus* membrane preparations show that within the range of PMS concentrations examined the reaction velocity is

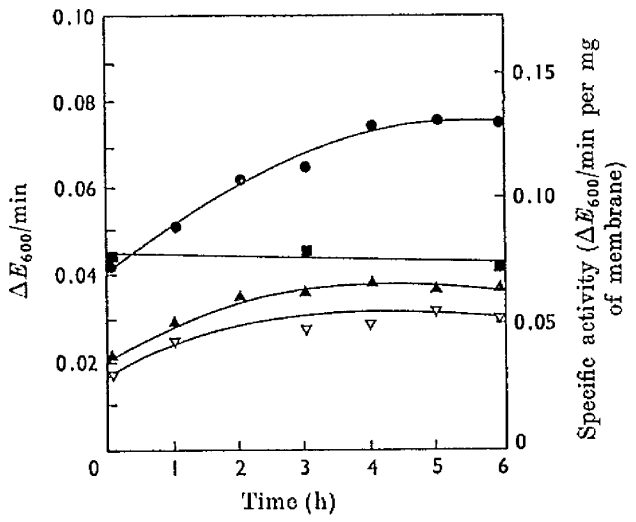


Fig. 9. Effect of Mg^{2+} and Ca^{2+} ions on the activity of succinate dehydrogenase in membrane suspensions. 'Standard' membrane suspensions (11.7 mg dry wt. of membrane/ml) were diluted in tris buffer (●), tris buffer containing 40 mM-$MgCl_2$ (▲) and tris buffer containing 40 mM-$CaCl_2$ (▽) to 366 μg dry wt. of membrane/ml. Enzyme activity was then recorded at intervals over the following 6 h. ■, Activity in undiluted, i.e. 'standard', membrane suspensions. All assays were performed on the same dry wt. of membrane.

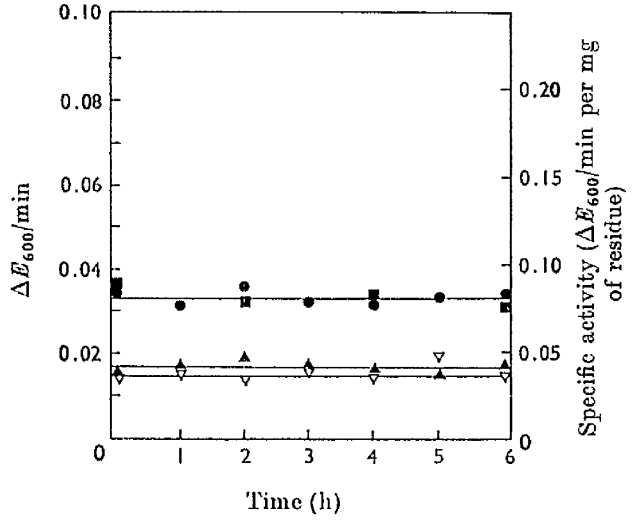


Fig. 10. Effect of Mg^{2+} and Ca^{2+} ions on the activity of succinate dehydrogenase in residues of deoxycholate-extracted membranes. Deoxycholate-extracted membranes (8.18 mg dry wt. of residue/ml) were diluted in tris buffer (●), tris buffer containing 40 mM-$MgCl_2$ (▲) and tris buffer containing 40 mM-$CaCl_2$ (▽) to 256 μg dry wt. of residue/ml. Enzyme activity was recorded at intervals over the following 6 h. ■, Activity in undiluted deoxycholate-extracted membrane suspensions. All assays were performed on the same dry wt. of residue.

directly proportional to the amount of membran added. Therefore, for estimates of comparati enzyme activity in such preparations, the assa system described by Ells (1959) and the modific tion used in this study, both using fixed concentr tions of PMS, are acceptable.

Sensitivity of succinate dehydrogenase from *M lysodeikticus* to temperatures greater than appro 10°C has considerable bearing on the preparatic methods adopted for membrane fractions. Sin relatively rapid loss of enzyme activity occurs room temperature (25°C), procedures involved the production of 'standard' membranes must l carried out at 0°C.

The role of tris buffer in the observed therm inactivation is not known. A diminished rate of i activation does occur with a 100-fold decrease in t molarity of the buffer, but the membrane itself known to undergo changes, resulting in the relea of proteins, as a response to such a decrease in buff molarity (Munoz, Nachbar, Schor & Salton, 196 Munoz, Salton, Ng & Schor, 1969). Tris has be shown to retard substrate activation of succina dehydrogenase in preparations obtained fro higher plants (Hiatt, 1961). Similar inhibito effects of tris may also be operative in the bacteri system described.

The 'activation by dilution' of succinate dehydr genase observed in the present study shows son superficial resemblance to the 'allotopic' enzym found in both bacterial (Munoz *et al.* 1969) a mitochondrial (Racker, 1967; Bruni & Racke 1968; Bulos & Racker, 1968) membrane system An important difference between the 'activation dilution' of bacterial succinate dehydrogenase a that of bacterial adenosine triphosphatase (Mun *et al.* 1969) is that the increase in activity of t latter enzyme was shown to be the result of transition from a 'bound' to a 'soluble' form. such 'soluble' form could be demonstrated in t present study. The activation observed on dilutio could be suppressed under conditions of m fixation or increased soluble protein concentratio Extraction of membranes with deoxychola resulted in the abolition of any 'activation dilution', yet promoted maximal enzyme activit These findings lend support to the possible presen of an 'inhibitor' associated with membrane-bou succinate dehydrogenase (cf. Warringa, Smit Giuditta & Singer, 1958). Dilution of 'standar membrane suspensions may result in the dissoci tion of 'inhibitor' leading to a correspondi activation of the membrane-bound enzyme. D oxycholate extraction may also promote enzy activity by removal of 'inhibitor' together wi other membrane components (*see* Salton *et al.* 196 Conversely, the presence of bovine serum albun or fixation with glutaraldehyde (Bensch & Kin

1961; Sabatini, Bensch & Barrnett, 1963; Bowes & Carter, 1966; Quiocho & Richards, 1966) may prevent the dissociation of inhibitor.

No evidence was found to indicate a role for bivalent cations in the observed activation of succinate dehydrogenase on dilution. Both Mg^{2+} and Ca^{2+} had no specific effect on the dilution phenomenon itself, although both of these cations caused an overall depression of enzyme activity. Since 'activation by dilution' of membranes was still evident in the presence of both Mg^{2+} and Ca^{2+}, it seems improbable that these ions play any role in the proposed association or dissociation of 'inhibitor'. Mg^{2+} or Ca^{2+} ions cause aggregation of *M. lysodeikticus* membranes dispersed by either ultrasound (Salton, 1967) or by detergents (Butler, Smith & Grula, 1967) and are involved in the binding of loosely associated membrane components (Munoz *et al.* 1968). Such mechanisms may account for the observed depression of enzyme activity in the presence of these cations. From the results presented above it is evident that careful consideration must be given to the history of membrane preparations used in comparative studies of membrane bound enzymes.

This investigation was supported by grants from the Medical Research Council. The authors wish to thank the Royal Society for the use of the Pye Unicam spectrophotometer and Dr M. R. J. Salton for help in preparing the manuscript.

REFERENCES

Bensch, K. G. & King, D. W. (1961). *Science, N.Y.*, **133**, 381.

Bowes, J. H. & Carter, C. W. (1966). *Jl R. microsc. Soc.* **85**, 193.

Bruni, A. & Racker, E. (1968). *J. biol. Chem.* **243**, 962.

Bulos, B. & Racker, E. (1968). *J. biol. Chem.* **243**, 3901.

Butler, T. F., Smith, G. L. & Grula, E. A. (1967). *Can. J. Microbiol.* **13**, 1471.

Ells, H. A. (1959). *Archs Biochem. Biophys.* **85**, 561.

Ferrandes, B., Chaix, P. & Ryter, A. (1966). *C. r. hebd. Séanc. Acad. Sci., Paris*, **263**, 1632.

Fitz-James, P. C. (1960). *J. biophys. biochem. Cytol.* **8**, 507.

Fitz-James, P. C. (1967). In *Protides of the Biological Fluids*, vol. 15, p. 289. Ed. by Peeters, H. Amsterdam: Elsevier Publishing Co.

Ghosh, B. K. & Murray, R. G. E. (1969). *J. Bact.* **97**, 426.

Hiatt, A. J. (1961). *Pl. Physiol., Lancaster*, **36**, 552.

Kearney, E. B., (1957). *J. Biol. Chem.* **229**, 363.

Munoz, E., Nachbar, M. S., Schor, M. T. & Salton, M. R. J. (1968). *Biochem. biophys. Res. Commun.* **32**, 539.

Munoz, E., Salton, M. R. J., Ng, M. H. & Schor, M. T. (1969). *Eur. J. Biochem.* **7**, 490.

Quiocho, F. A. & Richards, F. M. (1966). *Biochemistry, Easton*, **5**, 4062.

Racker, E. (1967). *Fedn Proc. Fedn Am. Socs exp. Biol.* **26**, 1335.

Reaveley, D. A. (1968). *Biochem. biophys. Res. Commun.* **30**, 649.

Reaveley, D. A. & Rogers, H. J. (1969). *Biochem. J.* **113**, 67.

Sabatini, D. B., Bensch, K. & Barrnett, R. J. (1963). *J. Cell Biol.* **17**, 19.

Salton, M. R. J. (1967). *Trans. N.Y. Acad. Sci.* **29**, 764.

Salton, M. R. J. & Freer, J. H. (1965). *Biochim. biophys. Acta*, **107**, 531.

Salton, M. R. J., Freer, J. H. & Ellar, D. J. (1968). *Biochem. biophys. Res. Commun.* **33**, 909.

Warringa, M. G. P. J., Smith, O. H., Giuditta, A. & Singer, T. P. (1958). *J. biol. Chem.* **230**, 97,

Succinate dehydrogenase. Properties of the membrane bound enzyme

During an initial study of several membrane fractions isolated from M.lysodeikticus St·O it was evident that fractions, isolated by apparently identical procedures, showed considerable variation in their content of succinate dehydrogenase (EC 1.3.99.1). Since this enzyme is a useful membrane "marker" in this organism and because of the considerable disagreement regarding the localisation of respiratory chain components within the bacterial membrane system (see Introduction and also Table 6), it was important that the factors influencing the activity succinate dehydrogenase in membrane preparations from M.lysodeikticus were fully understood. Consequently, an investigation of these factors was undertaken.

The results of this investigation were presented in part to the 57th General Meeting of the Society for General Microbiology (Owen & Freer, 1970a) and have been published in full (Owen & Freer, 1970b). I do not intend reiterating the arguments presented in these communications (Owen & Freer, 1970a,b. See reprints facing this page) but it is relevant at this point to briefly consider the implications of the results and their possible relationship to other recent findings.

The observation that the expression of membrane bound succinate dehydrogenase activity is influenced by a variety of parameters necessitates careful consideration being given both to the design of fractionation procedures and to the results obtained from

them. It is not valid to compare succinate dehydrogenase activity of membrane fractions a) prepared in the presence of different cations; b) prepared in the presence of differing concentrations of the same cations; c) prepared at, or exposed to, different temperatures; d) exposed to different washing procedures; e) assayed at different concentrations; f) of different "ages". For an accurate assessment of comparative enzyme activity in different membrane preparations it is essential that the different membrane fractions be prepared simultaneously and have essentially the same "history". Enzyme activity should only be monitored on membrane preparations "equilibrated" for at least 6h at $0^{o}C$ at a concentration of approx. 250ug dry wt. membrane /ml (the extinction at 446nm of solubilised membrane suspensions is a convenient method for estimating the concentration of membrane in suspension. See Fig.2).

Biochemical characterisation of the "inhibitor" of succinate dehydrogenase was not attempted. However, it is interesting to note that no component unique to the supernatant fraction, obtained following sedimentation (38 000g) of "equilibrated" membrane suspensions, was observed by polyacrylamide disc gel electrophoresis when compared with supernatant washes obtained during the preparation of "standard" membranes. The "inhibitor" may be a relatively loosely attached surface component of the membrane which can be dissociated during washing and equilibration procedures, but which remains membrane-bound after fixation with glutaraldehyde or in the presence of relative high protein concentrations. In this respect

the "inhibitor" responds to its environment in a similar manner to membrane-bound ATP-ase from M.lysodeikticus. This enzyme can also be released by manipulation of the washing technique (Munoz, Nachbar, Schor & Salton, 1968), a process which is dependent upon protein concentration (Vambutas & Salton, 1970a) and its release can be prevented by prior fixation with glutaraldehyde (Ellar, Munoz & Salton, 1971). Rapid activation of other bacterial enzymes by low concentrations of detergents (Lennarz & Talamo, 1966; Boll, 1969, 1970a,b; de Siervo & Salton, 1971; Patterson & Lennarz, 1971; Pollock et al.,1971) may also involve a similar but more efficient removal of "inhibitors" (Salton, 1971).

Pollock and coworkers (1971) have recently reported on the properties of partially purified succinate dehydrogenase from M.lysodeikticus membranes and have confirmed the reported inhibitory effects of divalent cations (Owen & Freer, 1970a,b). These authors also noted substrate activation of this enzyme (see also Kim & Bragg, 1971) when assayed in the presence of ammonium acetate buffer, a finding which would tend to confirm the suggestion (Owen & Freer, 1970b) that tris buffer inhibits substrate activation (Hiatt, 1961) in membrane preparations.

Partial characterisation of two strains of Micrococcus lysodeikticus NCTC 2665

During the course of this study two different strains of M.lysodeikticus NCTC 2665 have been utilised which differ in several important properties. The two strains could be readily distinguished by the ability of St.O to excrete a red pigment during stationary phase

growth in liquid medium (Fig. 6& 7). St. A had a slightly longer generation time (135min cf. 112min for St.O) as determined by optical density measurements and produced a slightly yellow pigment when cultured on solid medium. Major differences in the properties of "standard" membrane fractions of the two strains were also noted, differences which had important implications in the preparative procedure for membrane fractions from this organism.

Strain differences, initially noted in the yield of membrane (viz.approx. 23% and 13.5% of the dry wt. of cells of St.A and St.O respectively) were later observed in the gross chemical composition of the membrane preparations (Table 7 & 8). It should be noted that the chemical composition appeared virtually unaffected by the age of the culture and it is of interest that differences similar to those observed between membranes from St.A and St.O are also to be found by comparing the chemical composition of membrane fractions from this organism prepared by previous workers (Table 9). Membranes isolated from M.lysodeikticus contain mainly protein, phospholipid and carbohydrate (Tables 9 & 10) and major quantitative differences in the distribution of membrane phosphorus (mainly present as phospholipid - see Table 10) and protein (as estimated by the method of Lowry et al., 1951) have also been noted between the two strains. Although major qualitative differences in the amino acid composition of the two membrane preparations are suggested by the differing ratios obtained from protein values estimated by the methods of a) Gornall et al. (1949) and b) Lowry et al. (1951), they are not reflected in the pattern of polypeptides

observed by SDS-polyacrylamide disc gel electrophoresis (Plates 4 & 5), and during washing procedures (Nachbar & Salton, 1970b) designed to remove selectively components from the cell membrane (Plate 6).

These observed differences in gross chemical composition can be explained if the membranes isolated from St.A contain an additional component(s) accounting for approx. 40% of the dry wt. of the membrane and having the following theoretical composition

% Dry Wt.

Protein		Total		Total
a	b	hexose	Glucose	phosphorus
61	2	10	0.5	0.13

A component(s) with properties very similar to these was isolated during detergent fractionation of "standard" membranes from St.A. Whereas "standard" membranes from St.O showed a 95% reduction in turbidity (E^{1cm}_{620}) after addition of SDS to a final concentration of 0.1%, membrane fractions from St.A showed only a 57% reduction at SDS concentrations of 1% and 79% reduction at concentrations of 10% $^{W}/_{v}$ (Fig.8). The effect of SDS on the turbidity of membrane suspensions from St.A (Fig.8) is consistent with an initial solubilisation and/or dispersion of some membrane components at low concentrations of detergent, followed by a solubilisation and/or dispersion of a component(s) relatively insoluble in aqueous SDS. (The small rise in optical density following the initial drop in turbidity (Fig.8) is a reproducible feature of such

solubilisation/dispersion curves and may represent binding of SDS to the less soluble component(s).) The existence of a detergent-insoluble component in the membranes from St.A was confirmed by centrifugation of SDS-dispersed membrane preparations at 23 000g. At concentrations of SDS approaching 1%, the optically clear 23 000g supernatant fraction contained over 95% of the membrane-bound carotenoid (a useful "marker" molecule for membrane lipid. See Salton & Schmitt, 1967a) yet over 50% $^{w}/_{v}$ of the membrane fraction was sedimented as a white insoluble residue (Fig.9). Similar experiments conducted on membrane fractions from St.O did not indicate the presence of a sedimentable fraction at concentrations of SDS above 0.05%.

The optimum concentration of SDS for efficient preparation of this SDS-insoluble residue, free from carotenoid, appeared to be 1% (see Fig 8 & 9), although two further washes with buffered SDS were required for complete visual removal of residual carotenoid. The resultant white pellet, washed and dialysed free of detergent, gave a gross chemical composition (Table 11) remarkably similar to that of the theoretical additional component proposed for membranes prepared from St.A Experiments conducted in parallel using sodium deoxycholate (or Triton X-100) in place of SDS (Table 11) gave residues of similar composition to those obtained using SDS but visibly contaminated with the red, cytochrome-rich, lipid-depleted membrane residue reported by Salton and co-workers (1968).

Although displaying an infra-red spectrum characteristic of a polypeptide (hydrogen bonded O-H and N-H stretch, Amide 1 and Amide 2 absorption. See Fig.10 and Table 12) this SDS-insoluble, ninhydrin-positive residue showed no absorption at 280nm in the ultra-violet (Fig.11), nor any staining bands after SDS-polyacrylamide gel electrophoresis. The low value for protein (Table 11), when estimated using the Folin-Ciocalteu reagent (Lowry et al., 1951) and the lack of absorption in the ultra-violet (Fig.11) can be explained by the complete absence of aromatic amino acids in this insoluble residue (Table 13 & 14). Indeed only four amino acids viz. glutamic, glycine, alanine and lysine, in molar ratios 1.08; 1.05: 2.02: 1.00 respectively, together with the two amino sugars, muramic acid and glucosamine, were detected by automatic amino acid analysis. Serine in very minor quantities was also detected. (It should be noted that under the test conditions glucosamine and methionine co-elute. Methionine, however, could not be detected by paper chromatography even on overloaded chromatograms - see Fig.12).

The composition of this SDS-insoluble fraction (Tables 11, 13 & 14) suggests a cell wall derivation. The glycan portion of the rigid peptidoglycan of M.lysodeikticus consists of linear strands of alternating β-1-4-linked N-acetylglucosamine and N-acetylmuramic acid residues and the peptide portion of subunits of N^{α}- (L-alanyl-γ (α-D-glutamyl-glycine))-L-lysyl-D-alanine which substitute through their N-termini to the D-lactic acid group of N-acetylmuramic acid (Salton, 1956b; Salton & Ghuysen, 1960; Perkins, 1960; Sharon, Osawa, Flowers & Jealoz, 1966;

Leyh-Bouille, Ghuysen, Tipper & Strominger, 1966; Mirelman & Sharon, 1967). Linkage between peptide subunits can occur through either N^{ϵ}-(D-alanyl)-L-lysine bonds or D-alanyl-L-alanine bonds (Ghuysen, Bricas, Loche & Leyh-Bouille, 1966; Schleifer & Kandler, 1967). This rigid polymer is linked via a phosphodiester bond, emanating at C_6 of one of the N-acetylmuramic acid residues of the glycan fragment (Lui & Gotschlich, 1967; Campbell, Lehy-Bouille & Ghuysen, 1969) to a polysaccharide polymer of glucose and aminohexuronic acid (Perkins, 1963; Campbell et al.,1969). A comparison of the reported chemical composition of isolated cell walls from M.lysodeikticus (Table 15) with the composition found for the SDS-insoluble membrane residue (Table 11, 13 & 14) indicates close quantitative and qualitative similarity. Some differences were, however, noted. In contrast to isolated cell walls from M. lysodeikticus, which contain almost equivalent molar proportions of amino sugars and pentapeptide subunits (Table15), the SDS-insoluble membrane residue contained approx. one molecule of muramic acid per eight pentapeptide subunits (Table 13). The glucose content of the SDS-insoluble residue was also considerably lower although the figure for total hexose appeared similar to reported values for isolated cell walls. The value for total hexose is almost certainly an overestimate as samples containing residual cell-wall material consistently gave slightly turbid reaction mixtures when assayed using the anthrone reagent (Morris, 1948). Residual SDS accounted for approx. 5% of the dry weight of the detergent-insoluble fraction.

It should be noted that the insoluble

residue obtained from deoxycholate treatment of membranes isolated from St.A gave an amino acid pattern similar to that of the SDS-insoluble residue (Table 13) but contained minor amounts of other amino acids, the presence of which can be attributed to the contaminating cytochrome-rich, lipid-depleted membrane residue described by Salton et al., (1968. See also p.84).

A comparison of the molar proportions of amino acids in "standard" membranes from the two strains of M.lysodeikticus (Table 13 & 14) revealed a proportionality factor common to all amino acids, except for those present in the SDS-insoluble residue. Glucosamine and muramic acid were detected in membrane preparations from St.A as were increased concentrations of the pentapeptide amino acids. The results presented in Tables 13 & 14 and Tables 7, 8, & 11 are compatible with the hypothesis that the "standard" membranes from the two strains differ in that preparations obtained from St.A contain an additional component(s), separable by virtue of its insolubility in detergent and derived from the bacterial cell wall.

It is interesting to note that the membrane preparations from M.lysodeikticus isolated by Gilby et al. (1958) and Grula et al. (1967) display a spectrum of amino acids intermediate in molar proportions to those observed for membranes from St.A and St.O (Table 14). This tends to indicate that their preparations also contained components derived from the cell peptidoglycan.

The occurrence of material morphologically similar to cell wall in electron micrographs of thin-

sectioned membrane preparations isolated from St.A (compare Plate 7 & 8 with Plate 9) confirmed the hypothesis that these preparations contained incompletely digested cell wall. In this connection, electron micrographs showing essentially similar features were presented by Salton & Chapman (1962. See their Fig. 4 & 5). These authors acknowledged the presence of "fibrous" material but attributed it partly to oblique sectioning of the membrane. It thus appears probable that the membrane preparations isolated by Salton & Chapman (1962), Gilby et al. (1958), Grula et al. (1967) and also by Salton & Freer (1965. See Table 9) contained residual peptidoglycan components.

It was possible to prepare membrane fractions from St.A essentially free of SDS-insoluble components by manipulation of the lysozyme incubation conditions. Increasing the lysozyme incubation time and decreasing the cell concentration in the incubation mixture were shown to be effective in decreasing the yield of SDS-insoluble membrane residues, with a corresponding decrease in overall membrane yield (Table 16). The changes were paralleled by the expected alterations in the gross composition of membrane (Table 16) and also in their infra-red spectra (Fig.10). Thus for cells obtained from 12h cultures, incubation at a cell concentration of approx. 5mg/ml (10 fold less than that utilised for preparations of "standard" membranes from St.O) with lysozyme (100ug/ml) for approx. 2h (cf. 45min in "standard" procedure) would be required to produce "clean" membrane preparations, essentially free of wall components. The rather low yield

of membrane (8% dry wt. of cell cf. approx. 13% for St.O) obtained on prolonged incubation with lysozyme may be due to partial membrane degradation by autodigestive enzymes. Cells obtained from stationary phase cultures (36h) and digested under similar test conditions gave higher yields of membrane and of SDS-insoluble residue than those obtained from cells of 12h cultures. Even prolonged incubation (135min) with lysozyme at a cell concentration of 5mg/ml gave a membrane fraction containing 15% $^{w}/_{w}$ detergent-insol--uble residue. Wall thickening in cells of St.A during stationary phase of growth (Plate 9) may partly explain this phenomenon. It was found that cell walls of St.A increased in thickness from approx. 50nm during late logarithmic phase of growth (12h. See Fig.6) to approx. 85nm during stationary phase of growth (18h and 36h). The considerable sloughing of cell wall material during the logarithmic phase of growth (Plate 10) may partly explain the thinner wall in 12h cells. In contrast, the cell walls of St.O remained of constant thickness (approx. 45nm) during all stages of growth tested (Plate 9).

Although wall thickness in the two strains of M.lysodeikticus was similar after 12h growth, differences were noted in their lysozyme sensitivity curves (Fig.13). Incubation of cells of both St.A and St.O with lysozyme gave essentially a similar initial decrease in optical density, probably indicating a similar rate of cell disruption. However, the rates of reduction of the final 25% turbidity were different and may suggest a slower rate of digestion of residual peptidoglycan in disrupted cells of St.A. Similar results were also obtained with cells

from 18h and 36h cultures.

Development of a method for the fractionation of mesosomal and peripheral membranes from M.lysodeikticus St.O

In the Introduction to this thesis the importance of protoplast stability in any attempted mesosome fractionation procedure was emphasised. In this section of the Results, I deal with the development of a simple method for the efficient isolation of mesosomal membrane from stable protoplasts of M.lysodeikticus St.O. This strain was chosen for study because of the complications already documented in the preparation of membranes, free from residual peptidoglycan, from the cells of St.A. The values given in this results section are the mean of a single experiment performed in duplicate and are typical of those obtained in other similar experiments.

Protoplast stability. Numerous investigators have reported upon the requirement of divalent cations, in particular Mg^{2+}, for stability of bacterial protoplasts (Reaveley & Rogers, 1969). Table 17 illustrates the effects of different Mg^{2+} concentrations upon protoplast stability. Although there is clearly a requirement for Mg^{2+}, this is apparently satisfied at a level of Mg^{2+} greater than 2.0mM.

Membrane morphology. In all experiments reported, membrane preparations derived from both protoplasts and protoplast supernatant fractions were examined by negative staining in the electron microscope. The characteristic, smooth-surfaced, vesicular structures which are typical of extruded mesosomal membrane (Ryter et al., 1967; Ryter, 1968; Fitz-James, 1968; Ghosh & Murray, 1969; Thorne & Barker,

1969, 1972; Barker & Thorne, 1970; Ferrandes et al., 1970; Frehel et al., 1971a; Patch & Landman, 1971; Popkin et al., 1971; Wildermuth, 1971; Burdett & Rogers, 1972) accounted for all membranous material in protoplast supernatant fractions (Plate 11) except in those prepared below 10mM Mg^{2+} (see Plates 12-21). Particle-studded mesosomes have been observed for M.lysodeikticus (Simakova et al., 1968) and for B.stearothermophilus (Abram, 1965) in total membrane fractions from these organisms. There would, however, appear to be some doubt as to whether these structures represent mesosomes or fragmented peripheral membrane.

The diameter of mesosomal tubules isolated from M.lysodeikticus St.O was approx. 30nm. Their length varied from 50nm to 0.7um and appeared, in part, to be a function of the washing procedure (compare Plate 11 with Plate 22). Thus the extensive washing required to remove cytoplasmic contamination (Salton, 1967d) may cause mechanical fragmentation of mesosomal tubules (see also Burdett & Rogers, 1972). In many cases longer tubules were branched and appeared constricted at intervals along their length (Plates 22 & 23. See also Burdett & Rogers, 1972 for a similar observation). Some mesosomal vesicles had a "doughnut" appearance (see Plates 11 & 14) and may represent different morphological entities or merely products of tubule fragmentation. Structures of similar morphology can also be seen in electron micrographs of negatively stained mesosomal membranes presented by other workers (Ryter et al., 1967; Ryter, 1968; Ferrandes et al., 1970; Frehel et al., 1971a; Patch & Landman, 1971; Thorne & Barker, 1972). A "honeycombed"

membranous structure was observed in some mesosomal membrane fractions, although its frequency of occurrence was low compared with vesicles and tubules (see Plate 24). The origin of this structure remains obscure, although it is similar to mesosomal vesicles in possessing a particle-free surface and also in its staining properties (see also Fig.4C of Patch & Landman, 1971 for a similar feature).

In contrast to structures observed in mesosomal membrane fractions, peripheral membrane appeared as flat, particle-studded sheets (Munoz, Freer, Ellar & Salton, 1968) of variable shape and size, and appeared more permeable to stain than the mesosomal membrane vesicles (Plate 25). The presence of larger particles (Plate 25. See also Ellar, Munoz & Salton, 1970) on the surface of peripheral membrane appear to be dependent upon the presence of Mg^{2+} during the isolation procedure (compare Plate 25 with Plate 26).

A feature of peripheral membrane preparations was the occurrence of large (up to 1um in length), smooth-surfaced structures, similar in their staining properties to mesosomal membrane (see Plate 27). Their occasional bag-shaped morphology (Plate 28) suggests that they may represent the mesosomal sacculus, a structure which would be expected to co-fractionate with the remainder of the peripheral membrane under the preparation conditions used.

Mesosomal membrane vesicles were sometimes observed contaminating peripheral membrane preparations (Plates 25 & 27), although some vesicles may result from disruption of the bag-shaped structures (Plate 29).

Effect of plasmolysis on mesosome release. From Table 18 it can be seen that plasmolysis, prior to wall digestion, significantly increased the release of mesosomal membrane from protoplasts. At a level of 10mM Mg^{2+}, the membrane released from plasmolysed cells was approx. twice that of control cells. In a similar experiment carried out at a concentration of 40mM Mg^{2+}, the membrane release from plasmolysed cells, compared with control cells, was even more marked, although the amount of membrane released was, in fact, less than that at 10mM Mg^{2+}. Although there was an increased E_{260} value in protoplast supernatant fractions derived from plasmolysed cells, the degree of leakage was, nevertheless, extremely small. Indeed, leakage of a similar order occurred in cells which were not subjected to cell-wall digestion (see Table 18).

Effect of temperature, swirling and Mg^{2+} concentration on on release of mesosomal membrane. Ionic shock, involving a drop in Mg^{2+} concentration, has been used previously in mesosome isolation procedures involving M. lysodeikticus (Ellar & Freer, 1969). The influence of ionic shock and temperature on the yield of mesosomal membrane from protoplasts is summarised in Table 19. Membrane release was optimal at 30°C although in experiments involving ionic shock, a slightly higher optimum was observed. However, over the temperature range tested, the maximum difference in membrane yield was only twenty per cent. With ionic shock, there was an increase in membrane yield over control values at all temperatures tested.

The possibility that mild shearing forces

may promote the release of attached mesosomal membrane from protoplasts was investigated. From the results in Table 20, it is clear that swirling of protoplast suspensions under the conditions described had no effect on membrane yield in either suspensions subjected to ionic shock or in their corresponding controls. In instances where the rotation speed was increased to 150 rev./min, the only observable effect was to elevate the E_{260} values of protoplast supernatant fractions. A somewhat similar effect was observed in protoplast suspensions subjected to 50% reduction in Mg^{2+} concentration (see Table 21). At the Mg^{2+} concentrations tested, a sudden 50% reduction in Mg^{2+} concentration did not alter the amount of membrane released when compared with suspensions held at the lower value throughout. However, ionic shock did appear to increase the leakage from protoplasts as evidenced by increased E_{260} values. Sedimentation of protoplasts followed by gentle resuspension in tris buffer containing half the original Mg^{2+} concentration (Ellar & Freer, 1969) yielded protoplast supernatant fractions containing an increased E_{260} absorption (but similar E_{446} absorption) when compared with corresponding fractions obtained by ionic shock treatment alone. It thus appears that mechanical shear is not instrumental in effecting mesosome release from intact protoplasts.

The yield of released membrane increased with decreasing Mg^{2+} concentration in protoplast suspensions (see Table 21 and Fig. 14). Although membrane release increased appreciably below 10mM Mg^{2+}, it appeared that protoplast stability was considerably reduced below this

value (Fig.14). The occurrence of peripheral membrane fragments in negatively stained mesosomal membrane fractions prepared below 10mM Mg^{2+} supports this view (compare Plates 12-17 with Plates 18-21).

Effect of time on mesosomal membrane release. The time course of membrane release was investigated utilising protoplast suspensions prepared from cells plasmolysed in the presence of 10mM Mg^{2+}. Protoplast supernatant fractions from suspensions held for up to 2h after wall digestion all showed less than 3% increase in membrane release over supernatant fractions prepared immediately after wall digestion. However, sedimentation of protoplasts necessitates centrifugation for 2h, and therefore it is not possible to say whether or not release occurs spontaneously with wall digestion.

Sedimentation of mesosomal membrane. Efficient sedimentation of mesosomal membrane from protoplast supernatant fractions required centrifugation for 2h at 280 000g. Under these conditions 96% of the carotenoid absorption (E_{446}) in protoplast supernatant fractions was found to be sedimented. For reasons evident from the foregoing results and discussed more fully later, the preparation procedure outlined in Flow Diagram 2 was utilised for the preparation of mesosomal and corresponding peripheral membrane fractions.

Comparison of the properties of mesosomal membrane fractions prepared at different Mg^{2+} concentrations

Results had showed that pure mesosomal membrane preparations could be obtained from protoplast suspensions provided that the Mg^{2+} concentration was

maintained at or above 10mM. It was necessary, however, to consider the possibility that a population of mesosomal vesicles released at a particular Mg^{2+} concentration may differ functionally , yet be morphologically indistinguishable from that released at a different level of Mg^{2+}. The results of analysis of mesosomal membranes prepared at 10, 20 and 40mM Mg^{2+} are shown in Table 22. The yield of membrane increases with decreasing Mg^{2+} concentration and probably reflects a decrease in the stability of mesosomal membrane attachment to the protoplasts. Similar observations have also been reported for Bacillus spp. by Fitz-James (1967), Ryter et al. (1967) and by Reaveley & Rogers (1969). However, no significant differences were detectedin the protein, carotenoid pigments or total hexose content of the different preparations. A slight increase in the total phosphorus with decreasing Mg^{2+} concentration could be explained by an increased content of phospholipid and/or nucleic acid. The cytochrome content of the different mesosome preparations was quantitatively and qualitatively similar (Fig.15),,cytochrome b_{556} only being detected. By contrast, significant differences in the activity of succinate dehydrogenase (Table 22) and an unspecified autolytic enzyme(s) (Table 23) were detected in the different preparations. Although the activity of succinate dehydrogenase and the autolytic enzyme(s) increased with decreasing Mg^{2+} concentration, it is interesting that, in both cases, the ratio of the specific activity of mesosomal membrane to that of the corresponding peripheral membrane preparation remains practically constant. Thus, the partition of enzyme activity between mesosomal and peripheral membrane is independent of the concentration of Mg^{2+} in the

preparation procedures described. It has been shown previously that the observed activity of succinate dehydrogenase in membrane preparations from M.lysodeikticus is a function of the Mg^{2+} concentration (Owen & Freer, 1970b).

A comparison of the properties of mesosomal and peripheral membrane preparations

It is apparent from the above results that fractions with identical morphology (i.e. fractions free from peripheral membrane) are also very similar in their chemical composition. Major differences in constitution were, however, noted between peripheral and mesosomal membrane fractions.

Chemical analysis. Mesosomal membranes isolated in the presence of 10mM Mg^{2+} accounted for between 7% and 13% ($^{w}/w$) of the total membrane fractions from M.lysodeikticus. The results of chemical analysis and values for selected enzyme activities of both peripheral and mesosomal membranes are given in Tables 24 & 25.

The protein/lipid ratio for peripheral membrane is greater than that of mesosomal membrane, in large part a consequence of a low protein content in the latter. The very low levels of dehydrogenase activities found in mesosomal membranes correlates with the lower protein which is a feature of the mesosomal membrane. Apart from this quantitative difference in protein content between peripheral and mesosomal membranes, there also exists qualitative differences which can be demonstrated by disc gel electrophoresis (see Plate 30). Fifteen of the forty individual polypeptide components which could be

demonstrated for peripheral membrane preparations were either very much reduced in intensity or absent from mesosomal membrane fractions. Only two components showed an enhanced localisation in the mesosomal membrane fraction. Qualitative variation of protein in the two fractions would also account for the different ratios observed when comparing values for protein obtained by both Biuret (Gornall et al., 1949) and the Folin-Ciocalteu reagents (Lowry et al., 1951).

Although mesosomal membranes are lower in carotenoid content, the value for extractable lipid in mesosomal membrane fractions agrees closely with that of peripheral membrane. The figures for total phosphorus, however, suggest appreciable differences in the phospholid components of the two types of membranes. It should be noted, however, that figures for extractable lipid do not necessarily represent total lipid content of membrane fractions. Preliminary studies (Table 26) indicated that only 40%(^{W}/w) and 50%(^{W}/w) of the total phosphorus of peripheral and mesosomal membrane respectively were detected in the extractable lipid fraction. Mild acid hydrolysis of the defatted membrane, to release bound lipid, followed by extractions with ether and acetone/methanol gave two solvent-soluble fractions. The ether-soluble fraction accounted for approx. 10% (^{W}/w) of the membrane and contained less than 1% (^{W}/w) of the membrane phosphorus. The acetone/methanol-soluble, ether-insoluble fraction accounted for approx. 8% (^{W}/w) of the membrane and approx. 40% (^{W}/w) of the membrane phosphorus (Table 26).

Unlike intact membrane preparations, extractable lipid or the ether-insoluble, acetone/methanol-soluble fraction, the ether-soluble fraction displayed no absorption characteristic of O-H stretch, of P=O stretch or of Amide 1 & 2 modes (Fig.16 and Table 12). Indeed, the presence of strong absorption peaks indicative of C-H stretch and bend, of ester carbonyl stretch, of C-O stretch and of CH_2 "rock" are suggestive of an esterified fatty acid. In contrast the acetone/methanol-soluble "bound" lipid fraction showed strong absorption characteristic of P=O stretch, Amide 1 & 2 modes and of O-H and/or N-H stretch. No CH_2 "rock" mode was detected although there was some ester carbonyl absorption (Fig.16 and Table 12). It seems probable that the procedure adopted to release "bound" lipid (Yudkin, 1967) hydrolysed bound phospholipid(s) to give ether-soluble methyl esters of the fatty acids. Glyceric acid phosphates, together with unhydrolysed lipid and components containing peptide residues, constitute the acetone/methanol-soluble, "bound" lipid fraction. Indeed absorption characteristic of the peptide linkage (Amide 1 & 2) was detected in the infra-red absorption spectrum of extractable lipid (Fig.16 compare A & B. See also Sofronova et al., 1971 for a similar observation.).

Mesosomal membrane preparations showed over a fourfold increase in hexose content compared with peripheral membrane (Table 24). In neither preparation was hexose detectable in the extractable lipid but hydrolysis and paper chromatography of whole membrane preparations showed it to consist mainly of mannose plus small amounts of glucose.

Mesosomal membrane preparations possessed an autolytic enzyme activity, which was demonstrated by reduction of optical density of whole cell suspensions and confirmed by observing cell disruption under phase contrast optics. The differences in distribution of autolytic enzyme activity shown in Table 23 correlate with the observations of Ellar (personal communications). Mesosomal membranes showed an increase of approx. fifteenfold in autolytic enzyme activity when compared with that found in peripheral membrane fractions. The contribution of lysozyme to this activity is not known at present. When assayed under identical conditions crystalline lysozyme was shown to have an activity of 96 500 Units/mg. If one assumes no binding to membrane, then the extensive washing procedure adopted would result in a muralytic activity due to residual lysozyme of less than 10^{-3} Units/mg membrane assayed. However, if the autolytic activity is a consequence of exogenous lysozyme, then preferential binding to mesosomal membrane must occur, and the extent of the binding is inversely related to the Mg^{2+} concentration used in the preparative procedure (see Table 23).

Cytochromes. A most striking and interesting difference between the two membrane preparations was found in their cytochrome content. It can be seen that cytochromes a_{601}, b_{560} and c_{550} (Lukoyanova & Taptykova, 1968) clearly present in peripheral membrane (Fig.4) are not detectable in mesosome fractions (Fig.17). Cytochrome b_{556} was the only cytochrome apparent in the latter preparation (Fig.17), although a slight inflection was noted in the region of 600nm.

In peripheral membrane preparations the absorption due to cytochrome b_{556} is masked by absorption peaks of cytochrome b_{560} and cytochrome c_{550} (Fig.4). This problem can be overcome by selective reduction with $NADH_2$ or malate in the presence of detergent (Gel'man et al., 1970). Fig. 18 indicates that addition of substrate ($NADH_2$ or malate) to peripheral membranes which had been clarified by Triton X-100, unlike those clarified by ultrasound, caused visible selective reduction of cytochrome b_{556}. This is followed by reduction of the other cytochromes in the electron transport system. The incomplete reduction of cytochrome b_{560} by substrate confirms a similar observation by Lukoyanova & Taptykova (1968). The kinetics of substrate reduction of cytochromes a_{601}, b_{560} and c_{550} in membrane preparations dispersed with Triton X-100 can be altered by addition of Vitamin K_3 (Fig.19), a compound very similar in structure to the menaquinones reported in membranes isolated from M.lysodeikticus (Bishop & King, 1962; Salton & Schmitt, 1967a). $NADH_2$ and malate also reduced cytochrome b_{556} in mesosomal membrane preparations (Fig.17) although at a much reduced rate. This probably reflects the lower level of the corresponding dehydrogenase activities in mesosomal membrane compared with peripheral membrane (Table 25).

Quantitative determination (Simakova et al., 1969) of cytochromes a_{601}, b_{560} and c_{550} in peripheral membrane (Table 27) gave results in close agreement with those of Lukoyanova & Taptykova (1968. See Table 9). Determination of cytochrome b_{556} in peripheral membrane is, as noted above, inherently difficult. An estimate can be obtained from spectra of selectively reduced membrane

preparations. This method is not totally satisfactory, as the spectra monitored may not represent fully reduced cytochrome b_{556}. However, values for the cytochrome b_{556} content of peripheral membrane varying from 0.11-0.16 umoles/g dry wt. membrane are approx. one third of the estimated values for mesosomal membrane (Table 27).

A similar fractionation of cytochromes was observed after treatment of total membrane preparations with sodium deoxycholate. Salton and co-workers (1968) indicated previously that treatment of total membranes from M.lysodeikticus with detergent gave a lipid-depleted 30 000g residue, which accounted for approx. 15% of the dry weight of the membrane and contained most of the cytochrome and succinate dehydrogenase activity of the membrane. This present investigation showed that consecutive centrifugation of deoxycholate-treated membranes for 28 500g x h, 200 000g x h and 1400 000g x h gave three insoluble residues (see Flow Diagram 1). DO1, DO2 and DO3 accounted for approx. 18%, 17% and 3.5% of the dry weight of the membrane respectively (Table 28). DO1 appeared morphologically similar (see Plate 31) to the smooth-surfaced membranous sheets observed by Salton et al. (1968) and gave a similar cytochrome spectrum (Fig.20). In contrast DO3 contained cytochrome b_{556} only (Fig.20) and appeared in negatively stained preparations as loosely aggregated lengths of membranous material (6.5nm in width, axial ratio approx. 6-8) having little visible substructure (Plate 33). DO2 appeared both from electron micrographs (Plate 32) and from its cytochrome spectrum (Fig.20) to be a mixture of the two components. These differences are also reflected in the

pattern of polypeptides observed after SDS-polyacrylamide disc gel electrophoresis (Plates 34 & 35).

It is interesting to note that Pollock et al. (1971) have since reported on an essentially similar fractionation procedure which gives a deoxycholate-insoluble residue sedimentable at 130 000g and which contains cytochrome b and enhanced succinate dehydrogenase activity. Similar fractionation of total membranes from M. lysodeikticus, after treatment with Triton X-100, was shown to give a fraction not sedimentable at 144 000g and containing cytochrome b_{556} activity (Gel'man et al., 1970). It is tempting to speculate, as have Gel'man et al. (1970), that detergent treatment of membranes from M.lysodeikticus disrupts the electron transport chain, splitting it into two blocks. However, the morphological similarity between DO3 (Plate 33) and deoxycholate extracted mesosomal membrane (Plate 36) together with their similar cytochrome composition (compare Fig.17 and Fig.20. See also Fig.21) may serve to complicate this interpretation.

Ultrastructure of M.lysodeikticus as revealed by thin sectioning

The anatomy of cells of St.O and St.A as revealed from thin sections were found to be basically similar. Thus in this chapter, the results presented for cells from an 18h culture of St.O also pertain to cells of St.A, unless otherwise specified.

Cells (approx.1um in diameter) are bounded by a limiting peripheral (plasma) membrane approx. 9.0nm across and displaying the familiar triple-track staining

properties (see Plate 44). The outer leaflet of the peripheral membrane appears thicker (approx. 4.0nm) and to be more densely stained than the inner leaflet (approx.1.0nm) which is often difficult to see (for example Plate 37). The cell wall has uniform staining properties, the freshly cleaved cell wall displaying a smooth profile compared with the irregular profile of the wall derived from the parent cell (see Plate 44).

In most of the sections examined, only one mesosome per cell was observed. This was usually associated with the developing septum (Plates 37, 38, 40 & 44) although occasionally appeared as a complex invagination of the peripheral membrane (Plate 44 & 45). Under the fixation conditions used, the mesosomes appear as large structures usually with convoluted or lamellar morphology (see Introduction). Other membranous structures of unknown significance were also a feature of many cells. These often appeared as lengths of double membrane (approx. 14.5nm in width) around the periphery of the cytoplasm, and occasionally as rings of double membrane (Plates 37-40 & 45). Similar features have been reported in a Streptomyces sp. (Moore & Chapman, 1959), in Vibrio marinus (Felter, Colwell & Chapman, 1969) and in Streptococcus lactis (Thomas et al., 1969), and may arise from mesosome unfolding (Thomas et al., 1969).

An interesting feature, which may be related to the enhanced autolytic activity of isolated mesosomal membrane (Table 25), was observed in sections of dividing cells of M.lysodeikticus St. O. Plate 37 shows a dividing cell at an early stage of cell septum formation.

The developing septum appears as an invagination of the peripheral membrane and cell wall, and is linked to a mesosome via a narrow membranous channel or "stalk". The transition point from developing septum to "stalk" is often well defined (Plates 37 & 38) and will be referred to as the "neck" of the septum. Bisecting the developing septum is an electron-transparent zone 7.0-9.5nm wide and stretching from the "neck" to the base of the septum. This electron-transparent zone is similar in density to and sometimes appears continuous with a region, 8.5-11.0nm across, between the peripheral cell membrane and the cell wall. This latter electron-transparent region is often crossed with "bridges" apparently connecting the peripheral membrane to the cell wall (see Plates 37 & 39). Both electron-transparent zones terminate at the "neck" of the septum (Plates 37 & 38). Essentially similar features are observed during later stages of septum development (Plates 39 & 40) until, after completion of division, an electron-transparent zone can be observed to bisect completely the fully developed cell septum (Plate 41). At this stage the septum is of similar thickness to that of native cell wall. It should be stressed that the central electron-transparent zone, which is suggested as representing a region of muralytic activity, does not appear to progress beyond the base of developing or developed septa i.e. across the original wall of the parent cell. The initial lesion in cell separation probably involves mechanical rupture of the wall at one, or sometimes both, of these regions (Plate 41), giving it a ragged cleavage profile (see also Plate 44). Cell cleavage then proceeds down the existing central electron-transparent zone (Plates

42 & 43) to give two daughter cells which often remain attached by an unruptured region of cell wall (Plates 44 & 45). Unlike the initial division lesion the newly divided cell septum has a smooth profile, wall thickening in this region occurring after cleavage (see Plates 43 &44).

Some process of septum cleavage is required for cell-separation in any micro-organism. It appears that, in M.lysodeikticus St.O, the septal mesosome is responsible for laying down a muralytic enzyme which effectively bisects the developing cell septum. It should be noted that, in dividing cells of M.lysodeikticus St.A, the electron-transparent zone is ill-defined and often not detectable (see Plate 10).

Comparative study by the freeze-fracture/freeze-etch technique of peripheral and mesosomal membrane from M.lysodeikticus

General Introduction. The freeze-etch technique was first applied to biological material by Steere (1957) and later developed by Moor, Muhlethaler, Waldner & Frey-Wyssling (1961). It has since proved particularly useful in the examination of biological membranes (for reviews see Branton, 1969, 1971) and artificial membranes (e.g. Deamer, Leonard, Tardiew & Branton, 1970; James & Branton, 1971). An essential part of the method is the quick freezing of the specimen in order to prevent damage by ice crystal growth (Moor, 1964). The fracture process breaks the specimen along planes of weak bonding, the etching process subliming away volatile materials, usually water, from between non-volatile components of the sample (for a critical review of

the etching process see Staehelin & Bertaud, 1971). The interpretation of the relation of the surface revealed by the technique to membrane ultrastructure has been controversial (Moor, 1966; Branton, 1969). The investigations of Branton and his colleagues (Branton, 1966; see also Branton, 1969) have demonstrated that the membrane faces revealed after freeze-fracturing represent the topography of the interior of the membrane, the fracture plane occurring in the region of the methyl end groups of fatty acids of the lipid bilayer. According to the original Moor-Muhlethaler hypothesis, the faces revealed by freeze-etching represent true outer* surfaces of membranes (Muhlethaler, Moor & Szarkowsky, 1965; Weinstein, 1969) i.e. that two potential cleavage planes exist; one along the outer* surface of the membrane and a second along its inner* surface. Recent evidence from studies of artificial membranes (Deamer & Branton, 1967) and from the use of the double-replica-freeze-cleave technique on chloroplasts (Wehrli, Muhlethaler & Moor, 1970), red cell membranes (Weinstein, Clowes & McNutt, 1970), liver cell membranes (Chalcroft & Bullivant, 1970) and bacteria (Nanninga, 1971a; Van Gool & Nanninga, 1971; Sleytr, 1970a; Veltri & McClear, 1971, 1972) has suggested a unique cleavage plane for freeze-fractured membranes. The elegant investigations of Nanninga (1971b) and later of Hereward & Northcote (1972)

---

* The terms inner and outer are made in respect to the centre of the cell.

have also indicated that membranes of B.subtilis (Nanninga, 1971b) and of the tonoplast (Hereward & Northcote, 1972) fracture along an internal cleavage plane and substantiate the theory of Branton (1969).

The object of this part of the investigation was to compare the topography of fracture faces of isolated peripheral and mesosomal membranes and to determine whether differences observed in the chemical properties and negatively stained preparations of the two membrane fractions were reflected in differing internal architectures. Comparison of fracture faces of isolated membrane preparations with fracture faces of corresponding membranes in situ would also assist in the assessment of any internal rearrangement of membrane components during the isolation procedure.

The results given in this section pertain to both M.lysodeikticus St.A and St.O.

Freeze-fractured whole cells. Plates 46-51 show replicas of freeze-fractured unetched whole cells of M.lysodeikticus St.A and St.O. (Although the term unetched is used throughout this report, a more correct description would be minimally etched. If a cutting or fracture stroke causes an irregular fracture through the frozen specimen, then it is possible that consecutive strokes of the cutting blade will not produce a fresh fracture through some areas of the surface. This process can result in unintentional etching of fracture surfaces, a phenomenon which is sometimes manifested by changes in the structure of the background ice table. See Staehelin & Bertaud, 1971). Clearly

evident in the cross fractured cell wall (Plates 46 &47) and in the developed septum (Plate 50) are intrawall particles approx. 9.0nm in diameter. Particles of similar dimensions can also be seen in the cytoplasm (Plates 46 & 47). No nuclear material can be defined, contrasting with the observation of Remsen (1968) for B.subtilis. A characteristic feature of cells is the presence of vesicles within the cytoplasm and often situated around the cell periphery (Plates 46-50). Both convex (Plates 48-50) and concave (Plates 46 & 47) fractures of vesicles reveal smooth faces devoid of particles. Only very occasionally are the vesicles cross-fractured (Plate 51).

Plates 52 & 53 show concave fractures of peripheral membrane in situ and reveal the inner surface of the outer half of the membrane. Characteristic features of such fractures are linear depressions (approx. 20nm x 4.0nm) and few intramembrane particles approx. 9-10nm in diameter. Convex fractures of peripheral membrane in situ (Plates 54-56) reveal the outer surface of the inner half of the membrane. Many intramembrane particles 9-10nm in diameter are evident together with numerous rod-shaped structures distributed over the surface. The rod-shaped structures often show clear continuity between the interior of the membrane and cell wall and also the cell septum (Plates 55 & 56). These structures are approx. 6nm across and vary in length from 20-70nm and may possibly be complementary features to the rod-shaped depressions observed on concave fractures. Similar structures have also been (or can be) observed in electron micrographs of replicas of some freeze-fractured bacteria presented by other workers

(Table 29) and also for other micro-organisms (Staehelin, 1968a; Streiblova, 1968). They do not, however, appear to be a common feature of all bacteria so examined (Table 29).

Vesicles, often observed in cross fractured cells (Plates 46-50), can occasionally be revealed where the fracture plane through the membrane suddenly departs into the cytoplasm. Plates 57 & 58 show smooth-surfaced vesicles revealed in this manner. Plates 59 & 60 serve to summarise the features of freeze-fractured whole cells so far encountered.

Freeze-fractured "standard" membranes. Replicas of freeze-fractured, unetched "standard" membranes isolated from M.lysodeikticus St.O and St.A and subsequently suspended in glycerol are shown in Plates 61-66. The distribution and dimensions of intramembrane particles, rod-shaped structures and linear depressions on fracture faces are identical with those observed in corresponding fracture faces in situ. The membrane fragments usually appear round and are often observed as a number of concentric vesicles (Plates 64 & 65). These latter structures appear to be artifacts induced by the antifreeze agent, glycerol (Moor, 1971) and can be avoided by its omission (cf. Plates 74-77).

Two different concave and two different convex fractures are theoretically possible for isolated membranes. This contrasts with the situation in the whole cell where the number of different concave and convex fractures of peripheral membrane (i.e. one only of each) is limited by the geometry of the cell. In isolated membrane both concave and convex fracture faces can reveal

either the inner surface of the outer half of the membrane or the outer surface of the inner half of the membrane, depending on the orientation of the membrane. In the special case of an isolated membrane vesicle, the occurrence of the four fracture faces described would indicate the presence of both rightside-in and inside-out vesicles (Steck, Weinstein, Straus & Wallach, 1970). The features described are illustrated in Plate 66.

The topography of fracture faces does not appear to be altered in whole cells undergoing obvious autolysis (Plate 67).

Freeze-fractured and etched protoplasts. Observation of the outer surface of the membranes may be achieved by the etching process, which sublimes volatile components from the fractured surface. In specimens suspended in water or dilute buffer this effectively lowers the ice table surrounding the specimen (Davy & Branton, 1970; Branton, 1971). However, to see the outer surface of the outer half of the peripheral membrane of the intact bacterial cell necessitates the prior removal of the cell wall and consequent stabilisation of the protoplast/spheroplast. The most effective way of achieving this stabilisation is with fixatives such as osmium tetroxide or glutaraldehyde. The topography of membrane fracture faces of B.subtilis have been shown to be unchanged in cells previously treated with osmium tetroxide (Nanninga, 1968; 1969) a fixative which appears to stabilise mesosome structure (Nanninga, 1971a). Glutaraldehyde has also been used to stabilise yeast protoplasts during the freeze-fracture process and causes no significant alteration of membrane topography (Necas, Kopecka

& Brickta, 1969). Both fixatives, however, have been shown to reduce the overall number of membrane fracture faces (Necas et al., 1969; James & Branton, 1971). During this study glutaraldehyde was used to stabilise protoplasts of M.lysodeikticus (derived from a 18h culture) in dilute buffer.

Plates 68-73 show replicas of freeze-fractured and etched fixed protoplasts of M.lysodeikticus St.O. Convex fracture faces of the protoplast peripheral membrane (Plates 68 & 69) reveal a similar dense distribution of intramembrane particles to that observed in corresponding fractures in whole cells. The rod-shaped structures are also apparent on this fracture face and often connect with the etched surface (Plates 68 & 69). The outer surface of the outer half of the protoplast peripheral membrane often appears relatively smooth but at low shadowing angles a rougher topography can often be observed (Plates 69 & 73). These protuberances, or raised ridges, may arise from the accommodation of intramembrane particles by this surface. Indeed rows of protuberances, reported on the etched surface of flagellated epithelial cells of the mollusc Cominella maculosa, have been attributed to the accommodation of intramembrane particles (Flower, 1971). Concave fractures of protoplast peripheral membrane were not observed, making it impossible to see the inner surfaces of either the inner or outer half of the membrane.

A notable feature of some freeze-fractured and etched fixed protoplasts was the occurrence of areas on the outer surface of the inner half of the membrane, sparsely covered with intramembrane particles (Plates 70 & 71. See also Table 29). These areas, which may be quite extensive

(Plate 71), clearly merge with areas possessing a normal distribution of particles, the transition often being quite marked (Plate 70). A noticeable feature of these "bare" areas is the absence of any rod-shaped structures either on the fracture surface or connecting with the etched surface (Plates 69 & 70). In direct contrast, areas of the same fractured membrane surface, densely covered with intramembrane particles, reveal the presence of these rod structures, both on the fracture face and connecting with the outer surface of the membrane (see Plate 69).

No structures resembling the vesicles observed in replicas of freeze-fractured whole cells are evident for freeze-fractured and etched protoplasts. Occasionally fractures through the peripheral membrane reveal a "cratered" fracture face topography below (Plate 70). An almost identical feature, observed in replicas of freeze-fractured whole cells of B.polymyxa (Holt & Leadbetter, 1969), has been attributed to a mesosome structure. This interpretation appears unlikely, at least for M.lysodeikticus protoplasts, as mesosomal vesicles would be expected to be extruded upon wall digestion.

Another feature which was observed very occasionally on the etched outer surface of protoplasts was a line of small depressions, apparently revealing the outer surface of the inner half of the membrane, although none were large enough to reveal the characteristic features of that face (Plates 72 & 73). The line of these depressions sometimes parallels the edge of the etched face (Plate 72), whereas at other times appears as an extension of the fracture edge (Plate 73).

Freeze-fractured and etched isolated "standard" membranes. Plates 74-77 show replicas of freeze-fractured and etched "standard" membrane preparations from M.lysodeikticus St.O and St.A. The features revealed on fracture faces and etched surfaces are identical to those already described for fixed protoplasts. It should be noted that concave fractures of peripheral membrane were seldom recognised.

The distribution of intramembrane particles and rod-shaped structures on membrane fracture faces also appeared unaffected by prior treatment of the membrane with ionic shock (Plates 78 & 79) and with EDTA (Plates 80 - 83). Both treatments are known to remove relatively loosely associated membrane components (Nachbar & Salton,1970b) and were shown to yield smooth-surfaced membrane residues when examined by the negative staining procedure (Plates 84 & 85).

Freeze-fractured isolated mesosomal membrane. Plates 86-89 show replicas of freeze-fractured, six times washed mesosomal membrane isolated from M.lysodeikticus St.O. Freeze-fractured mesosomal membrane appears as a uniform collection of spherical vesicles approx. 70nm in diameter. The inner surface of the outer half of the mesosomal vesicle membrane (concave fracture face) and the outer surface of the inner half of the mesosomal vesicle membrane (convex fracture face) reveal a distribution of intramembrane particles essentially similar to that observed on corresponding fracture faces in peripheral membrane. Unlike freeze-fractured peripheral membrane, however, fracture faces of isolated mesosomal membrane do not show either rod-shaped structures on convex fractures or linear depressions on concave fractures. Indeed

only ONE vesicle in the many hundred of fields examined showed rod-shaped structures on the convex fracture face (Plate 88). It appears probable that this particular vesicle is contaminating peripheral membrane.

No inside-out vesicles were seen although larger vesicular structures (between 0.1-0.15um in diameter), revealing smooth-surfaced convex and concave fracture faces, were occasionally found (Plates 88 & 89). These structures bear striking resemblance to the vesicles in freeze-fractured whole cells (cf. Plates 46-50).

Freeze-fractured and etched mesosomal membrane. Plates 90-95 show replicas of freeze-fractured and etched, six times washed, mesosomal membrane isolated from M.lysodeikticus St.O (see Flow Diagram 2). The specimen appears to have a gross morphology compatible with the collection of vesicles and tubules observed in negatively stained preparations (compare with Plates 11-17). The fracture faces of small spherical vesicles, however, appear less complex than those of the larger tubular structures (Plate 90). I shall, therefore, deal with these two entities separately.

The fracture face revealed in replicas of freeze-fractured and etched small vesicles is usually convex. It exhibits a population of intramembrane particles very similar to that observed on corresponding fracture faces of isolated peripheral membrane and also of mesosomal membrane after suspension in glycerol (Plates 90-92). In common with the latter, no rod-shaped structures were evident on the convex fracture face. Concave fractures of small vesicles are seen infrequently but appear relatively smooth-

surfaced with only the occasional intramembrane particle visible (Plate 90). The outer surface of the outer half of the mesosomal vesicle membrane, revealed by etching, exhibits a relatively smooth topography (Plate 91) similar to that shown by freeze-fractured and etched peripheral membrane. Some, although not all of the vesicles, however, exhibit a cross-fracture surrounding the etched surface (Plate 90). The significance of this cross-fracture will become apparent later.

In common with the etched surface of the small vesicles, the outer surface of the outer half of the tubular membrane appears smooth (Plates 91, 93 & 94) and devoid of surface particles. The small radius of curvature of both vesicles and tubules does not allow adequate areas of the etched surface to be viewed at low shadowing angles. Hence, the apparent absence of protuberances on these surfaces may not be a true presentation of the surface topography. The fracture revealed in the mesosomal tubules is very complex (Plates 90-96). The tubular membrane itself invariably appears to be cross fractured (Plates 91-96) and the fracture through the tubule contents to be very undulating (See, however, Plate 96). A noticeable feature of these latter fractures are convex regions carrying a distribution of particles very similar to that observed on convex fractures of small vesicles (see Plates93-96). Indeed, in several micrographs, fractures give the impression of small vesicles within the mesosomal tubule (Plates 94-96). Another feature of fractured tubule contents is illustrated on Plate 91. Several relatively smooth-surfaced depressions are seen along the length of the

tubule contents. These concave areas often combine with convex, particle-covered, regions to give a very undulating surface (Plates 93 & 95). Occasionally, smooth-surfaced convex areas are also revealed (Plate 95). Between these various intratubular regions is interspersed an area similar in topography to that of the ice table (Plates 91 & 95).

These features are compatible with the hypothesis that the mesosomal tubules themselves contain membranous vesicles, some of which inevitably leave the confines of the tubule during the isolation procedure. Accordingly, the convex ,particle-studded region observed within fractured tubules corresponds to the outer surface of the inner half of the intratubular vesicle membrane and the smooth depressions to either the inner surface of the outer half of the intratubular vesicle membrane or to the inner surface of the inner half of the tubular membrane revealed by etching. The presence of cross-fractures in the immediate vicinity of these depressions rules out the possibility that they represent the inner surface of the outer half of the tubular membrane (Plate 91). Indeed the close similarity in diameter of the depressions and of free spherical vesicles suggests that they represent the concave fracture face of intratubular vesicle membrane.

Freeze-fractured deoxycholate insoluble membrane residue. Plate 97 shows part of a replica of freeze-fractured membrane residue (DOl - see Flow Diagram 1). No obvious fracture faces were seen. Indeed, the only definite structures were striations which may represent cross-fractured membrane residue. They may also be artifacts caused by the knife

blade although their frequent bent (Plate 97) appearance makes this unlikely. The absence of membrane fracture faces for specimen devoid of lipid (Salton et al., 1968) is compatible with the mechanism of membrane freeze-fracture postulated by Branton (1966, 1969).

D I S C U S S I O N.

Two strains of M.lysodeikticus NCTC 2665

M.lysodeikticus St.A and M.lysodeikticus St.O differ in that membranes isolated from the former contain an additional component which can be isolated by its insolubility in aqueous detergent (SDS). From its chemical composition there seems little doubt that this additional component is derived from the cell wall.

It does not appear likely that the presence of residual peptidoglycan can be attributed solely to incomplete digestion of a thickened cell wall of basically similar structure to that of St.O. The observation that cells from exponentially growing cultures of St.A possess walls of similar thickness to those of St.O and yet "standard" membrane preparations from the former contain residual cell wall material (Table 13) suggests that the walls of the two strains are structurally dissimilar. Alternatively, cells of St.A may produce a substance which is antagonistic towards lysozyme. Although it is evident that almost complete removal of cell wall material from membranes isolated from cells of St.A harvested during early stationary phase (12h) can be achieved by manipulation of the incubation conditions, the same is not true for membranes isolated from cells of St.A after 36h of growth. This phenomenon may be attributed in part to either cell wall thickening, wall "maturation" or decreased autolytic activity, or a combination of these factors.

No attempt was made to determine the basis of the partial lysozyme resistance of the cell wall from St.A. Two features, however, are worthy of note in this

respect. Firstly, dividing cells of St.A did not possess the distinct electron-transparent zone observed bisecting developing septa of cells of St.O. This may indicate a reduced complement of autolytic enzyme for St.A, a feature which may also contribute to the thicker walls of this strain during stationary phase. Secondly, the isolated cell-wall residue (SDS-insoluble residue), although indicating an amino acid molar ratio (Table 13) typical of wall isolated from whole cells (Table 15), showed differing molar proportions of amino sugars. It might be expected that in cell-wall residues partially digested with lysozyme the molar ratio of either of the amino sugars to pentapeptide subunit would be less than one. The extensive cross linking of peptides in the wall requires that many of the N-acetyl muramic acid residues remain unsubstituted and that enzymic removal of lengths of glycan unsubstituted by pentapeptide is possible. However, it is difficult to explain why there exists almost three times the molar quantities of glucosamine to that of muramic acid. Recent reports on the composition of cell walls isolated from M.lysodeikticus show slightly higher values for glucosamine content than for muramic acid, differences which remain largely unexplained. A molar ratio of glucosamine/muramic acid of up to two is theoretically possible for cell wall partially digested with both lysozyme and an endo-N-acetylglucosaminidase, a possible autolytic enzyme. However, it would seem inappropriate to speculate further until confirmation of the observed experimental results is obtained.

Whatever the basis of the partial lysozyme resistance of St.A, whether it be due to inactivation of

lysozyme or to steric hindrance (Brumfitt, Wardlaw & Park, 1958; Brumfitt, 1959; Prasad & Litwack, 1965) its effect is to yield isolated membrane preparations contaminated with material which is very probably partially digested peptidoglycan. Since both St.A and St.O were obtained originally from the same source it is difficult to determine which, if either, represents the original parent strain. It is apparent, however, from this study, and also from the survey presented (Table 10), that precautions must be taken to ensure that membrane fractions prepared from this organism are free of residual peptidoglycan.

The differences observed in the sensitivities of the walls of the two strains to lysozyme were not reflected in the topography of membrane fracture faces revealed by the freeze-fracture technique. Identical features were observed on fracture faces of isolated peripheral membrane and peripheral membrane in situ. Indeed, only minor differences were detected in the pattern of polypeptides displayed by the different membrane fractions and also by their corresponding membrane-wash supernatant fractions.

The amino acid analysis of the membrane fractions of both organisms also appear very similar. They display a wide range of acidic, basic and hydrophobic amino acids. Cysteine, however, is present only in trace amounts (in agreement with the observations of Grula et al., 1967), a finding which rules out disulphide bonding as a major cohesive force for proteins in the basal membrane continuum. This is inconsistent with the observations of Estrugo, Larraga, Corrales, Duch & Munoz (1972), that membranes from

M.lysodeikticus contain approximately 45moles of SH group per 10^5g of reduced protein,compared with a value of 0.87 calculated for membrane proteins from St.O.

Isolation of mesosomal membrane

As outlined in the Introduction, a productive approach to the problem of mesosome function would appear to be the isolation of this intracytoplasmic organelle followed by its characterisation. Such a disruptive method requires that, during manipulation, membranes be placed in a somewhat artificial environment. Thus, as with all studies on fractionated biological membranes, it may not be valid to compare the isolated product with the native membrane in situ. However, the extensive investigations of Salton and his colleagues (1972) have been instrumental in establishing a suitable method for the examination of isolated membranes from M.lysodeikticus.

Fractionation of the "mesosome" from the remainder of the cell would appear to be a difficult task, as it entails separation of the mesosomal sacculus (as well as its contents) from the rest of the peripheral membrane. Such a process would probably necessitate separation of different membrane fractions from total cell lysates. From previous studies on the isolation of total membrane fractions from M.lysodeikticus (Salton & Chapman, 1962) it is apparent that extensive fragmentation and vesicularisation occurred. It is mainly due to this fact that no satisfactory method is available at present for the isolation of "total" mesosome fractions.

The fractionation of the contents of the mesosome is possible and has been achieved in a number of

instances (see Table 4). Such fractions do not, however, help to clarify the role played by the mesosomal sacculus, since properties associated with the mesosome contents are not *per se* those of the membrane invagination itself. Bearing these problems in mind, I have devised a simple method for the efficient fractionation of mesosome contents (mesosomal membrane) free from contaminating peripheral membrane.

In order to minimise contamination of mesosomal membrane with peripheral membrane fragments, a method of isolation retaining protoplast stability was adopted. Several authors have recognised this requirement although only Rogers and coworkers (Rogers *et al.*, 1967; Reaveley & Rogers, 1969) studying *B.licheniformis*, have attempted to define in any detail the conditions under which concomitant mesosome release and protoplast stability are achieved. The release of mesosomal membrane from *M.lysodeikticus* was greatly enhanced if cells were plasmolysed prior to wall digestion, although protoplasts from cells treated in this way required a higher Mg^{2+} concentration for stability than unplasmolysed cells (compare Table 17 with Fig.14). Plasmolysis has been shown to cause extrusion and release of mesosomal membrane into the periplasmic space (Van Iterson, 1961; Fitz-James, 1964; Ferrandes *et al.*, 1966; Frehel *et al.*, 1971a) a process which probably involves breaks in the membrane, the resealing of which is facilitated by Mg^{2+} ions. Indeed Mg^{2+} ions have been shown to cause reaggregation of membrane fractions isolated from *M.lysodeikticus* and previously solubilised with SDS (Butler *et al.*, 1967).

Several authors have reported that the most efficient release of mesosomal membrane from parent protoplast occurs at Mg^{2+} concentrations below 10mM (Ryter et al., 1967; Van Iterson & Op den Kamp, 1969; Popkin et al., 1971; Thorne & Barker, 1971; 1972). This present study showed that release of mesosomal membrane was inversely related to the Mg^{2+} concentration, a phenomenon probably reflecting the degree of adherence of extruded mesosomal membrane to the protoplast surface (Rogers et al., 1967). Since the stability of protoplasts is impaired by lowering the divalent cation concentration in the suspending fluid, the choice of Mg^{2+} concentration during the protoplast stage is critical.

As noted previously (Fitz-James, 1968; Reaveley, 1968; Ghosh & Murray, 1969; Ferrandes et al.,1970; Patch & Landman, 1971; Thorne & Barker, 1972) characteristic differences were observed in the appearance of negatively stained preparations of mesosomal and of peripheral membrane. These consistent anatomical differences provided a convenient means of monitoring for cross contamination in different membrane fractions. By experiment the optimum concentration of Mg^{2+} for concomitant mesosome release and protoplast stability was found to be 10mM. It is perhaps not surprising that this critical value differs for different organisms. Rogers and coworkers found an optimum concentration of 20mM for B.licheniformis (Rogers et al.,1967).

The observation that mild shearing forces do not effect mesosomal membrane release from protoplasts, but only serve to increase protoplast leakage, necessitates

fairly gentle handling procedures for protoplast suspensions. The method of Ellar & Freer (1969), which entails resuspension of pelleted protoplasts, should therefore be modified to eliminate this step. Similarily, a 50% reduction in Mg^{2+} concentration (Ellar & Freer, 1969) can be replaced by a procedure in which protoplast suspensions are held at the lower level of Mg^{2+} throughout isolation. Of the parameters tested for their effect on the amount of membrane released from protoplasts, only variations in Mg^{2+} ion concentration greatly influenced yield. Temperature affected mesosomal membrane release only slightly, the optimum temperature appearing to be between 30°C and 35°C. The method outlined in Flow Diagram 2 thus appears to give a simple scheme for the preparation of mesosomal membrane free from detectable contamination with peripheral membrane. It is apparent that mesosomal membrane is not quantitatively released from protoplasts under these conditions since occasional mesosomal membrane vesicles can be detected in peripheral membrane preparations (see also Fig.14).

Mesosomal membrane fractions free from peripheral membrane are necessary before they can be chemically characterised and differentiated from peripheral membrane by virtue of any exclusively localised components (if indeed any exist). The presence of a mesosomal membrane "marker" molecules and their successful isolation would provide a means of labelling, via ferritin-conjugated specific antibody, the origin of the vesicles in whole cells. Evidence for a mesosomal origin of the vesicle fractions isolated as described in Flow Diagram 2 is overwhelming even

if indirect. Many workers (see Introduction) have shown from electron-microscopic evidence that plasmolysis of bacterial cells is accompanied by a simultaneous loss of internal membranous structures and the appearance in the periplasmic space of membranous tubules and vesicles similar to mesosome contents *in situ*. Fractionation of morphologically similar structures after cell-wall digestion (Ferrandes *et al.*, 1966; Ryter *et al.*, 1967; Ghosh & Murray, 1969; Frehel *et al.*, 1971a; Popkin *et al.*, 1971; Burdett & Rogers, 1972; Thorne & Barker, 1972) is confirmatory evidence that they do in fact represent the mesosome contents.

The collection of vesicles, tubules and tubulovesicles obtained from *M.lysodeikticus* are strikingly similar in morphology to mesosomal membrane fractions obtained from other bacteria (see Table 6 for references). The observed viability of plasmolysed cells and protoplasts of *B.subitilis* (Ryter & Landman, 1964) suggests that little mechanical damage to either the cell or its extruded contents occurs during the initial part of the fractionation procedures such as those outlined in Flow Diagram 2. The slight fragmentation of mesosomal tubules observed during washing is probably an inevitable consequence of this important procedure and may be a response to washing in buffer free from Mg^{2+}. It is unlikely that alteration of the Mg^{2+} concentration is the sole cause of this effect, as Lastras & Munoz (1971) showed that Mg^{2+} associated with membranes of *M.lysodeikticus* was retained after washing in buffer free from this cation. Hence the presence of Mg^{2+} in the protoplasting medium may be sufficient to stabilise mesosomal

S U M M A R Y.

The role of the mesosome in M.lysodeikticus has been studied using both biochemical and electron-microscopic techniques. During the developement of membrane fractionation procedures, unexpected variations in the specific activity of succinate dehydrogenase (EC 1.3.99.1) were observed in similar membrane preparations. Since this enzyme is an important membrane "marker", a detailed study of the factors affecting its activity was considered an essential preliminary investigation.

The membrane-bound enzyme was inactivated at temperatures above 10°C and its specific activity found to increase between two- and three-fold in diluted membrane preparations equilibrated at 0°C for 6h. Membranes treated with sodium deoxycholate showed no activation by dilution but displayed maximal activity. The increase in specific activity observed on dilution could be partially inhibited by fixation with glutaraldehyde, or by the presence of bovine serum albumin, whereas divalent cations caused an overal depression of membrane-bound succinate dehydrogenase activity. The significance of these observations are discussed in relatic to the developement of a reproducible method of assay.

Two strains of M.lysodeikticus N.C.T.C. 2665 have been detected and partially characterised. A strain which gave no detectable contamination of membrane preparations with residual wall peptidoglycan was chosen for a study of the properties of isolated mesosomal membrane.

By precise manipulation of the conditions prior to and during protoplast formation, it was possible

to obtain highly purified preparations of mesosomal membrane. Plasmolysis of cells, before wall digestion was necessary for effective mesosome release. The effects of mild shearing forces, divalent cation concentration, temperature and time upon the release of mesosomal membrane from protoplasts were also investigsted. The optimal yield of mesosomal membrane from stable protoplasts was achieved at 10mM Mg^{2+}, mesosomal membrane fractions prepared at differing Mg^{2+} above 10mM being similar in chemical composition. There was no evidence from freeze-fracturing and etching that structural rearrangement or autodigestion occurred during the adopted fractionation procedure.

Evidence from freeze-fracturing and etching indicated that the mesosome was a metabolically important organelle, the mesosomal sacculus possibly representing a region of localised insertion of some membrane components. Both biochemical and electron-microscopic studies suggested that the mesosome was a region of localised autolytic activity and may thus play a role in wall morphogenesis and in cell-separation. A four- to five-fold increase in the mannose content of mesosomal membrane compared with peripheral membran may also suggest a role for the mesosome in polymannan biosynthesis.

Isolated mesosomal membrane contained cytochrome b_{556} only, whereas isolated peripheral membrane was shown to possess a full complement of cytochromes. The complement of succinate, $NADH_2$ and malate dehydrogenases in mesosomal membrane was also much reduced compared with peripheral membrane. These results serve to elimate the

mesosome as the centre of respiratory activity in this organism.

Evidence from freeze-fracturing and etching and also from negative staining have suggested the presence of membranous vesicles within mesosomal tubules. These results are discussed in relation to membrane growth and to transport and secretion of extracellular products.

An additional investigation into the multiple forms displayed by staphylococcal α-toxin was also undertaken, and a report of this study is included.

vesicles during subsequent washing procedures. (The instability of staphylococcal mesosome membrane prepared in the absence of Mg^{2+} supports this suggestion (Popkin et al., 1971)) It seems probable that fragmentation is, to a large extent, a function of mechanical shear during the washing procedure.

"Doughnut" structures, observed in mesosomal membrane fractions after negative staining, are not evident in freeze-fractured and etched preparations, suggesting that they are artifacts of the negative staining procedure. It appears probable that forces exerted during the drying of the stain cause spherical vesicles to collapse and to form indented spheres. Breakage and fusion of apposing membrane faces could then result in the "doughnut" structures observed. The observation of negatively stained images compatible with indented spheres supports this suggestion. By a similar mechanism the negative staining procedure itself may be responsible for the fragmentation of mesosomal tubules to vesicles. However, the presence of vesicles in freeze-fractured and etched preparations indicates that artifacts of the type mentioned and induced by negative staining are of minor importance in tubule fragmentation.

The significance of "honeycombed" structures observed occasionally in mesosomal membrane preparations is obscure. Burdett & Rogers (1972) noted the occurance of "smooth membranous sheets" in mesosomal membrane fractions isolated from B.licheniformis. These sheets tended to roll up to give structures similar to tubules. It is not clear whether the "honeycombed " structures represent similar sheets of membrane that have been severely disrupted.

Certainly, "honeycombed" structures were never seen attached to tubules as were the smooth membranous sheets from B.licheniformis (Burdett & Rogers, 1972). Alternatively, the fact that these structures occur predominantly in mesosomal membrane fractions prepared below 10mM Mg^{2+} may suggest that they are derived, in part, from the protoplast peripheral membrane, possibly the mesosomal sacculus.

The morphology of the discrete mesosomal sacculus has not hitherto been described. In M.lysodeikticus, however, this structure, in common with its contents, appears smooth-surfaced and devoid of enzyme particles, such as the ATP-ase which Munoz, Freer, Ellar & Salton (1968) found on peripheral membrane fragments. Since the sacculus itself is an invagination of part of the bounding membrane of the cell, this implies a regional localisation of components such as ATP-ase on the membrane surface.

As noted earlier, a decrease in Mg^{2+} concentration in the mesosomal membrane preparation procedure increases the yield. The possibility that a selective release of functionally dissimilar vesicles and tubules occurred at different Mg^{2+} concentrations appears unlikely in view of the similarity in morphology and chemical composition of mesosomal membrane prepared at levels of Mg^{2+} above 10mM. This does not, however, rule out the possibility of simultaneous release of vesicles and tubules possessing different functions. It would seem more appropriate, however, to consider this possibility after a definition of the mesosome contents (in toto) in functional terms has been achieved.

Properties of mesosomal and peripheral membrane

Major differences in constitution were observed between peripheral and mesosomal membrane fractions. Mesosomal membrane had a lower protein and total phosphorus content than peripheral membrane. It should be noted, however, that the figures for extractable lipid were very similar, indicating differences in the phospholipid composition of the two fractions and also in the protein/lipid ratio. Interpretation is made more difficult by the observation that 40% and 50% of the total membrane phosphorus of peripheral and mesosomal membrane preparations respectively, is released with extractable lipid. Since the presence of sugar phosphates in membrane preparations from M. lysodeikticus has not been reported, it appears likely that the "non-extractable" phosphorus is present as bound lipid. Indeed, components soluble in organic solvent after mild hydrolysis of lipid extracted residues, accounted for approximately 17% of the dry weight of peripheral membrane preparations. However, the conditions used for release of "bound" lipid appear to cause hydrolysis of the product. Further investigations are therefore necessary for complete characterisation of the lipid components of these two membrane preparations.

From the close agreement between values obtained by amino acid analysis and by the use of the Biuret reagent (Gornall et al., 1949) peripheral membrane appears to contain approximately 50% $^{w}/_{w}$ protein. This value is lower than that obtained by Salton & Freer (1965. See Table 9). A figure similar to that observed by Salton & Freer (1965) was obtained if the contribution by membrane

pigments to the E_{560} was not taken into consideration prior to direct protein estimation by the method of Gornall *et al.* (1949).

The lower protein content of the mesosomal membrane when compared with peripheral membrane preparations was reflected in the diminished number of components detectable in the former by SDS-polyacrylamide disc gel electrophoresis. These observations of qualitative differences are not unexpected in view of the demonstrated partition of oxidative enzymes and respiratory pigments between the two fractions. They do, however, contrast with those of Reaveley (1968) and Patch & Landman (1971. See also Table 9) who showed only minor quantitative and qualitative differences in the protein components of peripheral and mesosomal membranes isolated from *B.licheniformis* and *B.subtilis* respectively. If, however, the mesosome of *M.lysodeikticus* represents a site of localised autolytic activity, as indeed appears probable, and also as a site of assembly of surface polymers *e.g.* polymannan, then it may be expected that several components unique to, or showing enhanced localisation in the mesosomal membrane fraction should be detected. However, only two components were detected which showed enhanced localisation in the mesosomal membrane fractions. It is probable that the technique of SDS-polyacrylamide dis gel electrophoresis is not sensitive enough to detect minor membrane polypeptides in the presence of a full complement of other membrane components. Indeed, if the autolytic enzyme(s) displays an autolytic activity to whole cells of *M.lysodeikticus* comparable to that of lysozyme, then, from the observed

autolytic activity of both mesosomal membrane and lysozyme, it may be expected to represent less than 0.5% of the dry weight of the mesosomal membrane.

The results obtained from SDS-polyacrylamide disc gel electrophoresis do not support the sugestion that the mesosome represents the site of membrane biosynthesis (Fitz-James, 1967) nor are they compitable with the theory that the mesosome contents represent an area of the peripheral membrane which accumulates because membrane synthesis occurs faster than wall extension. Both of these theories demand that mesosomal membrane possesses a similar distribution of components to that observed in peripheral membrane.

The subject of bacterial membrane biosynthesis is controversial. As already noted (see Introduction) membrane growth in the region of DNA attachment is an integral part of the hypothesis for nuclear segregation proposed by Jacob et al.(1963). Their proposed model for membrane growth has been supported by the use of markers for old membrane e.g. reduced tellurite (Jacob et al.,1963) and flagella (Ryter, 1971). Morrison & Morowitz (1970) concluded that membrane growth in B.megaterium was not restricted to equitorial zones (Jacob et al., 1963) but was primarily localised at the ends. However, their use of ^{3}H-palmitic acid to label membrane lipid has been criticised (Salton, 1971).

Different conclusions concerning membrane growth have been reached by several authors. Mindich (quoted by Morrison & Morowitz, 1970) found that a precursor, incorporated solely into membrane lipid of B.subtilis during pulse labelling, was randomly distributed about the membrane.

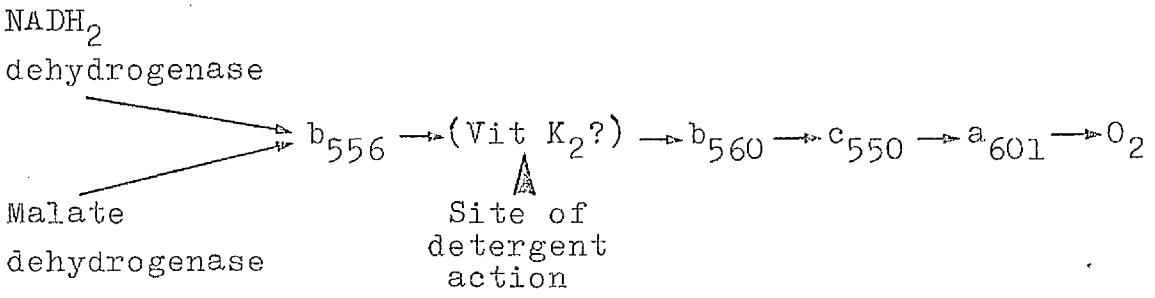
Fox and coworkers, using bromostearic acid as a density label for lipids (Fox, Law, Tsukagoshi & Wilson, 1970), have shown that incorporation of lipid and protein into E.coli membrane (Wilson & Fox, 1971a) does not occur at one or two fixed foci i.e. at the poles or at the equitorial band (Tsukagoshi, Fielding & Fox, 1971). Essentially similar conclusions were reached from a study of previously synthesised and newly synthesised membrane proteins in cells and newly formed mini-cells of a mini-cell producing strain of E.coli (Wilson & Fox, 1971b). These authors favoured the conclusion that newly synthesised lipid and protein were inserted together into the membrane matrix at many points. Thus there is evidence for both dispersive membrane growth and restricted-zone growth in bacteria.

Several theories can be invoked to explain the shortage of polypeptide components in mesosomal membrane. The mesosomal membrane vesicles may represent structures derived from "complete" peripheral membrane but which have lost components during this process. This seems unlikely. Alternatively, they may represent structures derived from areas of the peripheral membrane e.g. the sacculus, carrying a similar distribution of components to that of the mesosomal membrane vesicles. This would support the sugestion that the mesosomal membrane vesicles are derived from further invagination of the mesosomal sacculus (Fitz-James, 1960; Tomasz et al., 1964; Pate & Ordal, 1967; Kats & Kharatyan, 1969). Both these theories would require that membrane aquired an enhanced expression of autolytic enzyme activity upon invagination to give mesosomal membrane vesicles and tubules. Perhaps the most attractive suggestion is that mesosomal membrane

vesicles (lacking a full complement of membrane components) are synthesised *in situ* within the mesosomal sacculus. The membrane is envisaged as replicating by a dispersive type of growth, the mesosomal membrane vesicles possessing a reduced complement of polypeptide components (*e.g.* dehydrogenases and cytochromes). Indeed, evidence has been presented to indicate that components of mesosomal and peripheral membrane of both *L. monocytogenes* (Ghosh & Murray, 1969) and of *B. subtilis* (Patch & Landman, 1971) are synthesised independently. Incorporation of mesosomal vesicles into peripheral membrane may occur *e.g.* following septum formation. Addition of the remaining complement of membrane components could be made after its incorporation into peripheral membrane. This suggestion is not incompatible with the observations of Ellar and coworkers (Ellar, Thomas & Postgate, 1971). These authors indicated, from both pulse and pulse-chase experiments with ^{32}P and ^{14}C-acetate, that the specific radioactivities of total lipid and of individual phospholipids in both peripheral and mesosomal membrane were very similar. Thus the mesosome did not appear to be the preferential site for phospholipid synthesis in this bacterium or in fact in other microorganisms (Ghosh & Murray, 1969; Daniels, 1971; Ellar, Thomas & Postgate, 1971). If, however, membrane synthesis occurs by a dispersive type growth mechanism, then the ability to detect transfer of membrane lipid from mesosomal to peripheral membrane would not only depend upon the rate of such transfer but also upon the rates of membrane synthesis in the different regions of the cell. Alternatively, the results of Ellar and coworkers (Ellar, Thomas & Postgate, 1971) may be explained by a rapid lateral diffusion of membrane

lipid through the monolayer of the bilayer structure into which it is incorporated. Certainly, if lateral diffusion occurs during the lengthly mesosome isolation procedure at a value approaching that observed for phospholipids in liposomes (Kornberg & McConnell, 1971) then randomisation of labelled phospholipid would be expected. Obviously more experimental evidence is required before the validity of the proposed mechanism of vesicle incorporation into peripheral membrane can be assessed.

Respiratory chain components. Four cytochromes have previously been demonstrated in total membrane preparations isolated from M.lysodeikticus (Lukoyanova & Taptykova, 1968; Gel'man et al., 1970), the sequence of components in the electron transport chain being postulated as:-



Whereas a full complement of cytochromes was detected in peripheral membrane, cytochrome b_{556} was the only detectable cytochrome in mesosomal membrane. It seems unlikely that the point of inflection at approximately 600nm in the difference spectra of mesosomal membrane preparations (Fig. 17 and Table 27) is due to cytochrome a_{601}, as a similar phenomenon was noted in difference spectra of selectively reduced cytochrome b_{556} in peripheral membrane preparations (Fig.18. See also Fig 6 of Lukoyanova & Taptykova, 1968, for a similar phenomenon). It is

possible that the weak uncharacterised peak observed at 590nm in low temperature difference spectra of (total) membrane cytochromes (Lukoyanova & Taptykova, 1968) may in part be responsible for this phenomenon. Low temperature ultra-violet spectroscopy should help to resolve the possible presence of trace amounts of components other than cytochrome b_{556} in mesosomal membrane fractions.

Whereas a depleted complement of dehydrogenase activities have been noted in mesosomal membrane preparations from bacteria other than M.lysodeikticus (see Table 6), this is the first conclusive report of cytochrome deficiencies in this fraction. (Minor differences have been noted in the reduced cytochrome spectra of mesosomal and peripheral membrane fractions isolated from B.subtilis (Patch & Landman, 1971. See Table 6) and from B.licheniformis (Reaveley & Rogers, 1969. See Table 6). Reference has also been made recently to a cytochrome unique to mesosomal membrane fractions isolated from B.subtilis (Frehel et al., 1971a. See Table 6).) The demonstration of a much depleted complement of cytochromes in the mesosomal membrane fractions isolated from M.lysodeikticus, together with the very low levels of dehydrogenase activity serve to eliminate this organelle as a centre of respiratory activity in this organism. A similar situation appears probable in those other bacteria which show less dehydrogenase activity in isolated mesosomal membrane (see Table 6).

The occurrence of cytochrome b_{556} in peripheral membrane preparations from M.lysodeikticus is in keeping with a complete respiratory chain and appears

unlikely to be accounted for in full by comtamination of peripheral membrane with mesosomal membrane vesicles. The fractionation of deoxycholate-treated "total" membranes from M.lysodeikticus into two distinct fractions, one of which is similar morphologically and in its cytochrome content to deoxycholate-treated mesosomal membrane fractions, may simply be a reflection of the heterogeneity of the initial membrane fraction (i.e. mesosomal plus peripheral membrane). However, Pollock and coworkers (1971) have indicated that a particulate fraction, isolated from "total" membranes of M.lysodeikticus by a very similar isolation procedure to that used in this study, contains both cytochrome b and a high specific activity of succinate dehydrogenase when compared with isolated membrane fractions. It therefore seems improbable that DO3 (the cytochrome b_{556} rich, deoxycholate-insoluble membrane residue) is derived exclusively from mesosomal membrane, as this latter preparation was shown to have a very low specific activity of succinate dehydrogenase when compared with peripheral membrane (Table 25). A plausible explanation is that fraction DO3 and that of Pollock et al. (1971) represent a mixture of morphologically similar entities derived from both peripheral and mesosomal membrane. However, the proportion derived from mesosomal membrane would be expected to have a reduced complement of dehydrogenase activities. The proportion derived from peripheral membrane would possess a full complement of dehydrogenase activities and would have been spatially uncoupled from the other components of the electron transport chain. A similar suggestion that the complete electron transport chain of

peripheral membrane could be split into two blocks was recently proposed by Gel'man and coworkers (1970), after experiments on fractionated Triton X-100 treated "total" membranes from M.lysodeikticus. However, it would be necessary to characterise detergent-insoluble fractions isolated from peripheral membrane free of any contaminating mesosomal membrane before these conclusions can be verified. Nevertheless, it does seem from the studies reported here and also those of Pollock et al. (1971) and of Gel'man et al. (1970), that detergents, such as deoxycholate and Triton X-100 disrupt the bonding between cytochrome b_{556} and cytochrome b_{560} in the complete electron transport chain, thus splitting it into two constituent blocks. Hence the mesosome may only possess the ability to synthesise part of the first block of the electron transport chain. Synthesis and addition of the remaining components of the first block (e.g. malate and $NADH_2$dehydrogenases) and of the complete second block may occur if and when the mesosomal vesicles fuse with peripheral membrane.

Gel'man and colleagues (1970) have indicated that Vitamin K_2 may be localised at a site between cytochrome b_{556} and cytochrome b_{560}. It thus seems probable that spatial uncoupling of components of the electron transport chain by detergent action involves disruption of bonding around Vitamin K_2. Detergent action may thus effectively serve to dilute the concentration of this component with respect to the two blocks of the electron transport chain. This suggestion is supported by results which indicate that the addition of Vitamin K_3, a compound

closely related to Vitamin K_2, to detergent-treated peripheral membrane preparations of M.lysodeikticus increases the efficiency of electron transfer from cytochrome b_{556} to other components of the electron transport chain.

Muralytic activity and peptidoglycan synthesis. The deficiency of components of the electron transport system in mesosomal membrane contrasts with the relative enrichment of autolytic enzyme(s) found in these fractions. It is, however, necessary to estimate the contribution of lysozyme to this activity, as preferential binding to the mesosomal membrane of this strongly cationic protein cannot be ruled out at the present time. An investigation of the specificity of the autolytic enzyme action by end product analysis or the availability and use of either specific antibody to lysozyme or labelled lysozyme itself should clarify this problem. Further experimentation is also necessary to determine whether the inverse relationship between autolytic enzyme activity and Mg^{2+} concentration during the mesosomal membrane isolation procedure is due to either inhibition or to inactivation or to displacement of enzyme by Mg^{2+} ions.

The mesosome does, however, appear to be a logical site for a muralytic enzyme capable of opening up peptidoglycan to facilitate insertion of new wall material during septum formation and also to function as a cell-separating enzyme. Confirmation of this latter suggestion was obtained from an electron-microscopic study of dividing cells of M.lysodeikticus St.O. It seems probable that the distinct, electron-transparent zone, observed completely

bisecting the developing septum in dividing cells, corresponds to wall which has been digested by a muralytic enzyme from the septal mesosome. However, the possibility that this zone represents separation of two cell-wall layers synthesised separately cannot be ruled out.

Other bacteria have been shown to possess autolytic enzymes associated with the cell wall (Shockman, Pooley & Thompson, 1967; Shockman & Martin, 1968; Pooley & Shockman, 1969; Fan, 1970). Both biochemical (Shockman et al., 1967; Pooley & Shockman, 1969) and electron-microscopic evidence (Shockman & Martin, 1968) suggested that in S.faecalis the enzyme was located very close to the most recently synthesised wall (the streptococcal wall extension process has been shown to originate at the leading edge of the nascent cross walls-Higgins & Shockman, 1970a,b). Electron-microscopic observations later revealed that the leading edge of the nascent cross wall was the primary site of autolytic activity in this organism (Higgins, Pooley & Shockman, 1970a). The streptococcal mesosome, which was observed to be attached at only one point to the septum (Higgins & Shockman, 1970b), was suggested as an organelle "..... that could initiate cross-wall formation as well as notching and splitting at one point of the cell surface." (Higgins et al., 1971). The isolation of a mutant which grew exponentially in long chains and possessed less autolysin (Pooley, Shockman, Higgins & Porres-Juan,1972) supported a role for this autolysin in cell separation. However, the suggested presence of an autolytic enzyme localised in the vicinity of the mesosome has, until now, not been confirmed by biochemical studies.

Unlike the case for S.faecalis (Higgins & Shockman, 1970a), the initial cleavage lesion or "notch" does not occur, in M.lysodeikticus, until septation is complete. The absence of an apparent zone of autolytic activity across the wall at the base of the septum and the ragged profile of the initial cleavage lesion make it unlikely that the autolytic enzyme(s) are responsible for the primary step in cell-cell separation. Indeed, the ragged profile of the cell wall of a mutant of B.subtilis, possessing a reduced complement of autolytic enzymes, was shown to become smooth and regular after incubation of cells with added autolysins (Fan, Beckman & Cunningham,1972). Thus mechanical disruption of the cell wall, caused by increase in cell volume, appears to be responsible for the initial step in the separation of dividing cells of M.lysodeikticus. Autolytic enzyme(s), however, may be responsible for slight weakening of the wall in this region, thus determining the site of mechanical cleavage.

Following the initial rupture of cell wall, cell cleavage follows the line of previously autolysed septal wall. The final step in cell-cell separation appears to be cleavage of unruptured wall derived from the parent cell. This process seems to occur relatively slowly and is manifested by the occurrence of cells in chains or aggregates, connected in the manner described. It may be that rupture of the parent cell wall in the region of the developed septum is also related to the pressure exerted by the two daughter cells increasing in volume. Thus cells at the final stages of cell-cell separation and exerting little pressure on each other will tend to stay attached by

remaining unruptured cell wall until mechanical agitation effects their separation. This, however, poses the question of why cell wall derived from the parent cell is not apparently enzymically cleaved. One possibility is that the activity of the autolytic enzyme is carefully regulated by the mesosome and that only wall in direct contact with the "neck" of the mesosome is cleaved. This suggestion is supported by the observation that the zone of autolytic activity always connects to the "neck" of the septum. Thus the parent cell wall is excluded from muralytic digestion. A second possibility is that freshly synthesised cell wall is more susceptible to autolytic enzymes than older, more "mature" wall derived from the parent cell, as indeed may be the case in S.faecalis (Shockman et al., 1968; Pooley & Shockman, 1969; Shockman & Martin, 1968; Higgins & Shockman, 1970a). This suggestion requires that the septum represents the region of freshly synthesised wall. With other cocci there is good evidence that wall growth can occur at a single zone i.e. at the developing anulus of a dividing cell (Briles & Thomasz, 1970; Higgins & Shockman, 1970a,b; Higgins et al.,1971) but that wall thickening can occur over the whole surface (Higgins & Shockman, 1970b). In the case of Gram-positive bacilli the situation appears more complex. The evidence for a single growth zone is not compelling (Cole, 1965; Hughes & Stokes, 1971), whereas that for multiple growth points is more convincing. Radioautography of cells of B.megaterium labelled with ^{3}H-diaminopimelic acid revealed a uniform distribution of label in the progeny, indicating that cell-wall growth occurred at many points distributed over the surface (Mauk, Chan, Glaser &

Williamson, 1972). A similar conclusion was reached recently by Mauk & Glaser (1972). These authors showed, that during cell-wall growth in B.subtilis there was a random intercalation of new and old peptidoglycan chains. Electron-microscopic studies of cells of B.licheniformis (Highton & Hobbs, 1971) and of B.cereus (Highton & Hobbs, 1972) treated with and recovering from penicillin, and also cells of B.subtilis and B.megaterium recovering from amino acid starvation or chloramphenicol addition (Frehel, Beaufils & Ryter, 1971), have suggested a uniform distribution of growing points along the length of the cells. However, a different mode of growth of cell septum was indicated in many cases (Highton & Hobbs, 1971; 1972; Fan, Beckman & Cunningham, 1972). Indeed evidence has been presented to suggest that isolated walls from polar regions of the cells of B.subtilis are MORE resistant to autolytic degradation than are isolated lateral cell walls (Fan, Pelvit & Cunningham, 1972). It should be noted that since lateral walls comprise a greater percentage of the total wall in bacilli than do polar walls, any bulk analysis of whole walls will reflect mainly lateral wall material and may thus mask recognition of zonal synthesis of septa (Highton & Hobbs, 1972). It seems reasonable to consider that cell-wall growth in cocci corresponds primarily to the mechanism of cell-wall synthesis responsible for septum formation in bacilli and that wall elongation, as such, does not take place.

The close association of the mesosome with the developing septum in M.lysodeikticus and in many other cocci and bacilli suggests a role for this organelle in septal peptidoglycan synthesis and assembly. Additional

evidence for such a role has been reviewed in the Introduction. However, it is apparent that the mesosome is not the sole site of peptidoglycan synthesis in M.lysodeikticus, as thickening of the wall in the vicinity of the septum occurs after septal cleavage, a phenomenon common to other bacteria (Ellar et al., 1967; Higgins & Shockman, 1970b; Higgins et al., 1971). It seems probable that both peripheral and mesosomal membrane are capable of peptidoglycan assembly, as indeed has been demonstrated for L.casei and L.plantarum (Thorne & Barker, 1972. See Introduction). However, the ability of peripheral membrane of cocci to synthesise peptidoglycan may be manifested primarily in wall thickening and not in wall extension. A combined role for the mesosome of M.lysodeikticus in septal peptidoglycan assembly and as a centre of autolytic activity is therefore suggested. Thompson (1971) has recently proposed a model for synthesis and morphogenesis of bacterial cell walls. The fundamental concepts of this theory are that the polysaccharide chains of the wall peptidoglycan are spun out continuously from a lipid-bound carrier in the mesosome and that localised autolytic splitting of the glycan strands occurs to separate the cross walls into two parts, which then grow towards the poles of the cell. Wall thickening is envisaged as occurring in regions away from the sites of autolytic enzyme activity. The results presented here appear to substantiate this theory for M.lysodeikticus St.O. It remains to be seen, however, whether the glycan chains are oriented parallel to the plane of the septum, as predicted by the theory of Thompson (1971), or in a direction perpendicular

to the surface. Indeed evidence has been presented for an arrangement of glycan chains perpendicular to the cell surface of B.subtilis (Mauk & Glaser, 1972). It is possible, however, that different mechanisms responsible for septum synthesis and wall extension in bacilli may result in structurally dissimilar macromolecules. X-ray diffraction analysis (Balyuzi et al., 1972) may be instrumental in detecting inherent structural differences between isolated walls from polar regions and isolated lateral walls of bacilli.

Whatever the mode of synthesis of the wall of M.lysodeikticus, it is apparent that the presence of much enhanced autolytic activity in the mesosomal membrane is worthy of further investigation. The low levels of autolytic enzyme activity in the peripheral membrane may be explained by the low degree of cross contamination in such fractions with mesosomal vesicles. Alternatively the low level of autolytic activity may be an inherent property of peripheral membrane. Isolated mesosomal membrane would appear to be a logical starting material for isolation, purification and characterisation of the autolytic enzyme(s). However, as the mesosomal membrane isolation procedure is so tedious, it may be more convenient to fractionate the enzyme(s) from total cell lysates. The use of ferritin-labelled antibody against the autolytic enzyme(s) should make possible an investigation of the distribution of the enzyme over the cell surface and clarify its participation in wall extension, wall thickening and cell separation. A combination of ferritin-labelling and ultrathin sectioning of frozen (and fixed) cells and protoplasts of M.lysodeikticus may also help

to elucidate a role for the sacculus and also of mesosomal vesicle incorporation into peripheral membrane. It may also be possible to isolate mutants of M.lysodeikticus which, although possessing the ability to undergo division,do not possess the ability to undergo cell-cell separation. Such mutants may be expected to display different colony morphologies on solid medium to that of the parent strain. This may aid the screening of mutants which itself would be presumably achieved by light-microscopic examination cultures for chaining or clumping of cells. A comparison of the mesosomal membrane fractions from these strains with those of the parent strain may provide a correlation of localised autolytic activity with cell-cell separation. Alternatively, it may be more convenient to isolate mutants showing an increased or decreased complement of autolytic enzyme(s). These may be detected by their ability (or not) to autolyse under adverse conditions (e.g. as reported by Pooley et al., 1972) or by their potential to display zones of clearing on agar plates containing parent cell wall.

Mesosomal membrane fractions contain approx. 20% ($^{w}/w$) hexose (mainly as mannose). This value is some four to five times that observed in peripheral membrane. Mannan appears to be the major form of mannose in membrane preparations from M.lysodeikticus and has been reported as accounting for up to 20% of the dry weight of the membrane (Gilby et al., 1958; MacFarlane, 1964). Mannosyl diglycerides and dimannosyl diglycerides may account for minor proportions of the mannose content (see Table 10). The observation that mesosomal membrane ,when compared with peripneral membrane ,contains a four to five fold increase

in hexose content is interesting in the light of the reports by Thorne & Barker (1969; 1971; 1972) that a large percentage of the membrane bactoprenol (a C_{55} polyisoprenoid alcohol carrier lipid) is found in the mesosomal membrane fraction isolated from L.casei and L.plantarum (see Table 6) and those of Lennarz and co-workers who showed that bactoprenol is involved in polymannan biosynthesis in M.lysodeikticus (Scher et al., 1968; Scher & Lennarz, 1969; Lahav et al., 1969). Further experimentation however is necessary to test the involvement of the mesosome in polymannan biosynthesis. Pulse and pulse-chase labelling experiments with ^{3}H-mannose or alternatively, assay of mannan synthesis using suitable cell-free synthesising systems (i.e. purified peripheral and mesosomal membranes as particulate enzyme and GDP-mannose-^{14}C as substrate) may be useful in establishing this point.

Bactoprenol is also involved in the biosynthesis of peptidoglycan in M.lysodeikticus (Higashi et al., 1967) although the linkage from the lipid moiety to the disaccharide pentapeptide involves a pyrophosphate group (Higashi et al., 1967), whereas linkage from the same lipid moiety to mannose involves a single phosphate group (Scher et al., 1968). The antibiotic bacitracin is known to prevent the mono-dephosphorylation of bactoprenol pyrophosphate, an essential step in the biosynthetic cycle for peptidoglycan (see Anderson, Hussey & Baddiley, 1972). Thus it should be possible, by use of suitable labelling experiments involving membrane fractions obtained from synchronously growing cells, to investigate the relationship between the synthesis of both membrane and wall polymers.

Freeze etching

Three main features are evident on the fractured surfaces of membrane systems from M.lysodeikticus St.A and St.O, viz. intramembrane particles, rod-shaped structures and linear depressions. Although there is no direct evidence on the nature of these features, it may be instructive to discuss their possible significance in the light of available circumstantial evidence.

The exact chemical nature of the intramembrane particles is not known. Similar particles, observed on fracture faces of red cell ghosts, have been shown to be susceptible to proteolytic digestion (Engstrom, 1970). Pinta da Silva, Douglas & Branton (1971) later showed, from a study of freeze-fractured erythrocyte membrane, previously labelled indirectly with ferritin conjugated to the IgG fraction of rabbit anti-human IgG, that the intramembrane particles were located at or directly below the A-blood group antigen site. The nature of intramembrane particles in other freeze-fractured membrane systems is less certain. They may represent micellar transitions within a predominantly lamellar system (Kavanau, 1966) or result from plastic deformations during fracture (Clark & Branton, 1968). There is, however, some evidence which suggests they may be protein that is embedded in the lipid bilayer of the membrane (Wallach & Zahler, 1966; Wallach, 1969).

Whatever their chemical nature, intramembrane particles appear to be a common feature of almost all freeze-fractured biological membrane systems. However,

the number and distribution of these particles seems to be a fixed characteristic of a particular membrane type. It has been suggested (Branton & Park, 1967) that the functional complexity of a membrane may be related directly to the number of particles visible on its fracture surfaces. Indeed myelin, which functions primarily as a metabolically inert insulator around the axon, has been shown to reveal fracture faces devoid of intramembrane particles (Branton, 1967), a phenomenon shared with artificial lipid bilayers (Staehelin, 1968; Deamer et al., 1970). In contrast, metabolically active membrane systems e.g. chloroplast lamellae, reveal a population of intramembrane particles covering approximately 80% of the membrane fracture face (Branton & Park, 1967; Branton, 1969). In view of the known metabolic activity of isolated membranes from M.lysodeikticus (see Introduction), the very dense population of intramembrane particles on the outer surface of the inner half of the peripheral membrane of this organism (and also of many other bacteria) is in keeping with the suggestion of Branton & Park (1967).

The similar distribution of intramembrane particles on fracture faces of isolated mesosomal membrane from M.lysodeikticus suggests that these membrane structures also display metabolic activity. This seems hardly surprising in the light of the displayed localisation of autolytic enzyme activity in isolated mesosomal membrane vesicles and its probable role in the regulation of synthesis of surface polymers such as peptidoglycan and polymannan. The presence of particles of similar dimensions in the wall and septum

of this organism may also indicate the presence of enzyme complexes responsible for regulation of wall thickening.

The rod-shaped structures observed on the outer surface of the inner half of the fractured peripheral membrane apparently serve to anchor cell wall and membrane. They may represent enzyme complexes responsible for polymerisation of peptidoglycan involved in the cell-wall thickening process. If the glycan strands remain attached to the enzyme complex during polymerisation, then a mechanism for membrane-wall association is achieved. This process may manifest itself in thin-sectioned M.lysodeikticus as"bridges" apparently connecting cell wall and membrane. These features are not unique to M.lysodeikticus. "Bridges" have been observed connecting membrane and wall in many thin-sectioned organisms (Glauert et al., 1961; Robinow, 1962; Tomasz et al., 1964; Bayer, 1968; Leadbetter & Holt, 1968 Hurst & Stubbs, 1969; Rogers, 1970), as have rod-shaped structures in many freeze-fractured bacteria (see Table 29). This may demonstrate a general, although not necessarily universal method for membrane-wall association.

The observation that the dimensions of similar rod-shaped structures in freeze-fractured guinea pig retina appeared to vary with tonicity of the suspending medium (Clark & Branton, 1968) may indicate that they are artifacts of the fracture process, although several observations would tend to make this interpretation unlikely.

Firstly, rod-shaped structures are not evident on fracture surfaces of isolated mesosomal membrane from M.lysodeikticus. It may be argued that the small radius of

curvature of isolated mesosomal vesicles compared with peripheral membrane fragments may not be conducive to rod structure formation. However, this suggestion is not substantiated by the observation of one vesicle of similar dimensions to those of mesosomal membrane which carried a distribution of rod-shaped structures on its fracture surface.

Secondly, linear depressions are observed on the inner surface of the outer half of the peripheral membrane. The fact that these are absent on corresponding fracture faces of isolated mesosomal membrane, together with the absence of rod-shaped structures from the fractured mesosomal membrane, suggests that they possibly result from the presence of rod-shaped structures on the opposite half of the membrane. Although it is by no means clear whether this interpretation is correct, it is feasible that structures on the inner half of the peripheral membrane and capable of spinning off peptidoglycan chains would traverse the outer half of the membrane. The presence of linear depressions and "protuberences", or raised ridges, on the inner and outer surfaces respectively of the outer half of the peripheral membrane is in keeping with this suggestion. "Protuberences", or raised areas, may of course arise in part from accomodation of intramembrane particles lying beneath the outer leaflet of the membrane. The absence of depressions on the inner surface of the outer half of the freeze-fractured peripheral membrane, compatible with the presence of intramembrane particles on the outer surface of the inner half of the membrane, may be a consequence of metal shadowing.

This process tends to accentuate structures in positive relief and to "fill in" structures in negative relief. Thus it may also be responsible for the incompatibility in dimensions between rod-shaped structures and linear depressions.

The absence of rod-shaped structures in freeze-fractured mesosomal membrane isolated from M.lysodeikticus and also in fracture faces of mycoplasma membrane (Tillack et al., 1970) and erythrocyte membrane (Branton, 1971) is compatible with a role for these structures in the anchoring of the membrane and the wall and also in wall thickening.

In common with most biological membrane systems examined by the freeze-etch technique, peripheral and mesosomal membranes in M.lysodeikticus display a marked asymetric distribution of intramembrane particles on the two opposing membrane fracture faces. It is perhaps not surprising that the cytoplasmic leaflet of the membrane reveals the dense population of intramembrane particles, since much of the metabolic activity of the membrane would be expected to be located on this half of the membrane. An asymetric membrane structure may thus be a universal feature of biological membranes. Indeed Bretscher (1972) has recently reviewed evidence for a asymetric distribution of both phospholipid and proteins in mammalian cell membranes.

Both intramembrane particles and rod-shaped structures appear to be components of the "basic" membrane, since washing techniques such as ionic shock or treatment with EDTA, designed to remove loosely associated membrane

components (Nachbar & Salton, 1970b), do not remove them. It thus seems unlikely that either structure represents, for example, ATP-ase, but rather membrane proteins buried in and possibly traversing the lipid bilayer structure of the membrane. (Some components of the erythrocyte membrane have recently been shown to exhibit these properties - Bretscher, 1971a,b,c; Phillips & Morrison, 1971). It is evident, however, from a comparison of the topography of fracture faces of peripheral membrane isolated from M.lysodeikticus and peripheral membrane in situ in the cell, that little structural rearrangement of the membrane occurs during the isolation and washing procedures, and also in whole cells undergoing obvious autolysis. It is perhaps not surprising that membrane fracture faces were not observed for deoxycholate-insoluble membrane residues. Deoxycholate is known to remove over 95% of the membrane phospholipid (Salton et al., 1968) and thus might be expected to remove the region of weak hydrophobic bonding in the membrane i.e. the potential fracture plane.

It is evident from a comparison of isolated mesosomal membrane, freeze-fractured after suspension in buffered glycerol or in buffer alone, that glycerol causes extensive vesicularisation of the membrane. It does not, however, appear to affect the distribution of intramembrane particles or of rod-shaped structures on the various fracture faces of the membrane (see also Moor, 1971). A similar vesicularisation has also been noted by Reaveley & Rogers (1969) for mesosomal membrane isolated from B.licheniformis in the presence of polyethylene glycol 600.

The tendency of membrane to vesicularise may explain the large vesicles observed in freeze-fractured whole cells of M.lysodeikticus impregnated with glycerol. If, as appears to be the case, the mesosome is connected to the cell septum (or peripheral membrane) by a narrow channel or "stalk", then incubation of cells in the presence of glycerol may sever this connection. This would result in a collection of mesosomal vesicles contained within a larger spherical vesicle which is derived from the mesosomal sacculus. This proposal requires the mesosomal sacculus itself to display relatively smooth fracture faces. Several other observations support this suggestion.

Van Iterson & Groen (1971) have indicated that the mesosomal sacculus together with its contents may occasionally be released from the cell during protoplast formation. Thus the smooth-surfaced vesicles which are observed in freeze-fractured mesosomal membrane preparations, and which are of comparable size to those observed in freeze-fractured whole cells, may well be vesicularised sacculus.

Although occasionally released during extrusion of mesosomal vesicle from protoplasts, it is apparent, from many studies, that the mesosomal sacculus usually becomes part of the bounding membrane of the protoplast. In this respect, observed areas of fracture faces of protoplast peripheral membrane, devoid of any rod-shaped structures and possessing very few intramembrane particles, are interesting. As similar regions were not observed to be continuous with peripheral membrane in situ in whole

cells of M.lysodeikticus, it seems logical to suggest that they represent mesosomal sacculus. As such they are obviously structurally dissimilar to either the peripheral membrane or to the mesosomal vesicles.

Areas of peripheral membrane devoid of intramembrane particles have also been noted for other organisms (see Table 29). In some bacteria the membrane fracture faces in situ reveal a network-like distribution of intramembrane particles and areas devoid of such structures (Table 29). These appear to be a feature of growing cells and can be altered by metabolic disturbances (Fiil & Branton, 1969). Meyer & Richter (1971) have recently noted that these network patterns of intramembrane particles appear predominantly in membrane fractures of growing cells and are only occasionally viewed in membrane fractures of cells isolated from a non-dividing culture. These authors concluded that the networklike pattern probably corresponds to one stage in the mechanism of growth of the membrane. Particle-depleted areas, of similar dimensions to those observed for protoplasts of M.lysodeikticus, have been noted in several bacteria (Table 29). Of special interest are the particle-free regions observed in the immediate vicinity of the cell septum and mesosome of freeze-fractured vegetative cells of B.cereus (Holt & Leadbetter, 1969) and those on the freeze-fractured membrane surface of protoplasts of B.stearothermophilus (Sleytr, 1970a). It seems probable that the particle-free areas in both organisms may be derived from the mesosomal sacculus.

Formation of septum membrane may involve rapid insertion of membrane components (possibly lipid) that are not associated with particles. Incorporation of other membrane components essential for fully functional membrane may be achieved by subsequent fusion of mesosomal vesicles with the sacculus. Indeed, the occasional intramembrane particle observed on these "bare" areas may be manifestations of such fusion. However, it is evident that there exists, at some stage in the cell-division cycle, regions of the bounding membrane of the cell that differ widely in structure and probably function.

The presence of regions of the peripheral membrane devoid of intramembrane particles may in part explain the conflicting reports concerning the topography of freeze-fractured mesosomes in situ (see Table 29). Some reports suggest that mesosomes possess membrane fracture faces having a similar distribution of intramembrane particles to that observed for peripheral membrane. Others suggest that mesosomes possess fracture faces bearing few intramembrane particles. However, it is often difficult to determine whether the fracture faces observed correspond to those of mesosomal vesicles, mesosomal sacculus, or to periplasmic mesosomal vesicles (Nanninga, 1971). The absence of cross walls in these areas may mean that they do not relate to mesosomal vesicles, at least to those within a sacculus. To the author's knowledge, this is the first report of the topography of fracture faces of isolated mesosomal membrane. It is evident that for M.lysodeikticus the vesicular contents of the mesosomal sacculus carry a similar distribution of intramembrane particles on their

membrane fracture faces to those observed on the corresponding fracture faces of peripheral membrane.

Compared with the fracture faces observed for isolated mesosomal membrane suspended in glycerol, those observed for isolated mesosomal membrane, frozen-fractured and etched in dilute buffer alone are complex. In many instances the presence of discrete particle-studded, convex regions within apparently cross fractured tubules suggest the presence of vesicles within tubular structures. The appearance of spherical, smooth-surfaced depressions (concave fractures) along the length of many tubules help to confirm this suggestion. Bulging of the mesosomal tubule around the vesicle may well account for the constrictions observed along the length of many tubules when visualised by the negative staining procedure (see also Burdett & Rogers, 1972).

From a study of sectioned protoplasts of B.subtilis, Matheson & Kwong (1972) have recently reported the presence of ribosomes in extruded mesosomal membrane vesicles. It seems unlikely that the particle-studded regions observed in freeze-fractured and etched tubules isolated from M.lysodeikticus represent areas rich in ribosomes. The topography of the surface and dimensions of the intramembrane particles are identical to those observed for freeze-fractured mesosomal membrane suspended in glycerol. It seems improbable that this relatively homogeneous collection of vesicles could be solely accounted for in terms of ribosomes or polyribosomes. Also, no comparable structures were observed in the

cytoplasm of freeze-fractured cells of M.lysodeikticus as might be expected if they did represent ribosomes.

The apparent inability of the membrane of the mesosome tubule to reveal fracture faces is curious. The forces involved in the fracture of small tubular structures may be such that cross-fractures are the preferred mode. Alternatively, the tubule membrane may not be structurally amenable to fractures other than cross-fractures. This would correlate with the poor resolution of discrete membranous structures in thin-sectioned cells. Indeed several workers have noted the apparent lack of triple track membrane in isolated mesosomal membrane when observed by thin sectioning (e.g. Sargent et al., 1969a). This may suggest a different composition for the tubule membrane and for the intratubular vesicle membrane. However, only one type of membrane is implicated from observed fracture faces of isolated mesosomal membrane suspended in glycerol. Thus glycerol may cause vesicularisation of two basically similar structures. This tends to substantiate the former suggestion that the inability of mesosomal tubules to reveal fracture faces is a function of their geometry. However, it could be argued that glycerol may first cause fusion and then vesicularisation of the two different structures (i.e. tubules and intratubular vesicles). Present results do not allow a distinction between these possibilities. Monitoring the effect of decreasing concentrations of glycerol on the morphology and topography of freeze-fractured and etched isolated mesosomal membrane should clarify the situation.

The significance of vesicles within tubules is unknown. They may serve merely to increase the effective

surface area of the mesosomal membrane and hence its metabolic activity. They may represent freshly synthesised mesosomal membrane capable of translocation along the mesosomal tubule and of fusion with it. Bulging of the tubular membrane around vesicles and the cosequent fusion of the two structures could thus provide a mechanism for tubule growth and branching and also for the secretion of extracellulau products (i.e. a function similar to that of Golgi) and for transport of precursors involved in polymer synthesis.

Caution should obviously be exercised in the interpretation of some images observed in freeze-fractured and etched preparations. For example, the spatial relationship of the line of small depressions on the etched surface of freeze-fractured and etched protoplasts to the fracture edge itself suggests that they i.e. the depressions, represent artifacts of the fracture process. Freeze-cleavage may cause localised stress lines in the frozen membrane in the vicinity of the fracture edge. These could be manifested after etching by the appearance of small depressions or tears on the outer membrane surface.

.....

A shortened account of the isolation procedure for and properties of mesosomal and peripheral membrane fractions from M.lysodeikticus has recently been accepted for publication in The Biochemical Journal. A proof copy of this manuscript is attached facing page 146.

.....

C O N C L U S I O N S.

The role of the mesosome of M.lysodeikticus has been studied using both biochemical and electron-microscopic techniques. By precise manipulation of the conditions prior to and during protoplast formation, it is possible to obtain highly purified preparations of mesosomal membranes. There is no evidence from freeze-fracturing and etching experiments that structural rearrangement or autodigestion occurs during the adopted fractionation procedure.

The mesosome contents do not appear to be the site of membrane biosynthesis in this organism, a finding in common with conclusions reached for other bacteria. However, evidence from freeze-fracturing and etching does confirm that they are metabolically important organelles. It is proposed that membrane biosynthesis occurs by a dispersive type of growth mechanism with the possible incorporation of mesosomal vesicles into peripheral membrane, this being followed by time sequential addition of other components. Evidence from freeze-fracturing and etching shows that the mesosomal sacculus is structurally dissimilar to either mesosomal membrane itself or to peripheral membrane. The mesosomal sacculus may thus represent a region of localised insertion of some membrane components in order to effect rapid membrane growth during the septation process.

The depleted complement of electron transport components in the mesosomal membrane when compared with peripheral membrane finally provides conclusive evidence against a localisation of respiratory function in this organelle. It seems probable that this phenomenon is common to mesosomes of many bacteria.

Results from both biochemical and electron-microscopic studies suggest that the mesosome is a region of localised autolytic activity. Although no direct evidence is available to indicate the mesosome as a site of peptidoglycan synthesis in M.lysodeikticus, anatomical evidence from thin-sectioning experiments shows a close association of this organelle with the developing septum. Thus the mesosome has an important role to play in the process of wall morphogenesis and in cell-separation. It seems probable that the peripheral membrane participates in wall thickening and also in the anchoring of the wall to the membrane. A role for the mesosome in the biosynthesis of another surface polymer, polymannan, is also indicated.

Electron-microscopic evidence indicates that mesosomal membrane growth may occur by translocation of membrane vesicles along the tubules of the mesosome, followed by membrane fusion at the distal end of the tubule. This proposal also provides a mechanism for the secretion of extracellular products and for the transport of precursors to the site of surface polymer growth.

Although this study has identified several functions for the mesosome, it has not confirmed (or refuted) all the previously proposed roles for this organelle. A role in DNA manipulation may be indicated but this possibility would be better explored by experiments with whole cells (i.e. where both mesosome and chromatin are present). High voltage transmission electron microscopy of intact synchronised cells may provide information on the association of chromatin and mesosome during cell division.

M.lysodeikticus, displaying random division planes, is probably not a logical choice for such a study.

Because of the use of a non-sporing organism in this study, it was not possible to investigate the role for the mesosome in fore-spore formation and germination. This important problem may be resolved by studying suitable strains of sporing bacilli. Comparison of mesosome fractions isolated from B.megaterium KM with those isolated from sporing strains of the same species and obtained at the relevant stages in the sporulation/germination cycle may be helpful in this respect. A study of this kind may also be instrumental in determining whether morphologically dissimilar mesosomes have different functions.

R E F E R E N C E S.

Abadie, M. (1968). C.r.hebd. Seanc. Acad. Sci., Paris, 267, 1538.

Abram, D. (1965). J. Bact. 89, 855.

Abram, D. and Davis, B.K. (1970). J. Bact. 104, 948.

Abram, D., Vatter, A.E. and Koffler, H. (1966). J. Bact. 91, 2045.

Allen, R.J.L. (1940). Biochem. J. 34, 858.

Allen, T.C., Stevens, J.O., Florance, E.R. and Hampton, R.O. (1970). J. Ultrastruct. Res. 33, 318.

Altenberg, B.C. and Suit, J.C. (1970). J. Bact. 103, 227.

Altenberg, B.C., Suit, J.C. and Brinkley, B.R. (1970). J. Bact. 104, 549.

Anacker, R.L., Pickens, E.G. and Lackman, D.B. (1967). J. Bact. 94, 260.

Anderson, O.R. and Roels, O.A. (1967). J. Ultrastruct. Res. 20, 127.

* Anderson, J.S., Matsuhashi, M., Haskin, M.A. and Strominger, J.L. (1965). Proc. natn. Acad. Sci. U.S.A. 53, 881.

Anderson, J.S., Matsuhashi, M., Haskin, M.A. and Strominger, J.L. (1967). J. biol. Chem. 242, 3180.

Ariji, F., Fukashi, K. and Oka, S. (1968). J. elect. Microscopy, 17, 139.

Aronson, J.N., Bowe, P.A. and Swafford, J. (1967). J. Bact. 93, 1174.

Ashe, G.B. and Steim, J.M. (1971). Biochim. biophys. Acta, 233, 810.

Baillie, A., Thomson, R.O., Batty, I. and Walker, P.D. (1967). J. appl. Bact. 30, 312.

Balyuzi, H.H.M., Reaveley, D.A. and Bargo, R.E. (1972). Nature, Lond., 235, 252.

Barker, D.C. and Thorne, K.J.I. (1970). J. Cell Sci. 7, 755.

Barksdale, L. (1970). Bact. Rev. 34, 378.

Bayer, M.E. (1968). Virology, 2, 346.

Bayer, M.E. and Remsen, C.C. (1970). J. Bact. 101, 304.

Beaman, B.L. and Shankel, D.M. (1969). J. Bact. 99, 876.

Beaton, C.D. (1968). J. gen. Microbiol. 50, 37.

Beer, M. (1960). J. Bact. 80, 659.

* Anderson, R.G., Hussey, H. and Baddiley, J. (1972). Biochem. J. 127, 11.

Bernstein, B.-Z. (1969). Can. J. Microbiol. 15, 1125.

Birdsell, D.C. and Cota-Robles, E.H. (1967). J. Bact. 93, 427.

Biryuzova, V.I., Lukoyanova, M.A., Gel'man, N.S. and Oparin, A.I. (1964). Dokl. Akad. Nauk SSSR, 156, 198.

Bishop, D.H.L. and King, H.K. (1962). Biochem. J. 85, 550.

Bladen, H.A. and Mergenhagen, S.E. (1964). J. Bact. 88, 1482.

Bladen, H.A. and Waters, J.F. (1963). J. Bact. 86, 1339.

Bladen, H.A., Nylen, M.U. and Fitzgerald, R.J. (1964). J. Bact. 88, 763.

Boatman, E.S. (1964). J. Cell Biol. 20, 297.

Boatman, E.S. and Douglas, H.C. (1961). J. biophys. biochem. Cytol. 11, 469.

Boatman, E.S. and Kenny, G.E. (1970). J. Bact. 101, 262.

Boll, M. (1969). Arch. Mikrobiol. 68, 191.

Boll, M. (1970a). Experimentia, 15, 957.

Boll, M. (1970b). Arch. Mikrobiol. 71, 1.

Bradley, D.E. and Dewar, C.A. (1967). J. gen. Virol. 1, 179.

Bradley, S.G. and Ritzi, D. (1968). J. Bact. 95, 2358.

Branton, D. (1966). Proc. natn. Acad. Sci. U.S.A. 55, 1048.

Branton, D. (1967). Expl. Cell Res. 45, 703.

Branton, D. (1969). A. Rev. Pl. Physiol. 20, 209

Branton, D. (1971). Phil. Trans. R. Soc. Ser. B., 261, 133.

Branton, D. and Park, R.B. (1967). J. Ultrastruct. Res. 19, 283.

Brenner, S. and Horne, R.W. (1959). Biochim. biophys. Acta, 34, 103.

Bretscher, M.S. (1971a). J. mol. Biol. 59, 351.

Bretscher, M.S. (1971b). J. mol. Biol. 58, 775.

Bretscher, M.S. (1971c). Nature Lond. 231, 229.

Bretscher, M.S. (1972). Nature Lond. 236, 11.

Brieger, E.M. (1963). In Structure and Ultrastructure of Microorganisms, p. 211. Ed. by Brieger, E.M. London : Academic Press Inc.

Brieger, E.M., Glauert, A.M. and Allen, J.M. (1959). Expl. Cell Res. 18, 418.

Briles, E.B. and Tomasz, A. (1970). J. Cell Biol. 47, 786.

Brinton, L.P. and Burgdorfer, W. (1970). J. Bact. 105, 1149.

Brock, T.D. and Edwards, M.R. (1970). J. Bact. 104, 509.

Brown, J.P., Edwards, M.R. and Van Demark, P.J. (1968). Can. J. Microbiol. 14, 823.

Brumfitt, W. (1959). Br. J. exp. Pathol. 40, 441.

Brumfitt, W., Wardlaw, A.C. and Park, J.T. (1958). Nature, Lond., 181, 1783.

Buckmire, L.A. and Murray, R.G.E. (1970). Can. J. Microbiol. 16, 1011.

Bulger, R.J. and Bulger, R.E. (1967). J. Bact. 94, 1244.

Burdett, I.D.J. and Rogers, H.J. (1970). J. Ultrastruct. Res. 30, 354.

Burdett, I.D.J. and Rogers, H.J. (1972). J. Ultrastruct. Res. 38, 113.

Burnham, J.C., Hashimoto, T. and Conti, S.F. (1968). J. Bact. 96, 1366.

Burnham, J.C., Hashimoto, T. and Conti, S.F. (1970). J. Bact. 101, 997.

Burrell, A.D., Feldschreiber, P. and Dean, C.J. (1971). Biochim. biophys. Acta, 247, 38.

Burton, K. (1956). Biochem. J. 62, 315.

Butler, T.F., Smith, G.L. and Grula, E.A. (1967). Can. J. Microbiol. 13, 1471.

Campbell, J.N., Leyh-Bouille, M. and Ghuysen, J.-M. (1969). Biochemistry, 8, 193.

Carbonell, L.M. (1967). J. Bact. 94, 213.

Carbonell, L.M. and Pollak, L. (1962). J. Bact. 83, 1356.

Cagle, G.D., Vela, G.R. and Plister, R.M. (1972). J. Bact. 109, 1191.

Carrick, L. and Berk, R.S. (1971). J. Bact. 106, 250.

Chalcroft, J.P. and Bullivant, S. (1970). J. Cell Biol. 47, 49.

Chapman, G.B. (1956). J. Bact. 71, 348.

Chapman, G.B. (1959). J. biophys. biochem. Cytol. 6, 221.

Chapman, G.B. and Hillier, J. (1953). J. Bact. 66, 367.

Chapman, G.B. and Kroll, A.J. (1957). J. Bact. 73, 63.

Chapman, G.B., Hanks, J.H. and Wallace, J.H. (1959). J. Bact. 77, 205.

Chen, P.L. (1964). Am. J. Bot. 51, 125.

Cho, K.Y. and Salton, M.R.J. (1964). Biochim. biophys. Acta, 84, 773.

Cho, K.Y., Corpe, W.A. and Salton, M.R.J. (1964). Biochem. J. 93, 26C.

Cho, K.Y., Doy, C.H. and Mercer, E.H. (1967). J. Bact. 94, 196.

Clark, A.W. and Branton, D. (1968). Z. Zellforsch. mikrosk. Anat. 91, 586.

Claus, G.W. and Roth, L.E. (1964). J. Cell Biol. 20, 217.

Cobet, A.B. and Jones, G.E. (1971). J. gen. Microbiol. 66, 185.

Cohen-Bazire, G. and Kunisawa, R. (1963). J. Cell Biol. 16, 401.

Cohen-Bazire, G. and Sistrom, W.R. (1966). In The Chlorophylls, p. 313. Ed. by Vernon, L.P. and Seely, R. London : Academic Press.

Cohen-Bazire, G., Kunisawa, R. and Poindexter, J.S. (1966). J. gen. Microbiol. 42, 301.

Cohen-Bazire, G., Phennig, N. and Kunisawa, R. (1964). J. Cell Biol. 22, 207.

Cole, R.M. (1965). Bact. Rev. 29, 326.

Cole, R.M., Popkin, T.J., Boylan, R.J. and Mendleson, N.H. (1970). J. Bact. 103, 793.

Conti, S.F., Jacobs, N.J. and Gray, C.T. (1968). J. Bact. 96, 554.

Costerton, J.W., Murray, R.G.E. and Robinow, C.F. (1961). Can. J. Microbiol. 7, 329.

Cota-Robles, E.H. (1963). J. Bact. 85, 499.

Cota-Robles, E.H. (1966). J. Ultrastruct. Res. 16, 626.

Criswell, B.S., Stenback, W.A., Black, S.H. and Gardner, H.L. (1972). J. Bact. 109, 930.

Cummings, C.S. and Harris, H. (1956). J. gen. Microbiol. 14, 583.

Cutinelli, C., Galdiero, F. and Tufano, M.A. (1969). J. Bact. 100, 123.

Czerkowski, J.W., Perkins, H.R. and Rogers, H.J. (1963). Biochem. J. 86, 468.

Danco-Moore, L. and Higgins, M.L. (1972). J. Bact. 109, 1210.

Daniels, M.J. (1971). Biochem. J. 122, 197.

D'aoust, J.Y. and Kushner, D.J. (1971). J. Bact. 108, 916.

Darling, S., Theilade, J. and Birch-Anderson, A. (1969). J. Bact. 98, 797.

Dart, P.J. and Mercer, F.V. (1963). Arch. Mikrobiol. 47, 1.

Davies, S.L. and Whittenbury, R. (1970). J. gen. Microbiol. 61, 227.

Davis, B.J. (1964). Ann. N.Y. Acad. Sci. 121, 404.

Davy, J.G. and Branton, D. (1970). Science, N.Y., 168, 1216.

De Boer, W.E. and Hazeu, W. (1972). Antonie van Leeuwenhoek J. Microbiol. Serol. 38, 33.

De Petris, S., Karlsbad, G. and Kessel, R.W.I. (1964). J. gen. Microbiol. 35, 373.

De Siervo, A.J. and Salton, M.R.J. (1971). Biochim. biophys. Acta, 239, 280.

De Vito, E. and Santome, J.A. (1966). Experientia, 22, 124.

DeVoe, I.W., Costerton, J.W. and MacLeod, R.A. (1971). J. Bact. 106, 659.

DeVoe, I.W., Thompson, J., Costerton, J.W. and MacLeod, R.A. (1970). J. Bact. 101, 1014.

Deamer, D.W. and Branton, D. (1967). Science, N.Y., 158, 655

Deamer, D.W., Leonard, R., Tardieu, A. and Branton, D. (1970). Biochim. biophys. Acta, 219, 47.

Dietrich, C.P., Colucci, A.V. and Strominger, J.L. (1967). J. biol. Chem. 242, 3218.

Dische, Z. and Schwarz, K. (1937). Mikrochim. Acta, 2, 13.

Dixon, R.O.D. (1964). Arch. Mikrobiol. 48, 166.

Eberle, H. and Lark, K.G. (1966). J. mol. Biol. 22, 187.

Edwards, M.R. and Stevens, R.W. (1963). J. Bact. 86, 414.

Ellar, D.J. (1969). J. gen. Microbiol. 57, vi.

Ellar, D.J. and Freer, J.H. (1969). J. gen. Microbiol. 58, vii.

Ellar, D.J. and Lundgren, D.G. (1966). J. Bact. 92, 1748.

Ellar, D.J., Lundgren, D.G. and Slepecky, R.A. (1967). J. Bact. 94, 1189.

Ellar, D.J., Munoz, E. and Salton, M.R.J. (1971). Biochim. biophys. Acta, 225, 140.

Ellar, D.J., Thomas, T.D. and Postgate, S.A. (1971). Biochem. J. 122, 44P.

Ellison, J.S., Mattern, C.F.T. and Daniel, W.A. (1971). J. Bact. 108, 526.

Ells, H.A. (1959). Archs Biochem. Biophys. 85, 561.

Engstrom, L.H. (1970). Reported by Branton, D. (1971). Phil. Trans. R. Soc. Ser. B. 261, 133.

Estrugo, S.F., Larraga, V., Corrales, M.A., Duch, C. and Munoz, E. (1972). Biochim. biophys. Acta, 255, 960.

Fan, D.P. (1970). J. Bact. 103, 488.

Fan, D.P., Beckman, M.M. and Cunningham, W.P. (1972). J. Bact. 109, 1247.

Fan, D.P., Pelvit, M.C. and Cunningham, W.P. (1972). J. Bact. 109, 1266.

Farshtchi, D. and McClung, N.M. (1967). J. Bact. 94, 255.

Felter, R.A., Colwell, R.R. and Chapman, G.B. (1969). J. Bact. 99, 326.

Felter, R.A., Kennedy, S.F., Colwell, R.R. and Chapman, G.B. (1970). J. Bact. 102, 552.

Ferrandes, B., Chaix, P. and Ryter, A. (1966). C.r.hebd. Seanc. Acad. Sci., Paris, 263, 1632.

Ferrandes, B., Frehel, C. and Chaix, P. (1970). Biochim. biophys. Acta, 223, 292.

Fiil, A. and Branton, D. (1969). J. Bact. 98, 1320.

Fischman, D.A. and Weinbaum, G. (1967). J. Cell Biol. 32, 524.

Fitz-James, P.C. (1960). J. biophys. biochem. Cytol. 8, 507.

Fitz-James, P.C. (1962). J. Bact. 84, 104.

Fitz-James, P.C. (1964a). J. Bact. 87, 1202.

Fitz-James, P.C. (1964b). J. Bact. 87, 1477.

Fitz-James, P.C. (1964c). J. Bact. 87, 1483.

Fitz-James, P.C. (1965). Symp. Soc. gen. Microbiol. 15, 369.

Fitz-James, P.C. (1967). In Protides of the Biological Fluids, vol. 15, p. 289. Ed. by Peeters, H. Amsterdam : Elsevier Publishing Co.

Fitz-James, P.C. (1968). In Microbial Protoplasts, Spheroplasts and L-forms, p. 124. Ed. by Guze, L.B. Baltimore : Williams and Wilkins Co.

Fitz-James, P.C. (1970). J. Bact. 105, 1119.

Fitz-James, P.C. and Hancock, R. (1965). J. Cell Biol. 26, 657.

Flower, N.E. (1971). J. Cell Sci. 9, 435.

Fox, C.F., Law, J.H., Tsukagoshi, N. and Wilson, G. (1970). Proc. natn. Acad. Sci. U.S.A. 67, 598.

Frasca, J.M. and Parks, V.R. (1965). J. Cell Biol. 25, 157.

Freer, J.H. and Levinson, H.S. (1967). J. Bact. 94, 441.

Freer, J.H., Kim, K.S., Krauss, M.R., Beaman, L. and Barksdale, L. (1969). J. Bact. 100, 1062.

Freese, E.B., Cole, R.M., Klofat, W. and Freese, E. (1970). J. Bact. 101, 1046.

Frehel, C. and Ryter, A. (1972). J. Microscopie, 12, 59.

Frehel, C., Ferrandes, B. and Ryter, A. (1971a). Biochim. biophys. Acta, 234, 226.

Frehel, C., Beaufils, A.-M. and Ryter, A. (1971b). Annls. Inst. Pasteur, Paris, 121, 139.

Friedman, B.A., Dugan, P.R., Pfister, R.M. and Remsen, C.C. (1969). J. Bact. 98, 1328.

Fukui, Y., Nachbar, M.S. and Salton, M.R.J. (1971a). J. Bact. 105, 86.

Fukui, Y., Nachbar, M.S. and Salton, M.R.J. (1971b). Biochim. biophys. Acta, 241, 30.

Garrison, R.G., Lane, J.W. and Field, M.F. (1970). J. Bact. 101, 628.

Gel'man, N.S., Lukoyanova, M.A. and Oparin, A.I. (1960). Biokhimiya, 25, 481.

Gel'man, N.S., Lukoyanova, M.A. and Ostrovskii, D.N. (1967). In Respiration and Phosphorylation of Bacteria. Ed. by Pinchot, G.B. New York : Plenum Press.

Gel'man, N.S., Zhukova, I.G. and Oparin, A.I. (1963). Biokhimiya, 28, 95.

Gel'man, N.S., Zhukova, I.G., Lukoyanova, M.A. and Oparin, A.I. (1959). Biokhimiya, 24, 481.

Gel'man, N.S., Tikhonova, G.V., Simakova, I.M. Lykoyanova, M.A., Taptykova, S.D. and Mikelsaar, H.M. (1970). Biochim. biophys. Acta, 223, 321.

Ghosh, B.K. and Murray, R.G.E. (1967). J. Bact. 93, 411.

Ghosh, B.K. and Murray, R.G.E. (1969). J. Bact. 97, 426.

Ghosh, B.K., Lampen, J.O. and Remsen, C.C. (1969). J. Bact. 100, 1002.

Ghosh, B.K., Sargent, M.G. and Lampen, J.O. (1968). J. Bact. 96, 1314.

Ghosh, B.K., Wouters, J.T.M. and Lampen, J.O. (1971). J. Bact. 108, 928.

Ghuysen, J.-M. (1968). Bact. Rev. 32, 425.

Ghuysen, J.-M. and Salton, M.R.J. (1960). Biochim. biophys. Acta, 40, 462.

Ghuysen, J.-M., Bricas, E., Lache, M. and Leyh-Bouille, M. (1968). Biochemistry, N.Y., 7, 1450.

Gibbs, S.P., Sistrom, W.R. and Warden, P.B. (1965). J. Cell Biol. 26, 395.

Gibson, K.D. (1965). J. Bact. 90, 1059.

Giesbrecht, P. (1960). Zentbl. Bakt. Parasitkde (Abt 1) 179, 538.

Giesbrecht, P. and Drews, G. (1962). Arch. Mikrobiol. 43, 152.

Giesbrecht, P. and Drews, G. (1966). Arch. Mikrobiol. 54, 297.

Gilby, A.R. and Few, A.V. (1958). Nature, Lond., 182, 55.

Gilby, A.R., Few, A.V. and McQuillen, K. (1958). Biochim. biophys. Acta, 29, 21.

Gilleland, H.E., Roth, I.L. and Eagon, R.G. (1971). Can. J. Microbiol. 71, 421.

Glauert, A.M. (1962). Br. med. Bull. 18, 245.

Glauert, A.M. and Glauert, R.H. (1958). J. biophys. biochem. Cytol. 4, 191.

Glauert, A.M. and Hopwood, D.A. (1959). J. biophys. biochem. Cytol. 6, 515.

Glauert, A.M. and Hopwood, D.A. (1960). J. biophys. biochem. Cytol. 7, 479.

Glauert, A.M. and Hopwood, D.A. (1961). J. biophys. biochem. Cytol. 10, 505.

Glauert, A.M., Brieger, E.M. and Allen, J.M. (1961). Expl. Cell Res. 22, 73.

Gol'dfel'd, M.G., Ostrovskii, D.N. and Rozantsev, E.G. (1970). Dokl. Akad. Nauk USSR, 191, 702.

Gordon, M.A. and Edwards, M.R. (1963). J. Bact. 86, 1101.

Gornall, A.G., Bardawill, C.J. and David, M.M. (1949). J. biol. Chem. 177, 751.

Granboulan, P. and Leduc, E.H. (1967). J. Ultrastruct. Res. 20, 111.

Green, D.H. and Salton, M.R.J. (1970). Biochim. biophys. Acta, 211, 139.

Griffith, I.P. (1972). Biochem. J. 126, 553.

Griffiths, D.A. (1971). Arch. Mikrobiol. 76, 74.

Grula, E.A. and King, R.D. (1971). Biochem. biophys. Res. Commun. 44, 1356.

Grula, E.A. and Savoy, C.F. (1971). Biochem. biophys. Res. Commun. 43, 325.

Grula, E.A., Luk, Sh.-K. and Chu, Y.-C. (1961). Can. J. Microbiol. 7, 27.

Grula, E.A., Butler, T.F., King, R.D. and Smith, G.L. (1967). Can. J. Microbiol. 13, 1499.

Haidle, C.W., Brinkley, B.R. and Mandel, M. (1970). J. Bact. 102, 835.

Hall, W.T. and Claus, G. (1961). Protoplasma, 54, 356.

Hard, G.C. (1969). J. Bact. 97, 1480.

Hatten, B.A., Schulze, M.L., Huang, S.Y. and Salkin, S.E. (1969). J. Bact. 99, 611.

Henning, U., Dennert, G., Rehn, K. and Deppe, G. (1969). J. Bact. 98, 784.

Hereward, F.V. and Northcate, D.H. (1972). J. Cell Sci. 10, 555.

Hess, R. and Dietrich, F.M. (1960). J. biophys. biochem. Cytol. 8, 546.

Hiatt, A.J. (1961). Pl. Physiol., Lancaster, 36, 552.

Hickman, D.D. and Frenkel, A.W. (1959). J. biophys. biochem. Cytol. 6, 277.

Hickman, D.D. and Frenkel, A.W. (1965). J. Cell Biol. 25, 261.

Higashi, Y., Strominger, J.L. and Sweeley, C.C. (1967). Proc. natn. Acad. Sci.U.S. 57, 1878.

Higgins, M.L. and Daneo-Moore, L. (1972). J. Bact. 109, 1221.

Higgins, M.L. and Shockman, G.D. (1970a). J. Bact. 101, 643.

Higgins, M.L. and Shockman, G.D. (1970b). J. Bact. 103, 244.

Higgins, M.L., Pooley, H.M. and Shockman, G.D. (1970). J. Bact. 103, 504.

Higgins, M.L., Pooley, H.M. and Shockman, G.D. (1971). J. Bact. 105, 1175.

Highton, P.J. (1969). J. Ultrastruct.Res. 26, 130.

Highton, P.J. and Hobbs, D.G. (1971). J. Bact. 106, 646.

Highton, P.J. and Hobbs, D.G. (1972). J. Bact. 109, 1181.

Hirota, Y., Ryter, A. and Jacob, F. (1968). In Cold Spring Harbor Symposium on Quantitative Biology, vol. 33, p. 677. New York : Long Island Biological Association.

Hoeniger, J.F.M. and Headley, C.L. (1968). J. Bact. 96, 1835.

Hoeniger, J.F.M., Stuart, P.F. and Holt, S.C. (1968). J. Bact. 96, 1818.

Hoeniger, J.F.M., Van Iterson, W. and Van Zanten, E.N. (1966). J. Cell Biol. 31, 603.

Hofstad, T. and Selvig, K.A. (1969). J. gen. Microbiol. 56, 23.

Hohl, H.R. (1965). J. Bact. 90, 755.

Holligan, P.M. (1971). New Phytol. 70, 239.

Holt, S.C. and Canale-Parola, E. (1967). J. Bact. 93, 399.

Holt, S.C. and Leadbetter, E.R. (1969). Bact. Rev. 33, 346.

Holt, S.C. and Marr, A.G. (1965). J. Bact. 89, 1402.

Holt, S.C., Conti, S.F. and Fuller, R.C. (1966a). J. Bact. 91, 311.

Holt, S.C., Conti, S.F. and Fuller, R.C. (1966b). J. Bact. 91, 349.

Hopwood, D.A. and Glauert, A.M. (1960). J. biophys. biochem. Cytol. 8, 267.

Hoshino, O., Zehavi, U., Sinay, P. and Leanloz, R.W. (1972). J. biol. Chem. 247, 381.

Huang, P.-Y. and Goodman, R.N. (1970). J. Bact. 102, 862.

Huang, P.-Y. and Goodman, R.N. (1971). J. Bact. 107, 361.

Hugget, A. St.-G. and Nixon, D.A. (1957). Biochem. J. 66, 12P.

Hughes, R.C. and Stokes, E. (1971). J. Bact. 106, 694.

Hurst, A. and Stubbs, J.M. (1969). J. Bact. 97, 1466.

Hyde, J.M. and Walkinshaw, C.H. (1966). J. Bact. 92, 1218.

Ikawa, M. and Nieman, C. (1949). J. biol. Chem. 180, 923.

Imaeda, T. and Convit, J. (1962). J. Bact. 83, 43.

Imaeda, T. and Ogura, M. (1963). J. Bact. 85, 150.

Ishiguro, E.E. and Wolfe, R.S. (1970). J. Bact. 104, 566.

Ishikawa, S. (1970). J. Biochem. 67, 297.

Ito, S. and Vinson, J.W. (1965). J. Bact. 89, 481.

Jackson, S. and Black, S.H. (1970). Arch. Mikrobiol. 76, 308.

Jackson, F.L. and Lawton, V.D. (1959). Biochim. biophys. Acta, 35, 76.

Jacob, F., Brenner, S. and Cuzin, F. (1963). In Cold Spring Harbor Symposium on Quantitative Biology, vol. 28, p. 329. New York : Long Island Biological Association.

Jacob, F., Ryter, A. and Cuzin, F. (1966). Proc. R. Soc. (Ser. B. biol. Sci.) 164, 267.

James, R. and Branton, D. (1971). Biochim. biophys. Acta, 233, 504.

Kakefuda, T., Holden, J.T. and Utech, N.M. (1967). J. Bact. 93, 472.

Kats, L.N. and Kharat'yan, E.F. (1969). Microbiology, 38, 229.

Kats, L.N. and Moroz, A.F. (1968). Microbiology, 37, 78.

Katz, W. and Strominger, J.L. (1967). Biochemistry, N.Y., 6, 930.

Kats, L.N. and Tordzhyan, I.Kh. (1968). Microbiology, 37, 745.

Katz, W., Matsuhashi, M., Dietrich, C.P. and Strominger, J.L. (1967). J. biol. Chem. 242, 3207.

Kavanau, J.L. (1966). Fedn. Proc. Fedn. Am. Socs. exp. Biol. 25, 1096.

Kawata, T. (1963). J. gen. appl. Microbiol., Tokyo, 9, 1.

Kawata, T. and Inoue, T. (1964). Jap. J. Microbiol. 8, 49.

Kawata, T. and Inoue, T. (1965a). J. Bact. 89, 1613.

Kawata, T. and Inoue, T. (1965b). Jap. J. Microbiol. 9, 101.

Kaye, J.J. and Chapman, G.B. (1963). J. Bact. 86, 536.

Kellenberger, E., Ryter, A. and Sechaud, J. (1958). J. biophys. biochem. Cytol. 4, 671.

Kim, K.S. and Barksdale, L. (1969). J. Bact., 98, 1390.

Kim, I.C. and Bragg, P.D. (1971). Can. J. Biochem. 10, 1098.

Kohiyama, M., Cousin, D., Ryter, A. and Jacob, F. (1966). Annls. Inst. Pasteur, Paris, 110, 465.

Koike, M. and Takeya, K. (1961). J. biophys. biochem. Cytol. 9, 597.

Kojima, M., Suda, S., Hotta, S., Hamada, K. and Suganuma, A. (1970). J. Bact. 104, 1010.

Kornberg, R.D. and McConnell, H.M. (1971). Biochemistry, N.Y. 10, 1111.

Kozar, F. and Weijer, J. (1969). J. Genet. Cytol. 11, 613.

Kusaka, I. (1971). J. gen. Microbiol. 63, 199.

Lahav, M., Chiu, T.H. and Lennarz, W.J. (1969). J. biol. Chem. 244, 5890.

Lampen, J.O. (1965). Symp. Soc. gen. Microbiol. 15, 115.

Lampen, J.O. (1967a). J. gen Microbiol. 48, 249.

Lampen, J.O. (1967b). J. gen. Microbiol. 48, 261.

Landman, O.E. and Halle, S. (1963). J. mol. Biol. 7, 721.

Landman, O.E., Ryter, A. and Frehel, C. (1968). J. Bact. 96, 2154.

Lane, J.W., Garrison, R.G. and Field, M.F. (1969). J. Bact. 100, 1010.

Langenberg, K.F., Bryant, M.P. and Wolfe, R.S. (1968). J. Bact. 95, 1124.

Lastras, M. and Munoz, E. (1971). FEBS Lett. no. 14, p.69.

Lastras, M. and Munoz, E. (1972). FEBS Lett. no. 21, p. 109.

Leadbetter, E.R. and Holt, S.C. (1968). J. gen. Microbiol. 52, 299.

Leene, W. and Van Iterson, W. (1965). J. Cell Biol. 27, 241.

Leiva, S. and Carbonell, L.M. (1970). J. gen. Microbiol. 62, 43.

Lennarz, W.J. (1964). J. biol. Chem. 239, PC3110.

Lennarz, W.J. and Talamo, B. (1965). Fedn. Proc. 24, 479.

Lennarz, W.J. and Talamo, B. (1966). J. biol. Chem. 241, 2707.

Leyh-Bouille, M., Ghuysen, J.-M., Tipper, D.J. and Strominger, J.L. (1966). Biochemistry, 5, 3079.

Lisenkova, L.L. and Mokhova, E.N. (1964). Mikrobiologiya, 33, 918.

Listgarten, M., Loesche, W. and Socransky, S. (1963). J. Bact. 85, 933.

Lui, T.Y. and Gotschlich, E.C. (1967). J. biol. Chem. 242, 471.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). J. biol. Chem. 193, 265.

Lucy, J.A. (1964). J. theor. Biol. 7, 360.

Ludyic, J. (1964). In Electron Microscopy, Czech. Acad. Sci., p. 533. Prague. From Gel'man, N.S., Lukoyanova, M.A. and Ostrovskii, D.N. (1967). In Respiration and Phosphorylation of Bacteria, p. 5. Ed. by Pinchot, G.B. Maryland : Plenum Press.

Luft, J.H. (1961). J. biophys. biochem. Cytol. 9, 409.

Lukoyanova, M.A. and Biryuzova, V.I. (1965). Biokhimiya, 30, 529.

Lukoyanova, M.A. and Taptykova, S.D. (1968). Biokhimiya, 33, 888.

Lukoyanova, M.A., Gel'man, N.S. and Biryuzova, V.I. (1961). Biokhimiya, 26, 916.

Lukoyanova, M.A., Biryuzova, V.I., Simakova, I.M. and Gel'man, N.S. (1967). Biokhimiya, 32, 816.

Lundgren, D.G. and Remson, C.C. (1966). J. Bact. 91, 2096.

MacFarlane, M.G. (1961a). Biochem. J. 79, 4P.

MacFarlane, M.G. (1961b). Biochem. J. 80, 45P.

MacFarlane, M.G. (1962). Nature, Lond., 196, 136.

MacFarlane, M.G. (1964). In Metabolism and Physiological Significance of Lipids, p. 399. Ed. by Dawson, R.M.C. and Rhodes, D.N. London : Wiley & Sons Ltd.

Mackey, B.M. and Morris, J.G. (1970). J. gen. Microbiol. 66, 1.

McNiven, A.C., Owen, P. and Arbuthnott, J.P. (1972). J. med. Microbiol. 5, 113.

Mahoney, R.P. and Edwards, M.R. (1966). J. Bact. 92, 487.

Matheson, A. and Donaldson, D.M. (1968). J. Bact. 95, 1392.

Mauk, J. and Glaser, L. (1972). J. biol. Chem. 247, 1180.

Mauk, J., Chan, L., Glaser, L. and Williamson, J. (1972). J. Bact. 109, 373.

Mazanec, K., Kocur, M. and Martinec, T. (1965). J. Bact. 90, 808.

Mazanec, K., Kocur, M. and Martinec, T. (1966). Can. J. Microbiol. 12, 465.

Meyer, H.W. and Richter, W. (1971). Z. allg. Mikrobiol. 11, 397.

Mirelman, D. and Sharon, M. (1967). J. biol. Chem. 242, 3414.

Mitchell, P. (1963). Symp. Biochem. Soc. 22, 142.

Mitruka, B.M., Costilow, R.N., Black, S.H. and Pepper, R.E. (1967). J. Bact. 94, 759.

Moberly, B.J., Shafa, F. and Gerherdt, P. (1966). J. Bact. 92, 220.

Montes, L.F. and Black, S.H. (1967). Experientia, 23, 345.

Montague, M.D. and Moulds, J.D. (1967). Biochim. biophys. Acta, 135, 565.

Moor, H. (1964). Z. Zellforsch. mikrosk. Anat. 62, 546.

Moor, H. (1966). J. Cell Biol. 29, 153.

Moor, H. (1969). Int. Rev. Cytol. 25, 391.

Moor, H. (1971). Phil. Trans. R. Soc. Scr. B, 261, 133.

Moor, H. and Muhlethalar, K. (1963). J. Cell Biol. 17, 609.

Moor, H., Muhlethaler, K., Waldner, H. and Frey-Wyssling, A. (1961). J. biophys. biochem. Cytol. 10, 1.

Moore, R.T. and Chapman, G.B. (1959). J. Bact. 78, 878.

Morgan, C., Rosenkranz, H.S., Carr, H.S. and Rose, H.M. (1967). J. Bact. 93, 1987.

Morris, D.L. (1948). Science, N.Y. 107, 254.

Morrison, D.C. and Morowtiz, H.J. (1970). J. mol. Biol. 49, 441.

Mudd, S. (1956). Bact. Rev. 20, 268.

Mudd, S., Winterscheid, L.C., De Lamater, E.D. and Henderson, H.J. (1951). J. Bact. 62, 459.

Mudd, S., Kawata, T., Payne, J.I., Sall, T. and Takagi, A. (1961). Nature, Lond., 189, 79.

Mudd, S., Brodic, A.F., Winterscheid, L.C., Hartman, P.E., Beutner, E.H. and McLean, R.A. (1951). J. Bact. 62, 729.

Muhlethaler, K., Moor, H. and Szarkowsky, J.W. (1965). Planta, 67, 305.

Munoz, E., Salton, M.R.J. and Ellar, D.J. (1969). In Membranes, Structure and Function, report of 6th Meeting FEBS, vol. 20, p. 51. Ed. by Villanueva, J.R. and Ponz, F. London : Academic Press.

Munoz, E., Freer, J.H., Ellar, D.J. and Salton, M.R.J. (1968). Biochim. biophys. Acta, 150, 531.

Munoz, E., Nachbar, M.S., Schor, M.T. and Salton, M.R.J. (1968). Biochem. biophys. Res. Commun. 32, 539.

Munoz, E., Salton, M.R.J., Ng, M.H. and Schor, M.T. (1969). Eur. J. Biochem. 7, 490.

Murray, R.G.E. (1962). Symp. Soc. gen. Microbiol. 12, 119.

Murray, R. (1963). From Gel'man, N.S., Lukoyanova, M.A. and Ostrovskii, D.N. (1967). In Respiration and Phosphorylation of Bacteria, p. 10. Ed. by Pinchot, G.B. New York : Plenum Press.

Murray, R.G.E. and Birch-Anderson, A. (1963). Can. J. Microbiol. 9, 393.

Murray, R.G.E. and Watson, S.W. (1965). J. Bact. 89, 1594.

Muscatello, U. and Horne, R.W. (1968). J. Ultrastruct. Res. 25, 73.

Nachbar, M.S. and Salton, M.R.J. (1970a). Biochim. biophys. Acta, 223, 309.

Nachbar, M.S. and Salton, M.R.J. (1970b). In Surface Chemistry of Biological Systems, p. 175. Ed. by Blank, M. New York : Plenum Press.

Nanninga, N. (1968). J. Cell Biol. 39, 251.

Nanninga, N. (1969). J. Cell Biol. 42, 733.

Nanninga, N. (1970). J. Bact. 101, 297.

Nanninga, N. (1971a). J. Cell Biol. 48, 219.

Nanninga, N. (1971b). J. Cell Biol. 49, 564.

Nauman, R.K., Holt, S.C. and Cox, C.D. (1969). J. Bact. 98, 264.

Neale, E.K. and Chapman, G.B. (1970). J. Bact. 104, 518.

Necas, O., Kopecka, M. and Brichta, J. (1969). Expl. Cell Res. 58, 411.

Nermut, M. and Ryc, M. (1964). Folia Microbiol. 9, 16.

North, R.J. (1963). J. Ultrastruct. Res. 9, 187.

Oelze, J. and Drews, G. (1972). Biochim. biophys. Acta, 265, 209.

Ohnishi, Y. (1971). J. Bact. 107, 918.

Ohnishi, Y. and Kuwano, M. (1971). J. Virol. 7, 673.

Ohye, D.F. and Murrell, W.G. (1962). J. Cell Biol. 14, 111.

Op den Kamp, J.A.F., Van Iterson, W. and Van Deenan, L.L.M. (1967) Biochim. biophys. Acta, 135, 862.

Oparin, A.I., Lukoyanova, M.A., Shvets, V.I., Gel'man, N.S. and Torkhovskaya, T.I. (1965). Zh. Evolyuts. Biokhim. Fiziol. 1, 7.

Oppenheim, J. and Marcus, L. (1970). J. Bact, 101, 286.

Ostrovskii, D.N. and Gel'man, N.S. (1963). Dokl. Akad. Nauk SSSR, 148, 945.

Ostrovskii, D.N. and Gel'man, N.S. (1965). Biokhimiya, 30, 772.

Ostrovskii, D.N., Tsfasman, I.M. and Gel'man, N.S. (1969). Biokhimiya, 34, 993.

Overman, J.R. and Pine, L. (1963). J. Bact. 86, 656.

Owen, P. and Freer, J.H. (1970a). J. gen. Microbiol. 60, xi.

Owen, P. and Freer, J.H. (1970b). Biochem. J. 120, 237.

Owen, P. and Freer, J.H. (1972). Biochem. J.

Pangborn, J., Marr, A.G. and Robrish, S.A. (1962). J. Bact. 84, 669.

Patch, C.T. and Landman, O.E. (1971). J. Bact. 107, 345.

Pate, K.L. and Ordal, E.J. (1967). J. Cell Biol. 35, 1.

Patterson, P.H. and Lennarz, W.J. (1971). J. biol. Chem. 246, 1062.

Park, R.B. and Branton, D. (1966). Reported by Branton, D. (1969). A. Rev. Pl. Physiol. 20, 209.

Partridge, S.M. (1948). Biochem. J. 42, 238.

Perkins, H.R. (1960). Biochem. J. 74, 182.

Perkins, H.R. (1963). Biochem. J. 86, 675.

Perkins, H.R. and Rogers, H.J. (1959). Biochem. J. 72, 647.

Petitprez, A., Roos, P. and Tacquet, A. (1967). J. Microscopie, 6, 229.

Pfister, R.M. and Lundgren, D.G. (1964). J. Bact. 88, 1119.

Phillips, D.R. and Morrison, M. (1971). Biochemistry, N.Y., 10, 1766.

Pinto da Silva, P., Douglas, S.D. and Branton, D. (1971). Nature, Lond., 232, 194.

Poindexter, J.L.S. and Cohen-Bazire, G. (1964). J. Cell Biol. 23, 587.

Pollock, J.J., Linder, R. and Salton, M.R.J. (1971). J. Bact. 107, 230.

Pontefract, R.D. and Thatcher, F.S. (1970). J. Ultrastruct. Res. 30, 78.

Pontefract, R.D., Bergeron, G. and Thatcher, F.S. (1969). J. Bact. 97, 367.

Pooley, H.M. and Shockman, G.D. (1969). J. Bact. 100, 617.

Pooley, H.M., Shockman, G.D., Higgins, M.L. and Porres-Juan, J. (1972). J. Bact. 109, 423.

Pope, L.M., Hoare, D.S. and Smith, A.J. (1969). J. Bact. 97, 936.

Popkin, T.J., Theodore, T.S. and Cole, R.M. (1971). J. Bact. 107, 907.

Prasad, A.L.N. and Litwack, G. (1965). Biochemistry, N.Y., 4, 496.

Proctor, H.M., Norris, J.R. and Ribbons, D.W. (1969). J. appl. Bact. 32, 118.

Qadri, S.M.H. and Hoare, D.S. (1968). J. Bact. 95, 2344.

Rancourt, M.W. and Lechevalier, H.A. (1964). Can. J. Microbiol. 10, 311.

Ranhand, J.M., Leonard, C.G. and Cole, R.M. (1971). J. Bact. 106, 257.

Ray, A., Reynolds, J.A., Polet, H. and Steinhardt, J. (1966). Biochemistry, N.Y., 5, 2606.

Raymond, J.C. and Sistrom, W.R. (1967). Arch. Mikrobiol. 59, 255.

Reaveley, D.A. (1968). Biochem. biophys. Res. Commun. 30, 649.

Reaveley, D.A. and Rogers, H.J. (1969). Biochem. J. 113, 67.

Reichenbach, H., Voelz, H. and Dworkin, M. (1969). J. Bact. 97, 905.

Remsen, C.C. (1966). Arch. Mikrobiol. 54, 266.

Remsen, C.C. (1968). Arch. Mikrobiol. 61, 40.

Remsen, C.C. and Lundgren, D.G. (1965). J. Bact. 90, 1426.

Remsen, C.C. and Lundgren, D.G. (1966). J. Bact. 92, 1765.

Remsen, C.C., Lundgren, D.G. and Slepecky, R.A. (1966). J. Bact. 91, 324.

Remsen, C.C., Valois, F.W. and Watson, S.W. (1967). J. Bact. 94, 422.

Remsen, C.C., Watson, S.W. and Truper, H.G. (1970). J. Bact. 103, 254.

Remsen, C.C., Watson, S.W., Waterbury, J.B. and Truper, H.G. (1968). J. Bact. 95, 2374.

Reynolds, J.A. and Tanford, C. (1970). Proc. nat. Acad. Sci. U.S.A. 66, 1002.

Reynolds, J.A., Herbert, S., Polet, H. and Steinhardt, J. (1967). Biochemistry, N.Y., 6, 937.

Richter, G.W. and Kress, Y. (1967). J. Bact. 94, 1216.

Ritchie, A.E. and Ellinghausen, H.C. (1965). J. Bact. 89, 223.

Roberts, I.M. (1970). J. Microscopy, 92, 57.

Robinow, C.F. (1962). Circulation, 26, 1092.

Rogers, H.J. (1970). Bact. Rev. 34, 194.

Rogers, H.J. and Perkins, H.R. (1968). In Cell Walls and Membranes, London : Spon Ltd.

Rogers, H.J., McConnell, M. and Burdett, I.D.J. (1968). Nature, Lond., 219, 285.

Rogers, H.J., Reaveley, D.A. and Burdett, I.D.J. (1967). In Protides of the Biological Fluids, vol. 15, p. 303. Ed. by Peeters, H. Amsterdam : Elsevier Publishing Co.

Rosenberg, B.H. and Cavalieri, L.F. (1963). In Cold Spring Harbor Symp. Quant. Biol. vol. 33, p. 65. New York : Long Island Biological Association.

Rothblat, G.H., Ellis, D.S. and Kritchevsky, D. (1964). Biochim. biophys. Acta, 84, 340.

Ryter, A. (1968). Bact. Rev. 32, 39.

Ryter, A. and Jacob, F. (1963). C.r.hebd. Seanc. Acad. Sci., Paris, 257, 3060.

Ryter, A. and Jacob, F. (1964). Annls Inst. Pasteur, Paris, 107, 384.

Ryter, A. and Jacob, F. (1966). Annls Inst. Pasteur, Paris, 110, 801.

Ryter, A. and Kellenberger, E. (1958). Z. Naturf. 13b, 597.

Ryter, A. and Landman, O.E. (1964). J. Bact. 88, 457.

Ryter, A. and Piechaud, M. (1963). Annls Inst. Pasteur, Paris, 105, 1071.

Ryter, A. and Pillot, J. (1963). Annls Inst. Pasteur, Paris, 104, 496.

Ryter, A., Frehel, C. and Ferrandes, B. (1967). C.r.hebd. Seanc. Acad. Sci., Paris, 265, 1259.

Salton, M.R.J. (1956a). Symp. Soc. gen. Microbiol. 6, 81.

Salton, M.R.J. (1956b). Biochim. biophys. Acta, 22, 495.

Salton, M.R.J. (1964a). Biochim. biophys. Acta, 86, 421.

Salton, M.R.J. (1964b). In The Bacterial Cell Wall, Amsterdam : Elsevier Publishing Co.

Salton, M.R.J. (1965). Lecture on Theoretical and Applied Aspects of Modern Microbiology, University of Maryland, Maryland.

Salton, M.R.J. (1967a). A. Rev. Microbiol. 21, 417.

Salton, M.R.J. (1967b). In The Specificity of Cell Surfaces, p. 71. Ed. by Davis, B. and Warren, L. New Jersey : Prentice-Hall, Inc.

Salton, M.R.J. (1967c). In Protides of the Biological Fluids, vol. 15, p. 279. Ed. by Peeters, H. Amsterdam : Elsevier Publishing Co.

Salton, M.R.J. (1967d). Trans. N.Y. Acad. Sci. Ser. II, 29, 764.

Salton, M.R.J. (1970). In Physical Principles of Biological Membranes, p. 259. Ed. by Snell, F., Wolken, J., Iverson, G.J. and Lam, J. New York : Gordon and Breach Science Publishers.

Salton, M.R.J. (1971). In Critical Reviews on Microbiology, vol. 1, p. 161. Ohio : Chemical Rubber Co.

Salton, M.R.J. and Chapman, J.A. (1962). J. Ultrastruct. Res. 6, 489.

Salton, M.R.J. and Ehtisham-ud-din, A.F.M. (1965). Aust. J. exp. Biol. med Sci. 43, 255.

Salton, M.R.J. and Freer, J.H. (1965). Biochim. biophys. Acta, 107, 531.

Salton, M.R.J. and Nachbar, M.S. (1970). In Anatomy and Biogenesis of Mitochondria and Chloroplasts, p. 42. Ed. by

Salton, M.R.J. and Netschey, A. (1965). Biochim. biophys. Acta, 107, 539.

Salton, M.R.J. and Schmitt, M.D. (1967a). Biochim. biophys. Acta, 135, 196.

Salton, M.R.J. and Schmitt, M.D. (1967b). Biochem. biophys. Res. Commun. 27, 529.

Salton, M.R.J., Freer, J.H. and Ellar, D.J. (1968). Biochem. biophys. Res. Commun. 33, 909.

Salton, M.R.J., Schmitt, M.D. and Trefts, P.E. (1967). Biochem. biophys. Res. Commun. 29, 728.

Samsonoff, W.A., Hashimoto, T. and Conti, S.F. (1971). J. Bact. 106, 269.

Santo, L.M., Hohl, H.R. and Frank, H.A. (1969). J. Bact. 99, 824.

Sargent, M.G. and Lampen, J.C. (1970). Archs Biochem. Biophys. 136, 167.

Sargent, M.G., Ghosh, B.K. and Lampen, J.O. (1969a). J. Bact. 96, 1329.

Sargent, M.G., Ghosh, B.K. and Lampen, J.O. (1969b). J. Bact. 97, 820.

Sasson, A. and Delaporte, B. (1969). C.r.hebd. Seanc. Acad. Sci., Paris, 268, 217.

Schaefer, W.B. and Lewis, C.W. (1965). J. Bact. 90, 1438.

Scher, M. and Lennarz, W.J. (1969). J. biol. Chem. 244, 2777.

Scher, M., Lennarz, W.J. and Sweeley, C.C. (1968). Proc. natn. Acad. Sci., U.S.A. 59, 1313.

Schliefer, K.H. and Kandler, O. (1967). Biochem. biophys. Res. Commun. 28, 965.

Schnaitman, C. and Greenawalt, J.W. (1966). J. Bact. 92, 780.

Schotz, F., Abo-Elnaga, I.G. and Kandler, O. (1965). Z. Naturf. 20B, 790.

Schwartz, F.M. and Zinder, N.D. (1968). Virology, 34, 352.

Sedar, A.W. and Burde, R.M. (1965a). J. Cell Biol. 24, 285.

Sedar, A.W. and Burde, R.M. (1965b). J. Cell Biol. 27, 53.

Serrano, J.A., Tablante, R.V., de Serrano, A.A., de San Blas, G.C. and Imaeda, T. (1972). J. gen. Microbiol. 70, 339.

Shands, J.W., Graham, J.A. and Nath, K. (1967). J. mol. Biol. 25, 15.

Shapiro, A.L. and Maizel, J.V., Jr. (1969). Anal. Biochem. 29, 505.

Shapiro, A.L., Vinuela, E. and Maizel, J.V. (1967). Biochem. biophys. Res. Commun. 28, 815.

Sharon, M., Osawa, T., Flowers, H.M. and Jeanloz, R.W. (1966). J. biol. Chem. 241, 223.

Shatkin, A.J. and Tatum, E.L. (1959). J. biophys. biochem. Cytol. 6, 423.

Shewan, J.M. (1963). In Symposium on Marine Microbiology, p. 499. Ed. by Oppenheimer, C.H., Springfield, Illinois : Thomas, C.C., Publisher.

Shilo, M. (1970). J. Bact. 104, 453.

Shinohara, C., Fukushi, K. and Suzuki, J. (1957). J. Bact. 74, 413.

Shinohara, Ch., Fukushi, K. and Suzuki, J. (1958). Jap. J. elect. Microscopy, 6, 47.

Shinohara, Ch., Fukushi, K., Suzuki, J., Sato, K., Suzuki, T. and Motomiya, M. (1959). Ann. Rept. Japan. Soc. Tuberc. 4, 10.

Shively, J.M., Decker, G.L. and Greenawalt, J.W. (1970). J. Bact. 101, 618.

Shockman, G.D. and Martin, J.T. (1968). J. Bact. 96, 1803.

Shockman, G.D., Pooley, H.M. and Thompson, J.S. (1967). J. Bact. 94, 1525.

Simakova, I.M., Lukoyanova, M.A., Biryuzova, V.I. and Gel'man, N.S. (1968) Biokhimiya, 33, 1047.

Simakova, I.M., Lukoyanova, M.A., Biryuzova, V.I. and Gel'man, N.S. (1969). Biokhimiya, 34, 1271.

Silva. M.T. (1967). Expl. Cell Res. 46, 245.

Silva, M.T. (1971). J. Microscopy, 93, 227.

Sleytr, U.B. (1970a). Protoplasma, 71, 295.

Sleytr, U.B. (1970b). Arch. Mikrobiol. 72, 238.

Sleytr, U.B. and Kocur, M. (1971). Arch. Mikrobiol. 78, 353.

Sleytr, U.B. and Krebs, B. (1971). Arch. Mikrobiol. 77, 377.

Smirnova, T.A., Kushnarev, V.M. and Tshaikovskaja, S.M. (1971). J. Ultrastruct. Res. 37, 269.

Smith, K.R. (1960). J. Ultrastruct. Res. 4, 213.

Smith, L. (1954). Archs Biochem. Biophys. 50, 299.

Smith, U. and Ribbons, D.W. (1970). Arch. Mikrobiol. 74, 116.

Smith, U., Ribbons, D.W. and Smith, D.S. (1970). Tissue Cell, 2, 513.

Snellen, J.E. and Raj, H.D. (1970). J. Bact. 101, 240.

Sofronova, M. Yu., Ostrovskii, D.N. and Gel'man, N.S. (1971). Biokhimiya, 36, 977.

Staehelin, L.A. (1968a). Proc. R. Soc. B. 171, 249.

Staehelin, L.A (1968b). J. Ultrastruct. Res. 22, 326.

Staehelin, L.A. and Bertaud, W.S. (1971). J. Ultrastruct. Res. 37, 146.

Steck, T.L., Weinstein, R.S., Straus, J.H. and Wallach, D.F.H. (1970). Science, N.Y., 170, 255.

Steed, P. and Murray, R.G.E. (1966). Can. J. Microbiol. 12, 263.

Steere, R.L. (1957). J. biophys. biochem. Cytol. 3, 45.

Stevenson, I.L. (1968). Can. J. Microbiol. 14, 1029.

Stoeckenius, W. and Rowen, R. (1967). J. Cell Biol. 34, 365.

Streiblova, A. (1968). J. Bact. 95, 700.

Stuart, D.C. (1959). J. Bact. 78, 272.

Suganuma, A. (1961). J. biophys. biochem. Cytol. 10, 292.

Suganuma, A. (1963). J. infect. Dis. 113, 179.

Suganuma, A. (1966). J. Cell Biol. 30, 208.

Suganuma, A. (1968). Jap. J. elect. Microscopy, 16, 315.

Swank, R.T. and Munkres, K.D. (1971). Anal. Biochem. 39, 462.

Szulmajster, J., Bonamy, C. and Laporte, J. (1970). J. Bact. 101, 1027.

Takagi, A., Abe, O. and Ueda, M. (1965). J. gen. appl. Microbiol. Tokyo, 9, 1.

Takagi, A., Nakamara, K. and Ueda, M. (1965). Jap. J. Microbiol. 9, 131.

Takagi, A., Ueyama, K. and Ueda, M. (1963). J. gen. appl. Microbiol., Japan, 9, 287.

Takeya, K., Koike, M., Mori, R. Yuda, Y. and Toda, T. (1959). J. Bact. 78, 313.

Tchan, Y.T., Birch-Anderson, A. and Jensen, H.L. (1962). Arch. Milkrobiol. 42, 50.

Theodore, T.S., Popkin, T.J. and Cole, R.M. (1971). Preparative Biochem. 1, 233.

Thomas, T.D., Lyttleton, P., Williamson, K.I. and Batt, R.D. (1969). J. gen. Microbiol. 58, 381.

Thompson, J.S. (1971). J. theor. Biol. 33, 63.

Thorne, K.J.I. and Barker, D.C. (1969). Eur. J. Biochem. 11, 582.

Thorne, K.J.I. and Barker, D.C. (1971). Biochem. J. 122, 45P.

Thorne, K.J.I. and Barker, D.C. (1972). J. gen. Microbiol. 70, 87.

Thorne, K.J.I. and Kodicek, E. (1962). Biochim. biophys. Acta, 59, 295.

Thornley, M.J. and Glauert, A.M. (1968). J. Cell Sci. 3, 273.

Tichy, P. and Landman, O.E. (1969). J. Bact. 97, 42.

Tillack, T.W., Carter, R. and Razin, S. (1970). Biochim. biophys. Acta, 219, 123.

Tokuyasu, K. and Yamada, E. (1969). J. biophys. biochem. Cytol. 5, 123.

Tomasz, A., Jamieson, J.D. and Ottolenghi, E. (1964). J. Cell Biol. 22, 453.

Tordzhyan, I. Kh. and Kats, L.N. (1968). Microbiology, 37, 880.

Tourtellotte, M.E., Branton, D. and Keith, A. (1970). Proc. natn. Acad. Sci., U.S.A., 66, 909.

Trentini, W.C. and Starr, M.P. (1967). J. Bact. 93, 1699.

Truper, H.G. (1968). J. Bact. 95, 1910.

Tsukagoshi, N., Fielding, P. and Fox, C.F. (1971). Biochem. biophys. Res. Commun. 44, 497.

Tung, T.-S. and Knight, C.A. (1971). Biochem. biophys. Res. Commun. 42, 1117.

Uffen, R.L. and Wolfe, R.S. (1970). J. Bact. 104, 462.

Valentine, A.F. and Chapman, G.B. (1966). J. Bact. 92, 1535.

Vambutas, V.K. and Salton, M.R.J. (1970a). Biochim. biophys. Acta, 203, 83.

Vambutas, V.K. and Salton, M.R.J. (1970b). Biochim. biophys. Acta, 203, 94.

Van Gool, A.P. and Nanninga, N. (1971). J. Bact. 108, 474.

Van Gool, A.P., Lambert, R. and Laudelout, A. (1969) Arch. Mikrobiol. 69, 281.

Van Iterson, W. (1961). J. biophys. biochem. Cytol. 9, 183.

Van Iterson, W. (1962). In Recent Progress in Microbiology, vol. 8, p. 14. Ed. by Gibbons, N.E. Canada : University of Toronto Press. (1963).

Van Iterson, W. (1965). Bact. Rev. 29, 299.

Van Iterson, W. and Groen, J.B. (1971). J. Cell Biol. 49, 553.

Van Iterson, W. and Leene, W. (1964a). J. Cell Biol. 20, 361.

Van Iterson, W. and Leene, W. (1964b). J. Cell Biol. 20, 377.

Van Iterson, W. and Op den Kamp, J.A.F. (1969). J. Bact. 99, 304.

Van Iterson, W., Hoeniger, J.F.M. and Van Zanten, E.N. (1966). J. Cell Biol. 31, 585.

Vanderwinkel, E. and Murray, R.G.E. (1962). J. Ultrastruct. Res. 7, 185.

Veltri, B.J. and McAlear, J.H. (1971). J. Microscopy, 93, 191.

Veltri, B.J. and McAlear, J.H. (1972). J. gen. Microbiol. 70, 31.

Venable, J.H. and Coggeshall, R. (1965). J. Cell Biol. 25, 407.

Voelz, H. (1965). Arch. Mikrobiol. 51, 60.

Voelz, H. and Ortigoza, R.O. (1968). J. Bact. 96, 1357.

Wallach, D.F.H. (1969). J. gen. Physiol. 54, 3S.

Wallach, D.F. and Zahler, P.H. (1966). Proc. natn. Acad. Sci., U.S.A., 56, 1552.

Walker, P.D. and Baillie, A. (1968). J. appl. Bact. 31, 108.

Walsh, B.L., O'Dor, J. and Warren, R.A.J. (1971). Can. J. Microbiol. 17, 593.

Warburg, O. and Christian, W. (1941). In Data for Biochemical Research, p. 625. Ed. by Dawson, R.M.C., Elliot, P.C., Elliot, W.H. and Jones, K.M., Oxford : Oxford University Press.

Watson, S.W. and Mandel, M. (1971). J. Bact. 107, 563.

Watson, S.W. and Remsen, C.C. (1969). Science, N.Y., 163, 685.

Watson, S.W. and Remsen, C.C. (1970). J. Ultrastruct. Res. 33, 148.

Weber, K. and Osborn, M. (1969). J. biol. Chem. 244, 4406.

Wehrli, E., Muhlethaler, K. and Moor, H. (1970). Expl. Cell Res. 59, 336.

Weibull, C. (1953). J. Bact. 66, 137.

Weibull, C. (1965a). J. Bact. 89, 1151.

Weibull, C. (1965b). J. Bact. 90, 1467.

Weibull, C. and Cyllang, H. (1965). J. Bact. 89, 1443.

Weigand, R.A. and Greenawalt, J.W. (1971). Bact. Proc. 158

Weigand, R.A., Shively, J.M. and Greenawalt, J.W. (1970). J. Bact. 102, 240.

Weinstein, R.S. (1969). In Red Cell Membrane Structure and Function, p. 36. Ed. by Jamieson, G.A. and Greenawalt, T.H., Philadelphia : Lippincott Co.

Weinstein, R.S., Clowes, A.W. and McNutt, N.S. (1970). Proc. Soc. exp. Biol. Med. 134, 1195.

Whiteside, T.L. and Salton, M.R.J. (1970). Biochemistry, 9, 3034.

Whiteside, T.L., de Siervo, A.J. and Salton, M.R.J. (1971). J. Bact. 105, 957.

Wiebe, W.J. and Chapman, G.B. (1968a). J. Bact. 95, 1862.

Wiebe, W.J. and Chapman, G.B. (1968b). J. Bact. 95, 1874.

Wildermuth, H. (1971). J. gen Microbiol. 68, 53.

Wildermuth, H. and Hopwood, D.A. (1970). J. gen. Microbiol. 60, 51.

Wildermuth, H., Wehrli, E. and Horne, R.W. (1971). J. Ultrastruct. Res. 35, 168.

Williams, J.G. and Gratzer, W.B. (1971). J. Chromat. 57, 121.

Wilson, G. and Fox, C.F. (1971a). J. mol. Biol. 55, 49.

Wilson, G. and Fox, C.F. (1971b). Biochem. biophys. Res. Commun. 44, 503.

Wolin, H.L. and Naylor, H.B. (1957). J. Bact. 74, 163.

Wolstenholme, D.R., Vermeulen, C.A. and Venema, G. (1966). J. Bact. 92, 1111.

Wyss, O., Neumann, M.G. and Socolofsky, M.D. (1961). J. biophys. biochem. Cytol. 10, 555.

Young, H.L., Chao, F.-C., Turnbill, C. and Philpott, D.E. (1972). J. Bact. 109, 862.

Yudkin, M.D. (1967). In Progress in Microbiological Techniques, p. 97. Ed. by Collins, C.H. London : Butterworth & Co. Ltd.

Zhukova, I.G., Ostrovskii, D.N., Gel'man, N.S. and Oparin, A.I. (1966). Biokhimiya, 31, 1209.

Zwillenberg, L.O. (1964). Antonie van Leeuwenhoek J. Microbiol. Serol. 30, 154.

ADDENDA TO REFERENCES

Allison, D.P. (1971) J. Bact. 108, 1390.

Bettinger, G.E. & Lampen, J.O. (1971). Biochem. Biophys. Res. Commun. 43,200.

Chalcroft, J.P. & Ballivant, S. (1970). J.Cell. Biol. 47, 49.

Drieger, A.A. (1970). Can.J. Microbiol. 16, 881.

Highton, P.J. (1970a). J.Ultrastruct. Res. 31, 247.

Highton, P.J. (1970b). J.Ultrastruct. Res. 31, 260.

Ivarie, R.D. & Pene, J.J. (1970). J.Bact. 104, 839.

Kushner, D.J. & Pollock, M.R. (1961). J.gen. Microbiol. 26, 255.

Matheson, A. & Kwong, M.C.M. (1972). J.Bact. 110, 747.

Partridge, S.M. (1948). Biochem. J. 42, 238.

Robertson, J.D. (1959). Symp. Biochem. Soc. 16, 3.

Ryter, A. (1971). Annls. Inst. Pasteur, Paris, 121, 271.

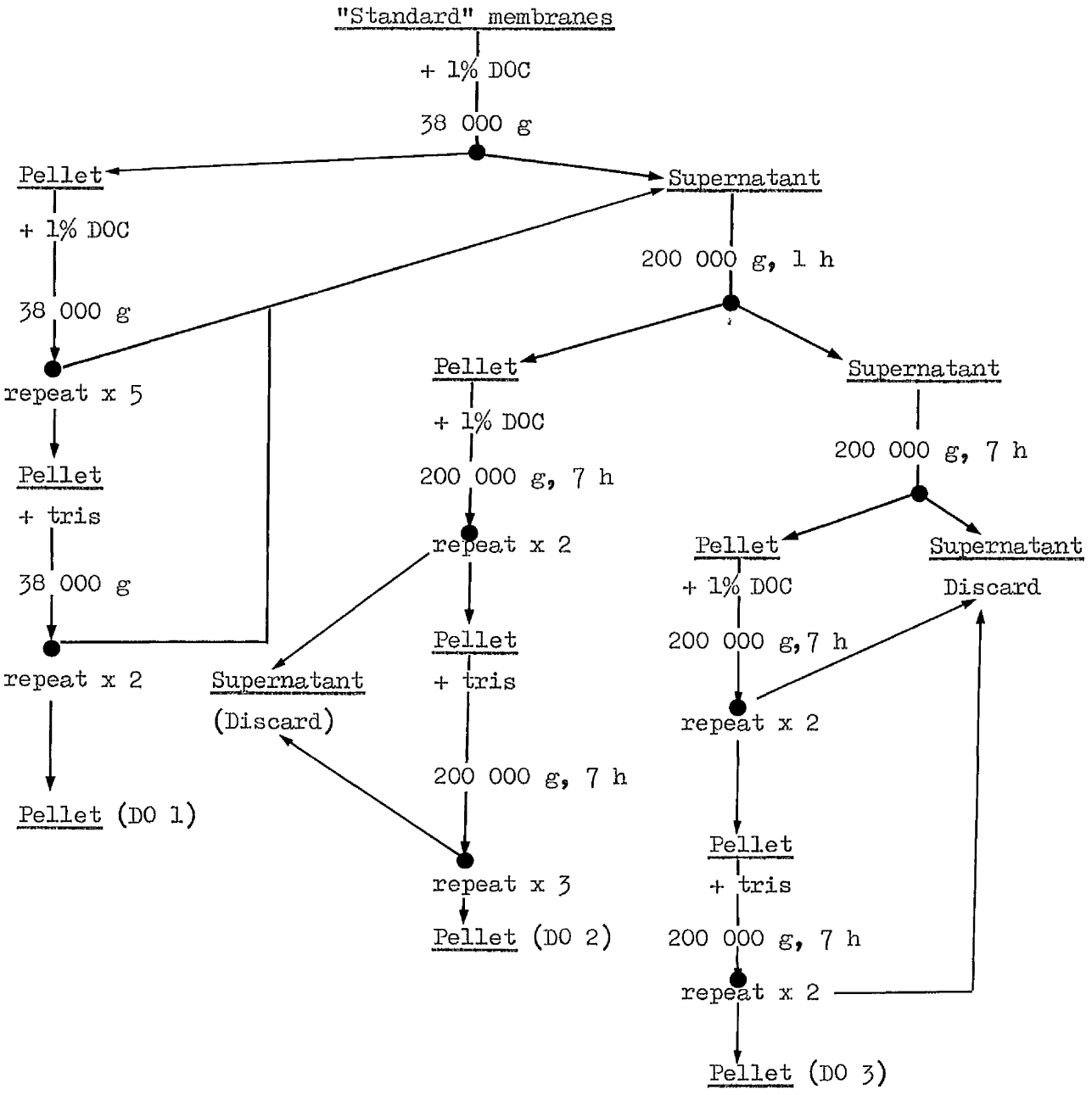
Salton, M.R.J. & Ghaysen, J.M. (1960). Biochim. Biophys. Acta 45, 355.

SUPPORTING PUBLICATION.

Table of Contents

	PAGE
Flow Diagrams 1 & 2	D1 & D2 resp.
Figures 1-21	Facing F1 - F21 resp.
Plates 1-97	Facing P1 - P97 resp.
Tables 1-29	T1 - T29 resp.

Flow Diagram 1. DOC extraction of "Standard" membranes of M. lysodeikticus St.0



Flow Diagram 2.

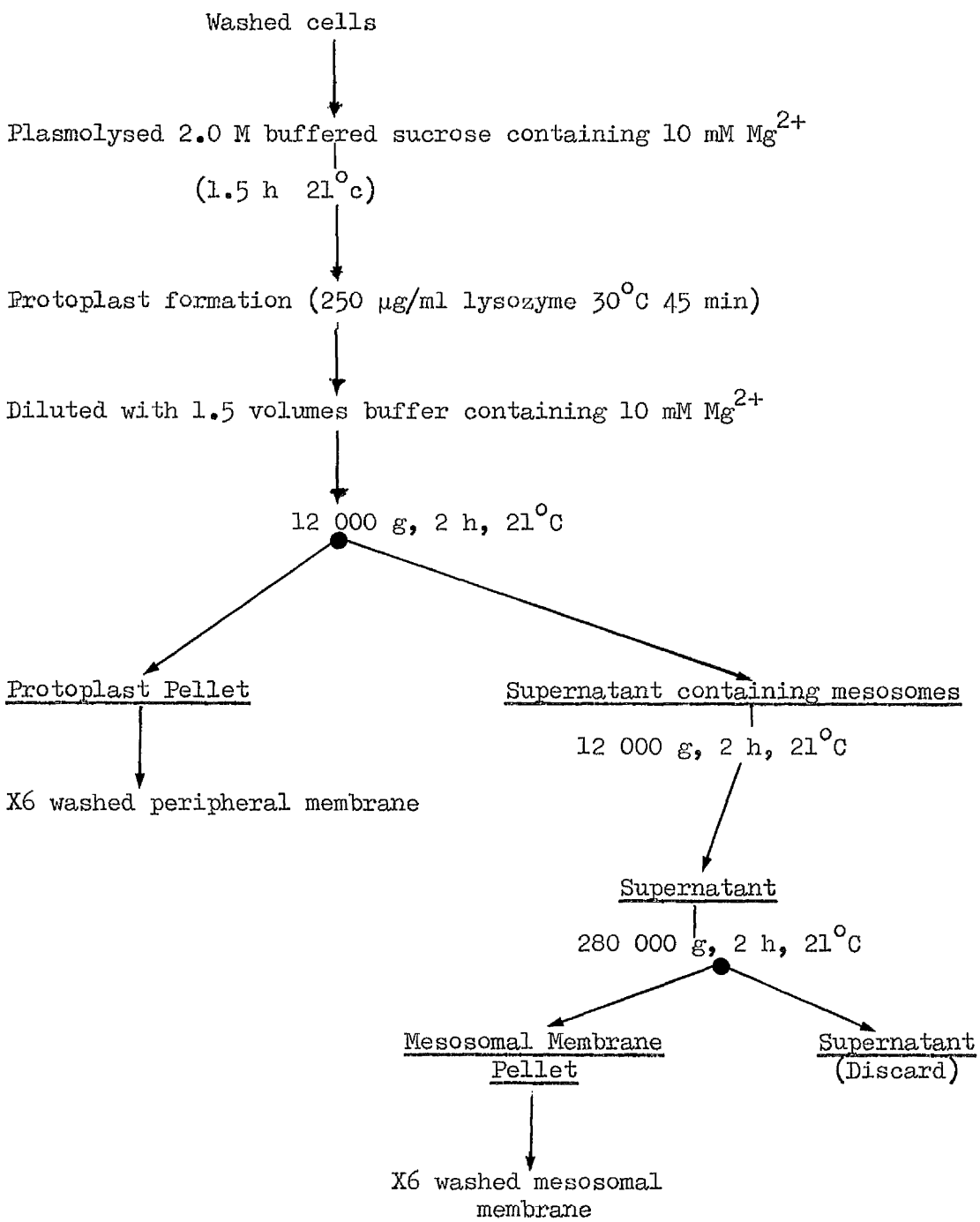


Fig. 1. Visible spectrum of membrane preparations isolated from M. lysodeikticus St.O. Peripheral membrane (a) and mesosomal membrane (b) at concentrations of 1.81 and 1.52 mg dry wt membrane/ml respectively were solubilized by the addition of SDS (final concentration 0.1%) prior to recording spectrum. Under those conditions the λ_{max} values for both preparations are 419 nm, 446 nm and 475 nm. Note the considerable absorption in the region of 550 nm due to presence of respiratory pigments (cytochromes).

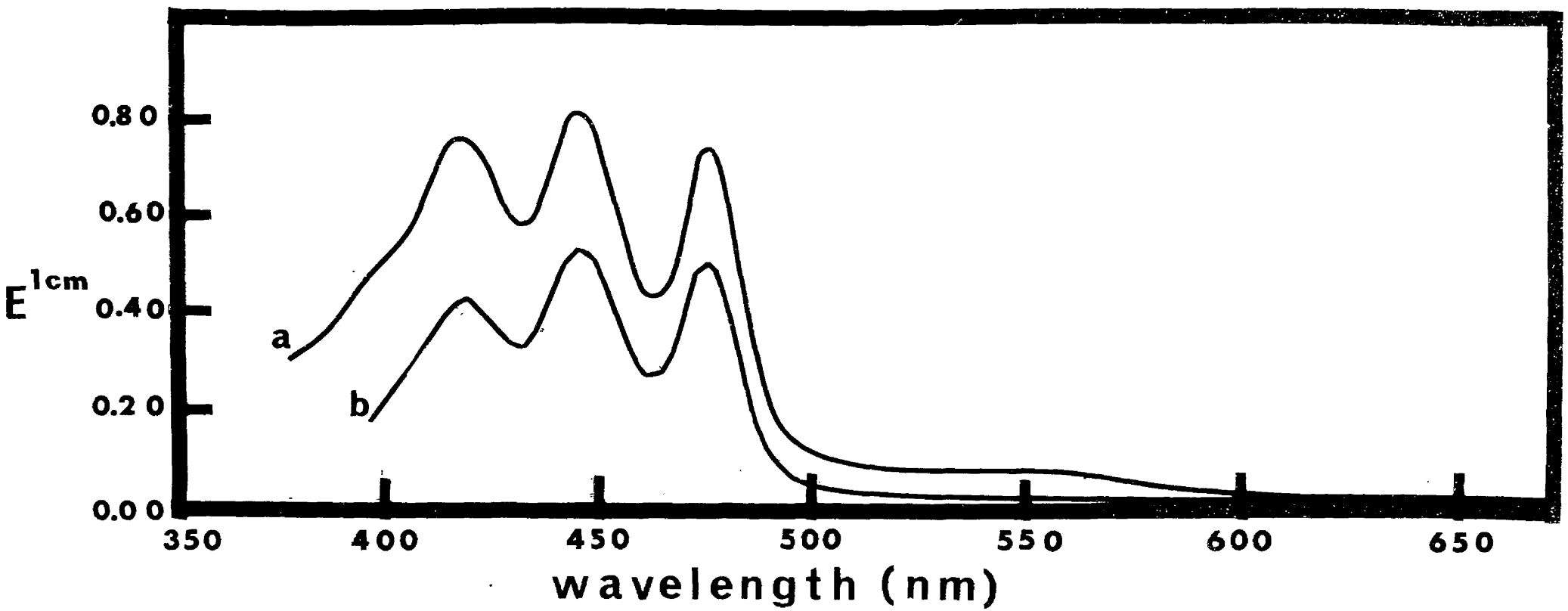

0.80
0.60
0.40
0.20
0.00
E 1cm
a
b
350
400
450
500
550
600
650
wavelength (nm)

Fig. 2. Standard curve of carotenoid absorption against the concentrations of membrane in suspension. Isolated peripheral membrane (O) and mesosomal membrane (Δ) preparations isolated from M. lysodeikticus St.0 were clarified by the addition of SDS to a final concentration of 0.1% and extinction at 446 nm recorded. Aliquots of membrane suspensions were dialysed against distilled water prior to freeze drying and gravimetric determination. Each point represents a unique membrane preparation. Note linear relationship between E_{446}^{1cm} and membrane concentration for both preparations and the similarity in their carotenoid content.

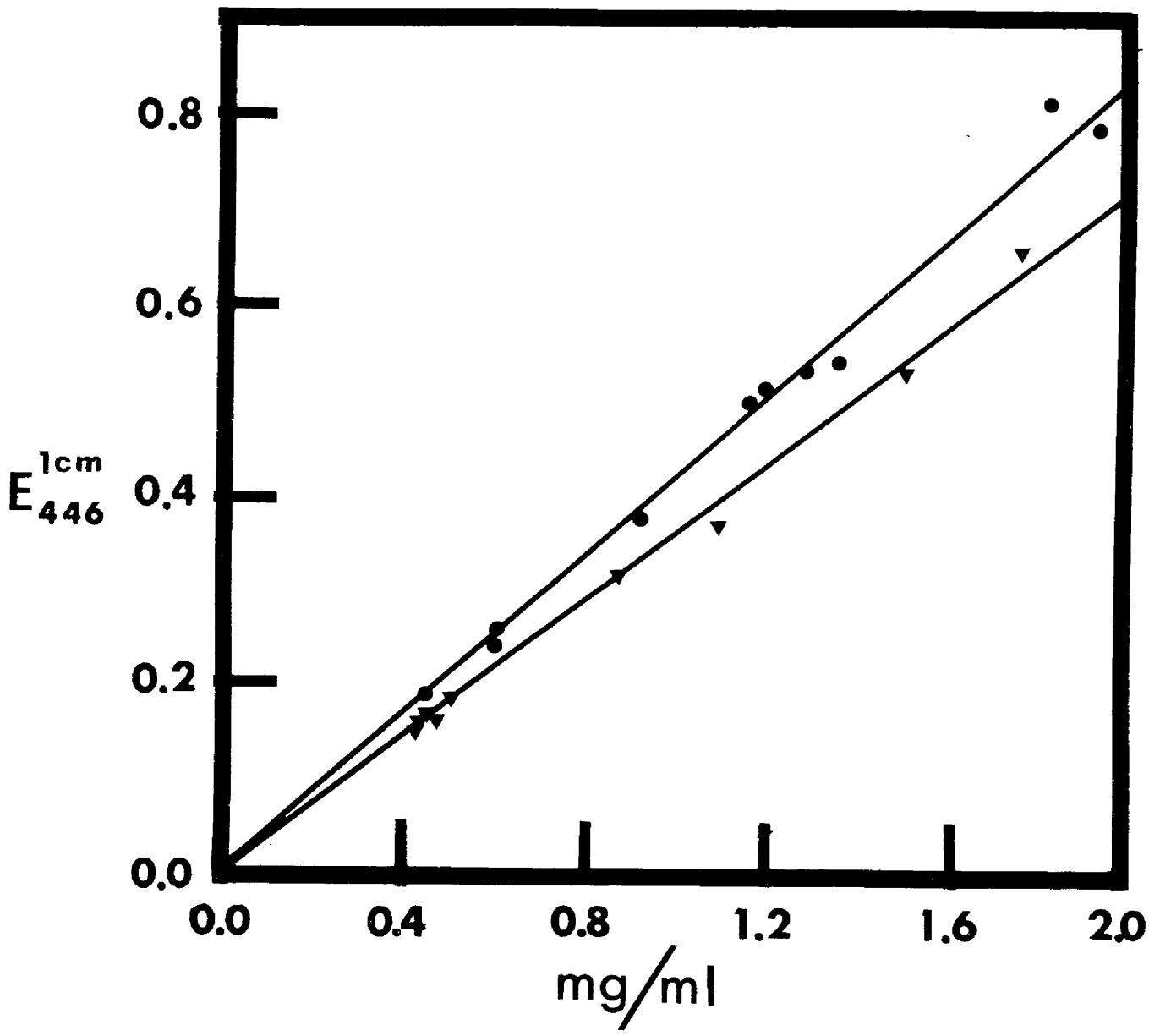

0.8
0.6
0.4
0.2
0.0
E_{446}^{1cm}
0.0
0.4
0.8
1.2
1.6
2.0
mg/ml

Fig. 3. Semi-log plot of molecular weight (M.W.) of marker proteins against their respective migration (Rf) relative to bromophenol blue. Bovine serum albumin (○), ovalbumin (△), hexikinase (□), chymotrypsinogen (●), myoglobin (■) and cytochrome c (▲) were subjected to polyacrylamide disc gel electrophoresis in the presence of 0.1% SDS and resolved in gels containing acrylamide at a concentration of 11.7% w/v and bis at a concentration of 0.153%.

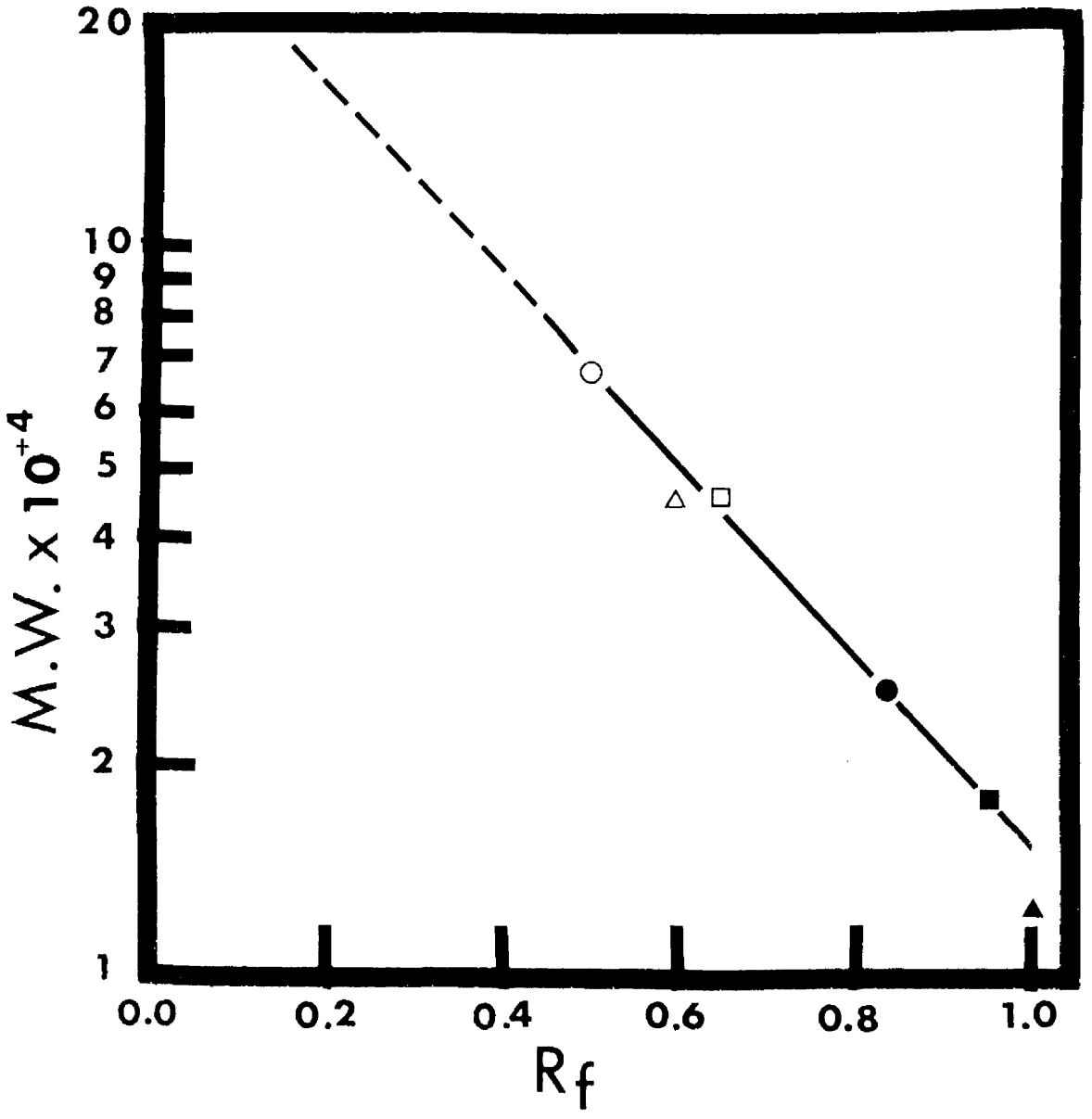

20
10
9
8
7
6
5
4
3
2
1
M.W. x 10+4
0.0
0.2
0.4
0.6
0.8
1.0
Rf

Fig. 4. Cytochrome difference spectra of isolated peripheral membranes from M. lysodeikticus St.O. Membrane suspensions (18.4 mg dry wt. membrane/ml) were clarified by ultrasound (a - d) or with 1% v/v Triton X-100 (e - f) and chemically reduced with sodium dithionite. Difference spectra were recorded immediately following addition of dithionite (a and e) and after 5 min (b and f), 10 min (c and g) and 15 min (d and h). Note that complete reduction of cytochrome b_{560}, unlike cytochromes a_{601} and c_{550}, is only achieved after approx. 10 min in suspensions treated with ultrasound or after approx. 5 min if treated with Triton X-100.

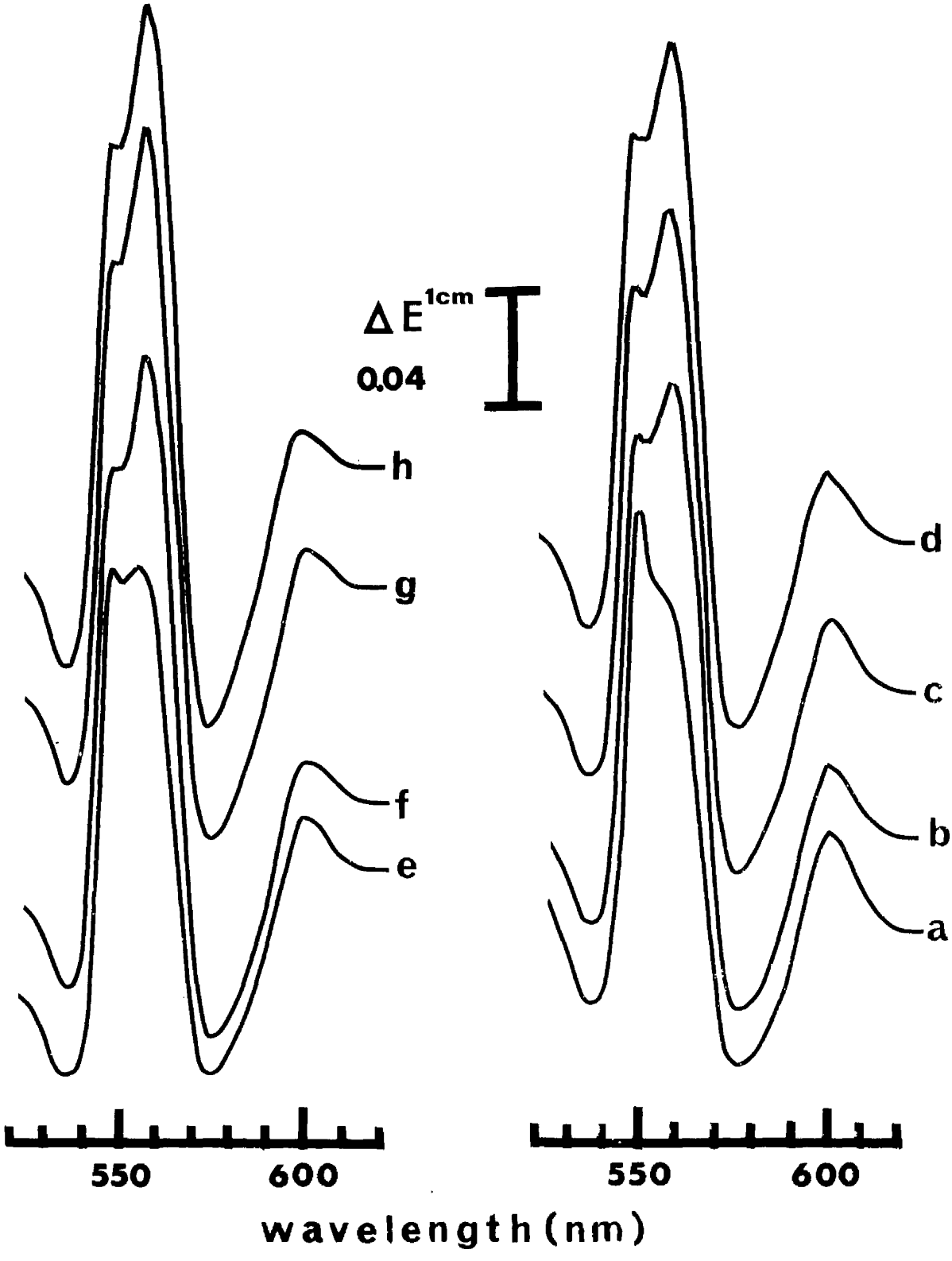
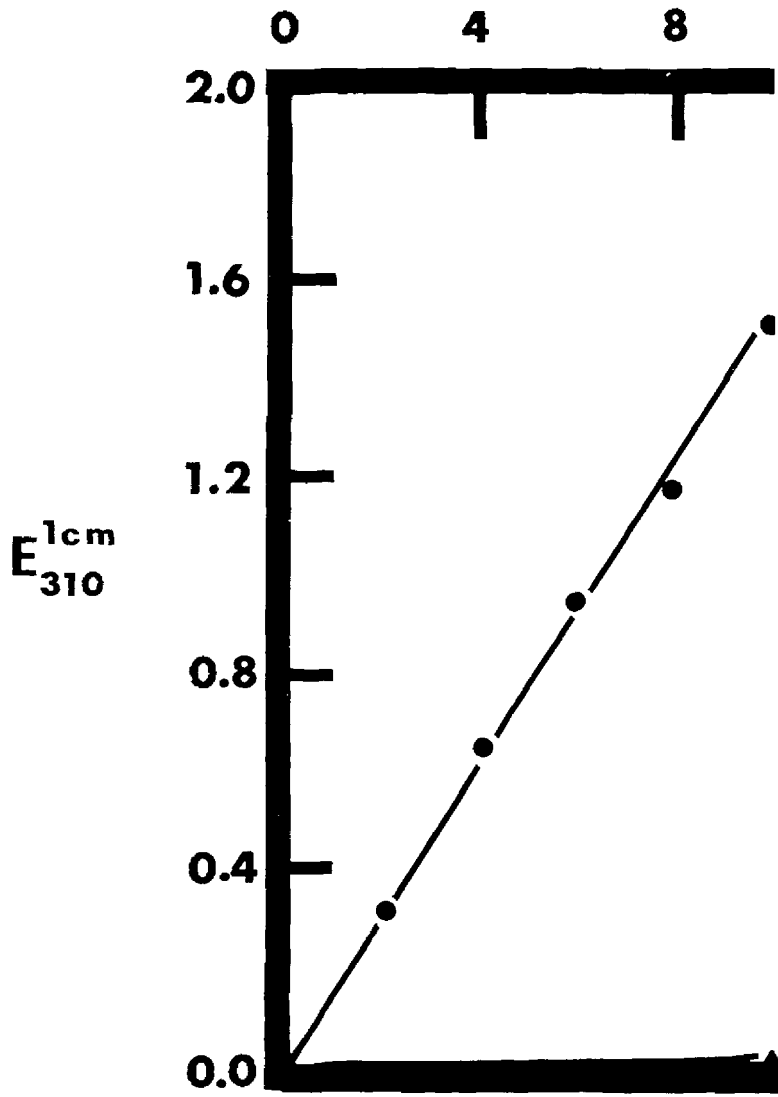

ΔE 1cm
0.04
h
g
f
e
d
c
b
a
550
600
550
600
wavelength (nm)

Fig. 5. Fructose, glucose and mannose in 79% sulphuric acid. 0 - 30 μg of fructose (●), mannose (▲) and glucose (■) were incubated at 25°C with 3.0 ml 79% w/w sulphuric acid (final concentration) and the E^{1cm}_{310} monitored after 2 h. Only fructose, a ketosugar, shows absorbance at 310 nm in this system.


0
4
8
2.0
1.6
1.2
0.8
0.4
0.0
E_{310}^{1cm}

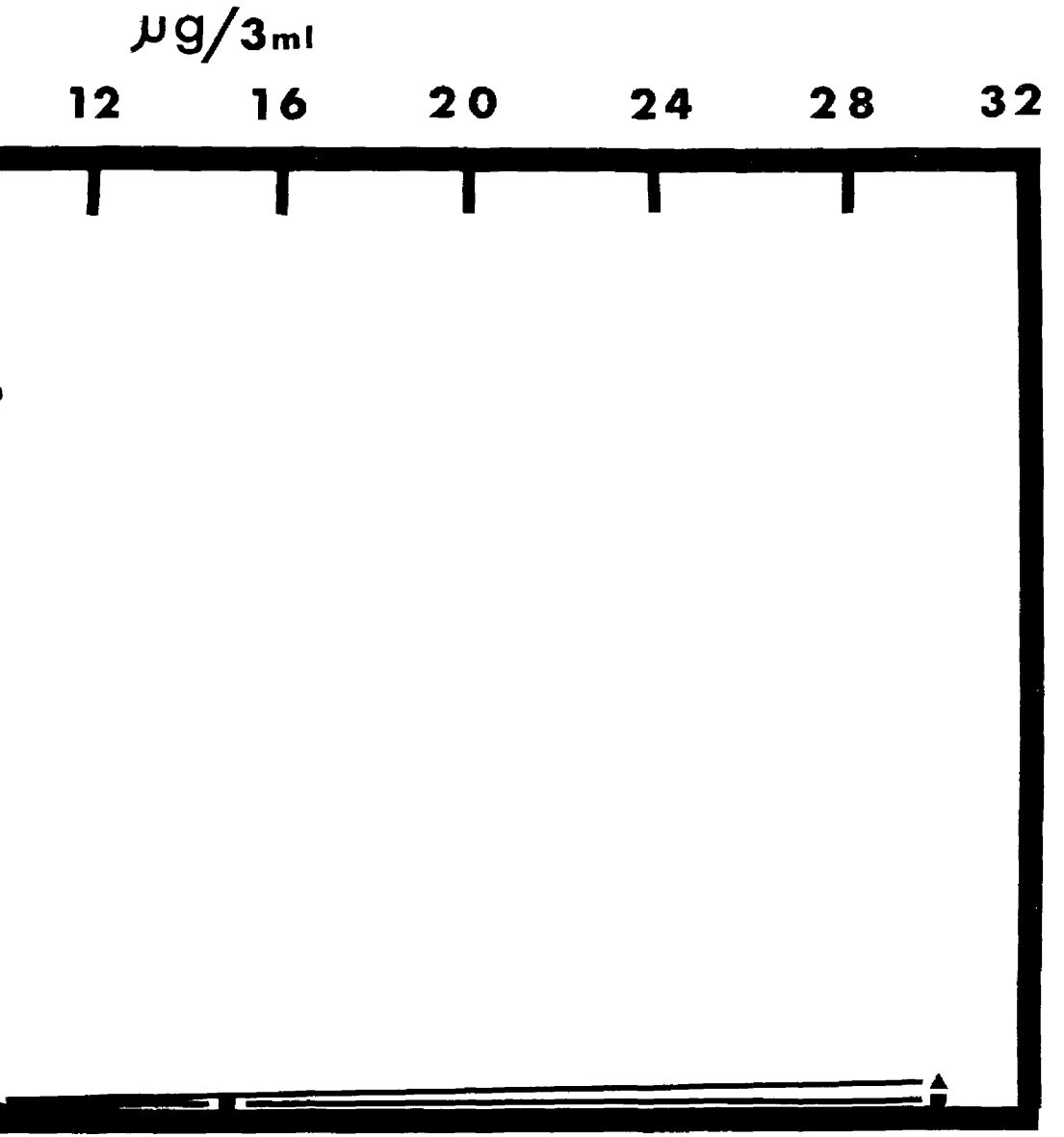

µg/3ml
12
16
20
24
28
32

Fig. 6. Growth and pigment production by M. lysodeikticus St.A. Cells were cultured from a 10% inoculum as described in Methods and growth estimated by E^{1cm}_{620} of the broth (●). Pigment production, estimated by E^{1cm}_{405} (■), and pH values (▲) were monitored in culture supernatant fluids. The generation time of 135 min was estimated by the time taken for doubling of E^{1cm}_{620} during exponential growth. Note the relatively small increase in pigment content of culture in the exponential phase of growth cf M. lysodeikticus St.0 (Fig. 7).

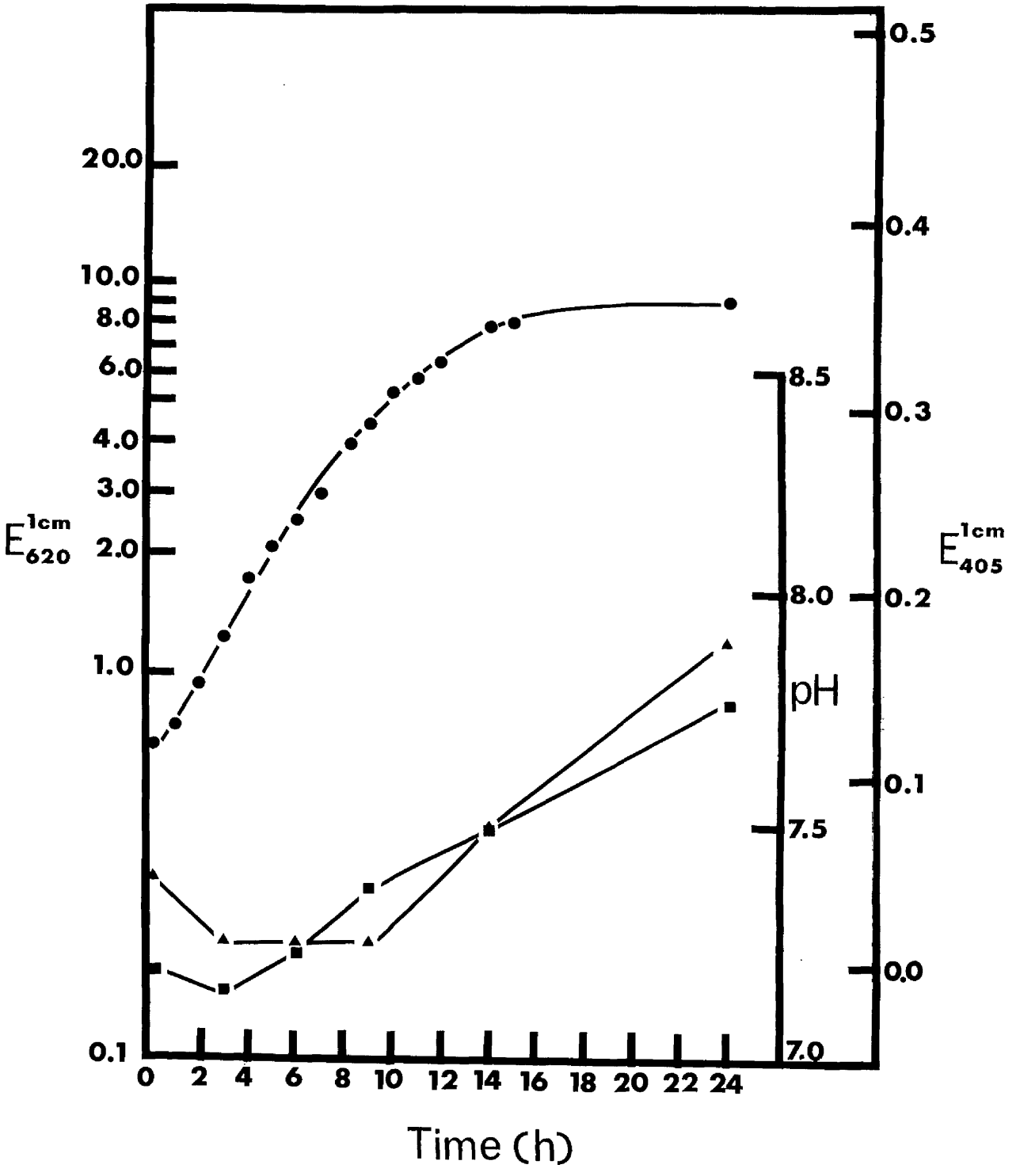

20.0
10.0
8.0
6.0
4.0
3.0
2.0
1.0
0.1
E^{1cm}_{620}
0.5
0.4
0.3
0.2
0.1
0.0
E^{1cm}_{405}
8.5
8.0
7.5
7.0
pH
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (h)

Fig. 7. Growth and pigment production by M. lysodeikticus St.O. Cells were cultured from a 10% inoculum as described in Methods and growth estimated by E_{620}^{1cm} of the broth (●). Pigment production, estimated by E_{405}^{1cm} (■), and pH values (▲) were monitored in culture supernatant fluids. The generation time of 112 min was estimated by the time taken for doubling of E_{620}^{1cm} during exponential growth. Note the characteristic increase in pigment production during stationary phase of growth.

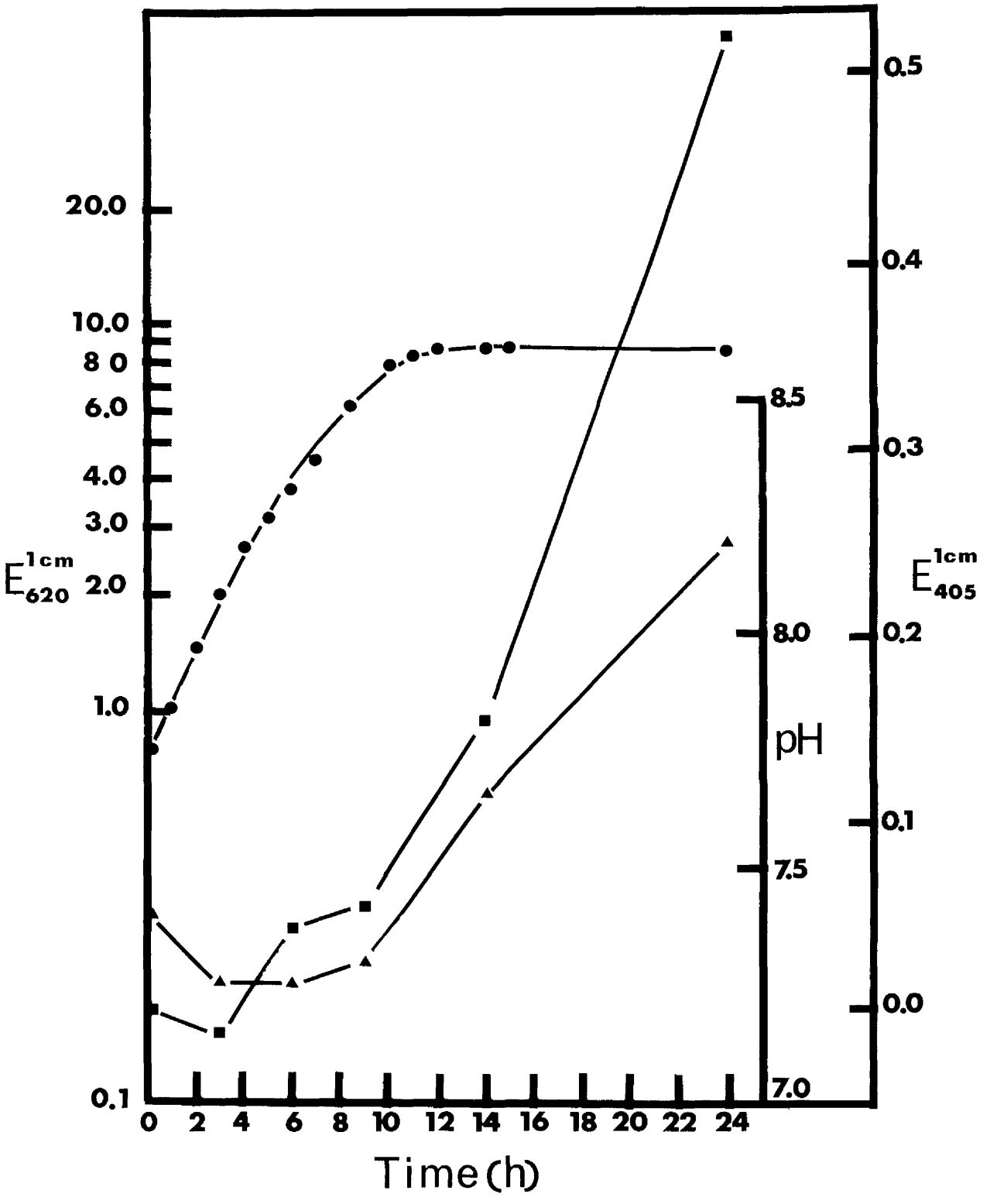

20.0
10.0
8 0
6.0
4.0
3.0
2.0
1.0
0.1
E^{1cm}_{620}
0.5
0.4
0.3
0.2
0.1
0.0
E^{1cm}_{405}
8.5
8.0
7.5
7.0
pH
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (h)

Fig. 8. Effect of SDS on the turbidity of "standard" membrane suspensions from M. lysodeikticus St.A. "Standard" membrane preparations (12.5 mg dry wt. membrane/ml) were diluted with an equal volume of tris buffered SDS to the final concentrations of SDS indicated. Turbidity was monitored by E^{1cm}_{620}. Inset is an expanded plot between 0.0% and 0.1% SDS.

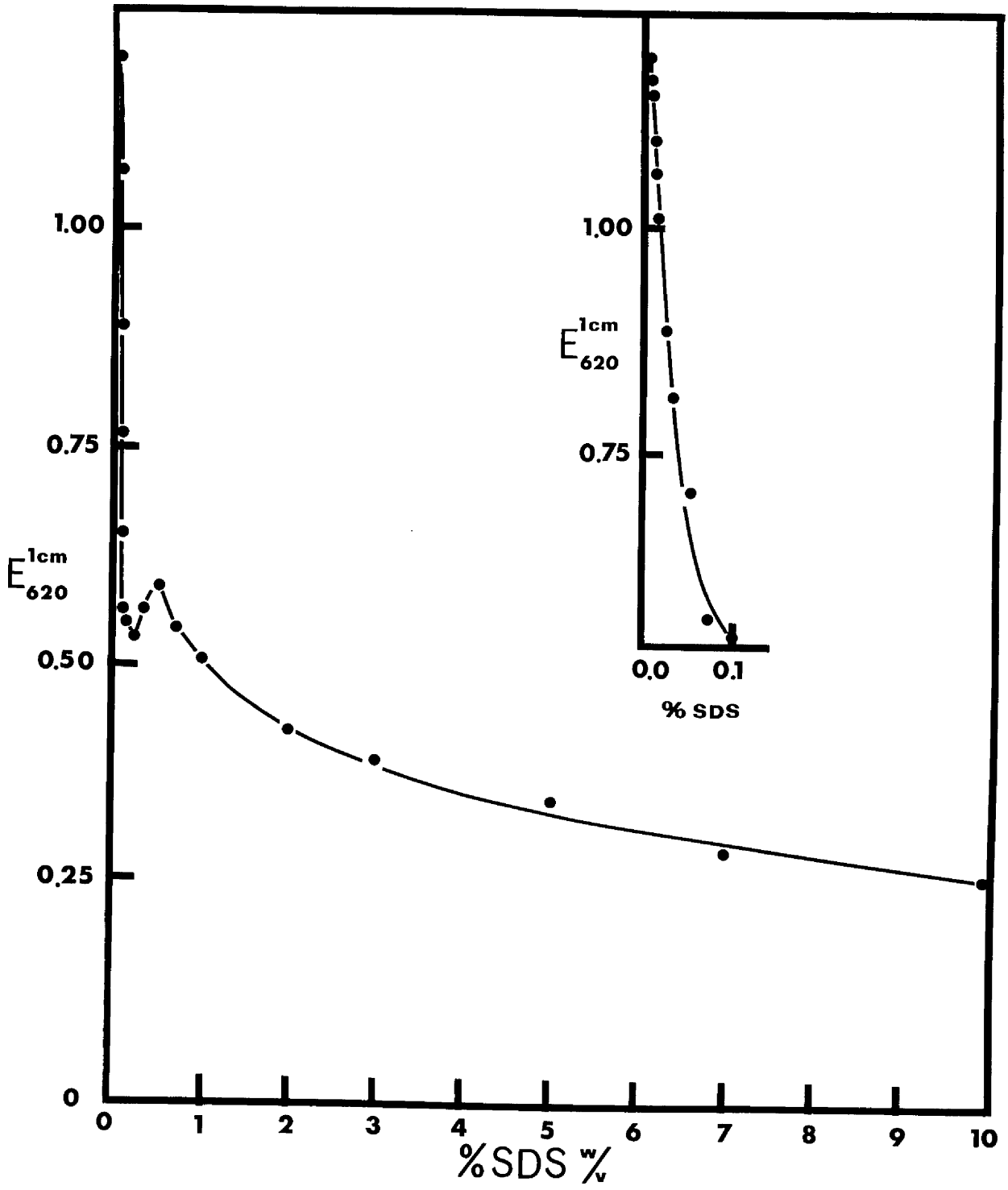

1.00
0.75
0.50
0.25
0
E_{620}^{1cm}
0 1 2 3 4 5 6 7 8 9 10
%SDS w/v
1.00
0.75
E_{620}^{1cm}
0.0 0.1
% SDS

Fig. 9. Extraction of "standard" membranes of M. lysodeikticus St.A with SDS. "Standard" membranes were diluted to 1.39 mg dry wt. membrane/ml with tris buffer and extracted with an equal volume of buffer containing SDS at the concentrations indicated. Extracted carotenoid (■) was monitored by extinction at 446 nm in the 23 000 g supernatant fluid and compared with the total carotenoid absorbance. Gravimetric determination of the 23 000 g pellet allowed on estimation of the percentage total wt. of membrane extracted (●). Note the concomitant extraction of 95% of the membrane carotenoid and 30% of the total wt. of the membrane with a concentration of detergent (SDS) of 0.25%; also the relatively low "solubility" of the sedimented fraction in higher concentrations of SDS.

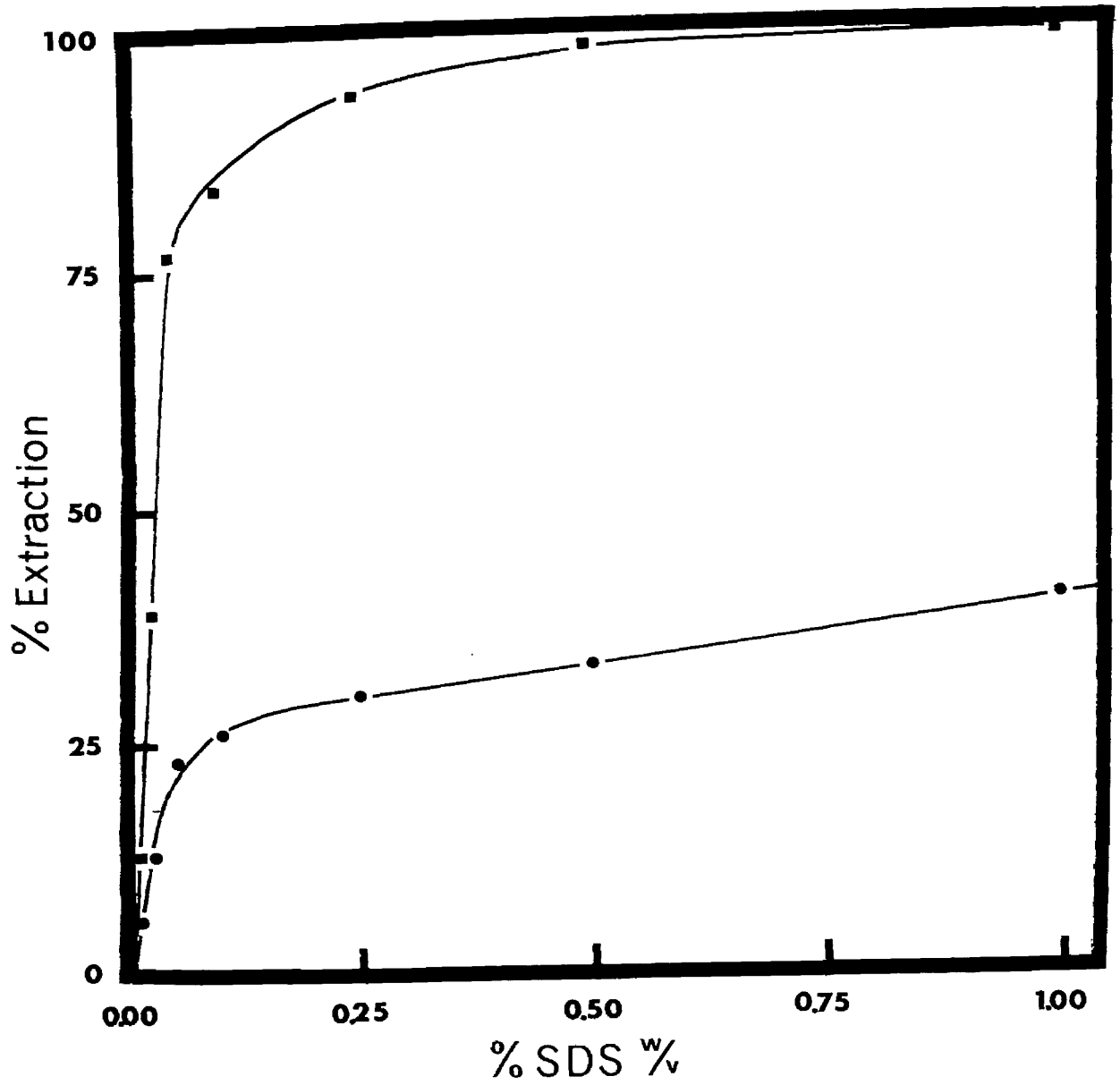

100
75
50
25
0
% Extraction
0.00
0.25
0.50
0.75
1.00
% SDS w/v

Fig. 10. Infra red absorption spectra of membranes and membrane residues from M. lysodeikticus St.A. "Standard" membranes were prepared from late logarithmic phase cells (12 h) which had been resuspended to 1/20th (a) or 1/2 (b) the original culture volume and subjected to incubation with lysozyme (100 μg/ml) for 45 min (a) or 120 min (b) at 30°C. Membrane preparations were extracted with 1% SDS to give insoluble residue (c) (see Methods). The spectrum of residue (c) has been arbitrarily displaced by a unit of 20% transmittance at 4000 cm^{-1} and at 1800 cm^{-1}. Note the different ratio of band intensities for $\frac{2930\ cm^{-1}}{3300\ cm^{-1}}$ and for $\frac{1735\ cm^{-1}}{1655\ cm^{-1}}$ between preparations a and b, indicating increased lipid content of membrane preparations (b). (See Table 12 for band assignments).

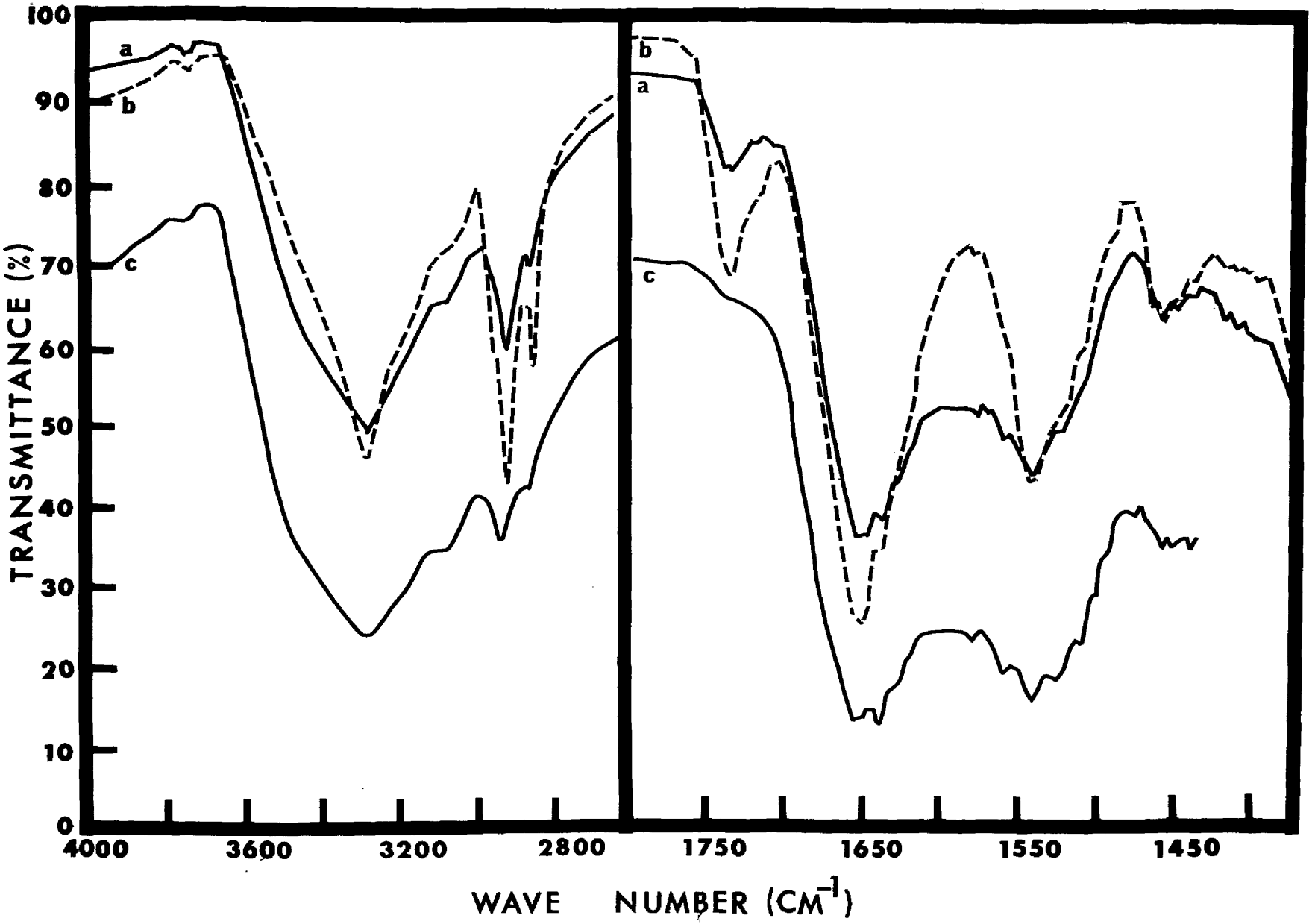

100
90
80
70
60
50
40
30
20
10
0
TRANSMITTANCE (%)
a
b
c
4000
3600
3200
2800
1750
1650
1550
1450
WAVE NUMBER (CM^{-1})

Fig. 11. Ultraviolet absorption spectra of insoluble residue isolated from "standard" membranes of M. lysodeikticus St.A by extraction with sodium dodecyl sulphate. The washed insoluble residue was suspended in tris buffer to 1.0 mg dry wt. residue/ml and the suspension clarified with ultrasound prior to recording spectrum. Note the absence of absorption at 280 nm characteristic of aromatic amino acids.

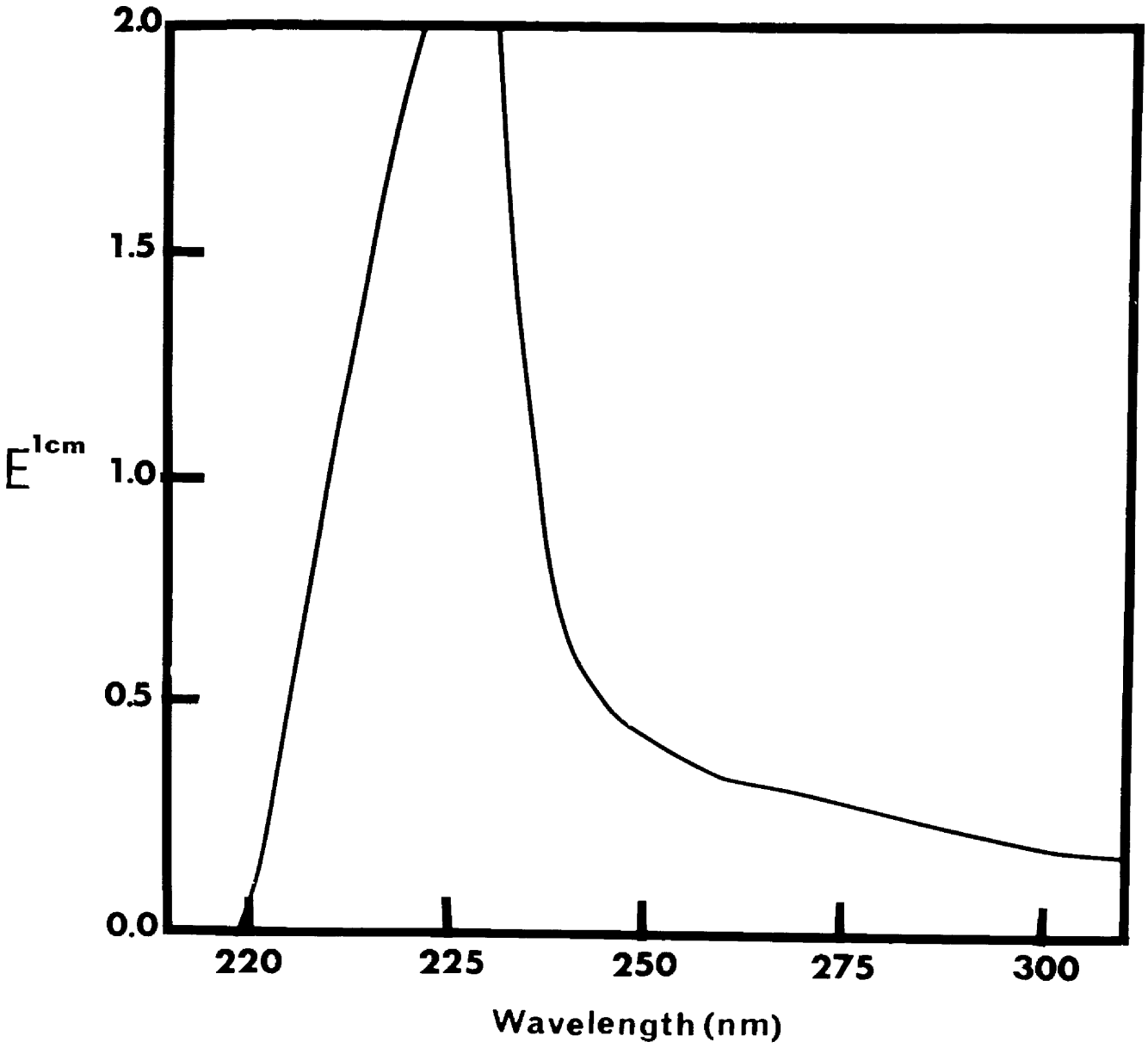

2.0
1.5
1.0
0.5
0.0
E^{1cm}
220
225
250
275
300
Wavelength (nm)

Fig. 12. Paper chromatogram of amino acid hydrolysate of the SDS insoluble residue from membranes of M. lysodeikticus St.A. SDS residue was prepared from a "standard" membrane suspension and hydrolysed as described in Methods. Neutralised hydrolysate was applied to Whatman No. 1 paper (25 cm x 25 cm) at origin (o) and amino acids separated by two dimensional ascending chromatography using solvents A. butanol-acetic acid-water (120:30:50 by vol.) followed by B. water saturated phenol-ammonia (200:1 by vol.). (.:::.) indicates the positions occupied by amino acids after chromatography of approximately 200 µg of hydrolysed residue and (=) the addition area occupied on overloaded chromatograms (approximately 1 mg of hydrolysed residue). Amino acids were identified by comparison with known standards run under identical conditions. Note the absence of methionine (position indicated by star) even on overloaded chromatograms.

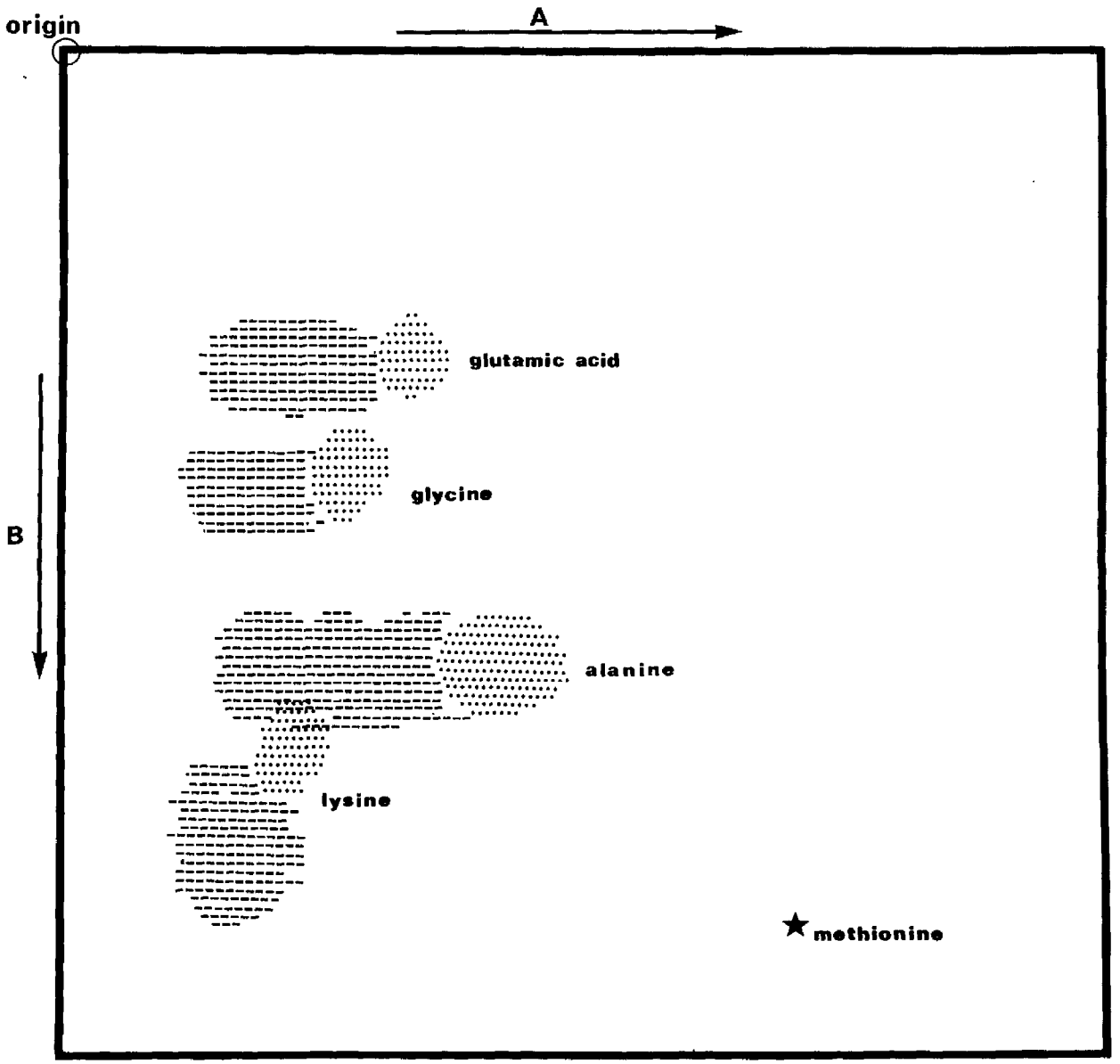

origin
A
B
glutamic acid
glycine
alanine
lysine
methionine

Fig. 13. Lysozyme sensitivity of M. lysodeikticus St.A and M. lysodeikticus St.O. Cells were grown for 12 h (from a 10% inoculum), washed once with buffer and resuspended to an E_{620}^{1cm} of 1.90. The change in E_{620}^{1cm} of suspensions of St.A (•–•–•) and St.O (——) after addition of lysozyme (100 µg/ml) was recorded automatically at 30°C. Note the slower rate of decline of optical density for cell suspensions of St.A during the later stages of the lysis curve.

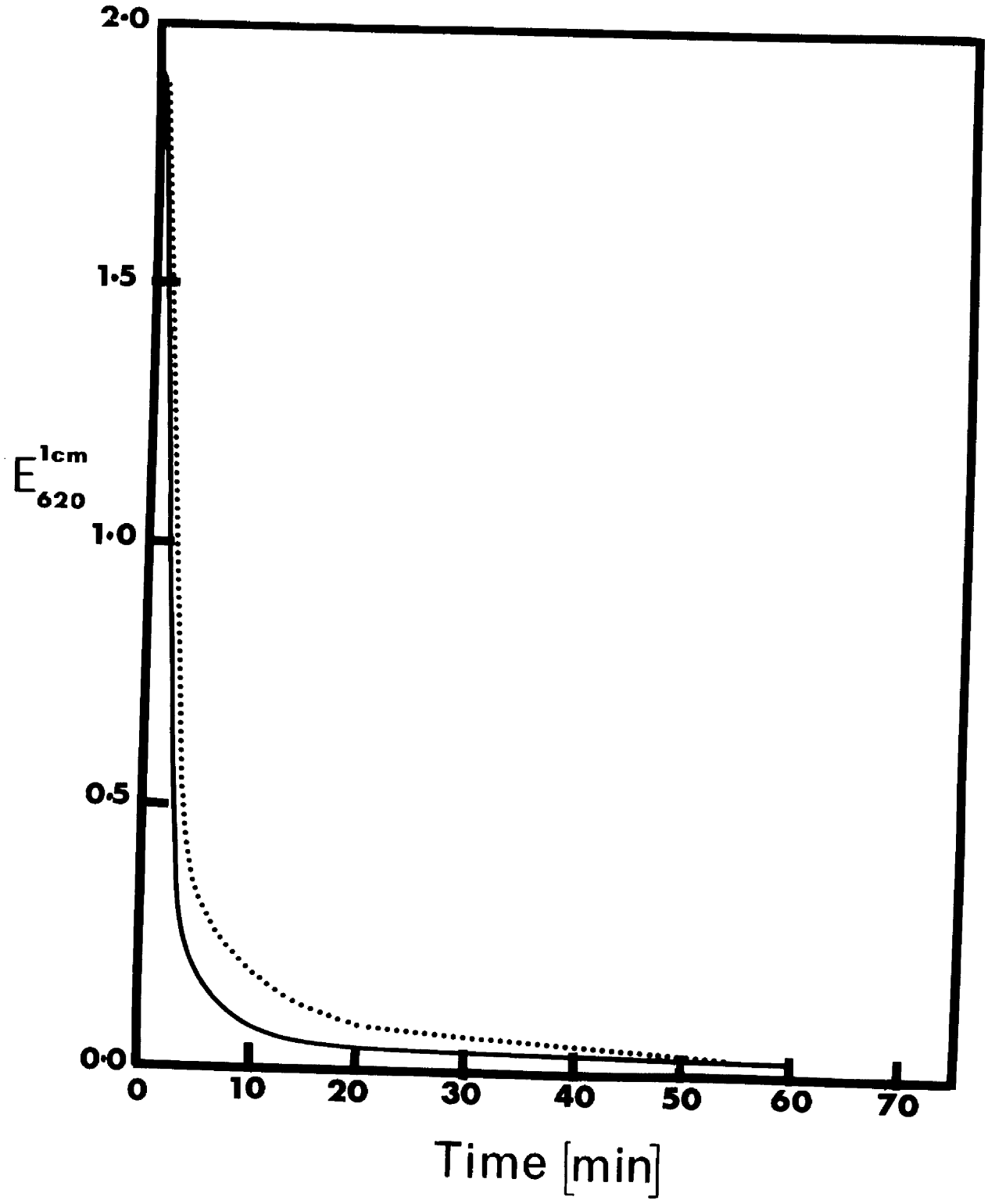

2·0
1·5
1·0
0·5
0·0
E_{620}^{1cm}
0
10
20
30
40
50
60
70
Time [min]

Fig. 14. The effect of Mg^{2+} concentration on membrane release and protoplast stability. Protoplast suspensions were prepared from plasmolysed cells at various Mg^{2+} concentrations and held for 2 h at 30°C. E_{260} (■) and E_{446}(●) values were determined for each supernatant fraction.

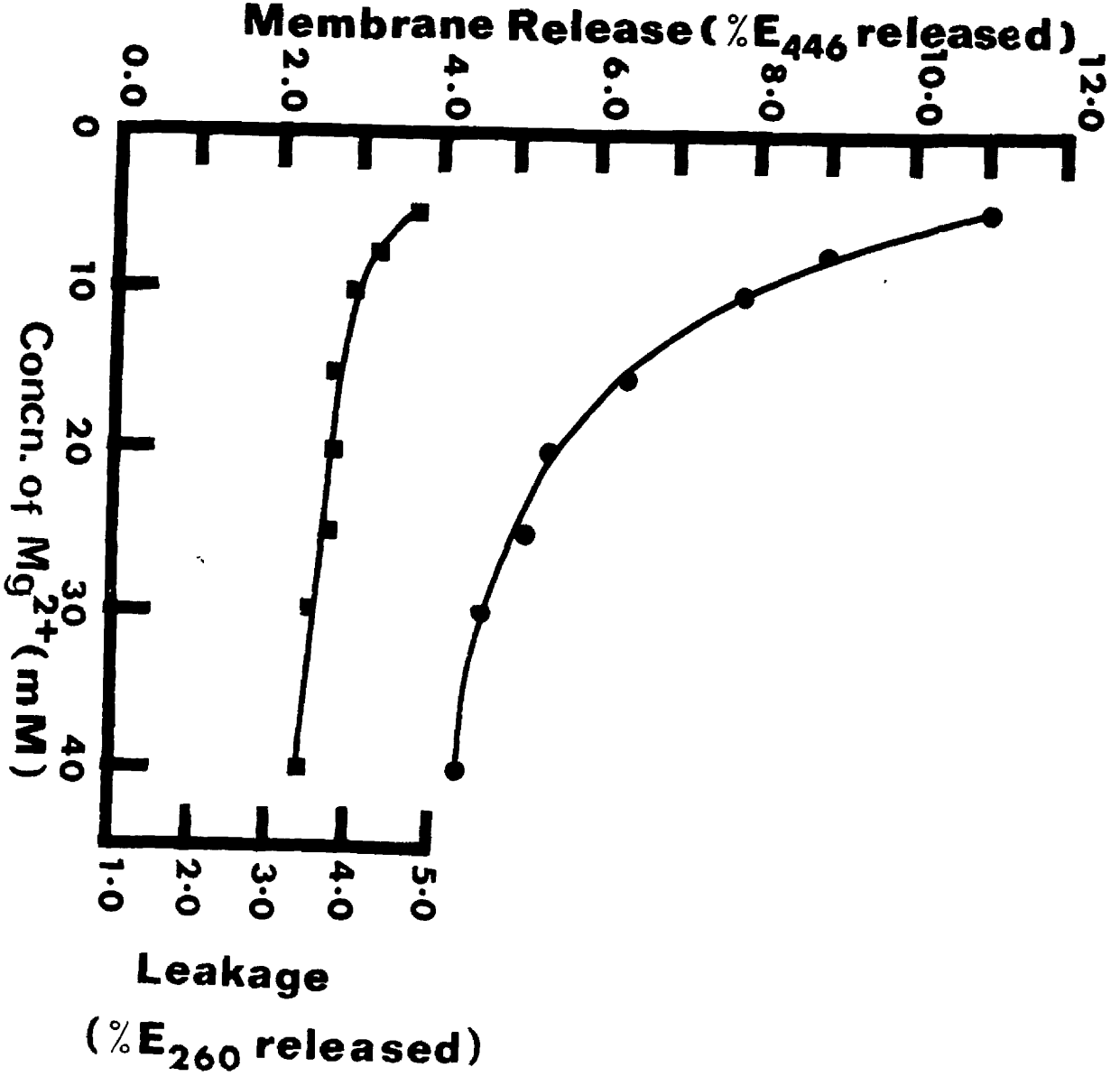

Membrane Release (%E446 released)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
0
10
20
30
40
Concn. of Mg2+ (mM)
1.0
2.0
3.0
4.0
5.0
Leakage
(%E260 released)

Fig. 15. Cytochrome difference spectra of isolated mesosomal membranes from M. lysodeikticus St.O. Mesosomal membranes, prepared in the presence of (a) 10 mM Mg^{2+} (b) 20 mM Mg^{2+} (c) 40 mM Mg^{2+}, were resuspended to final concentrations of (a) 8.91 (b) 6.80 and (c) 4.81 mg dry wt. membrane/ml. Sonicated suspensions were chemically reduced by the addition of sodium dithionite and difference spectra recorded after 10 min. Calculation of the cytochrome content by the method of Lisenkova and Mokhova (1964) using the parameters given in Table 5 (from Simakova, Lukoyanova, Biryuzova and Gel'man, 1969) gives values for cytochrome b_{556} content of preparations a, b and c of 0.45, 0.51 and 0.49 μ moles/g of membrane respectively. Note that cytochrome b_{556} is the only cytochrome detected in these preparations.

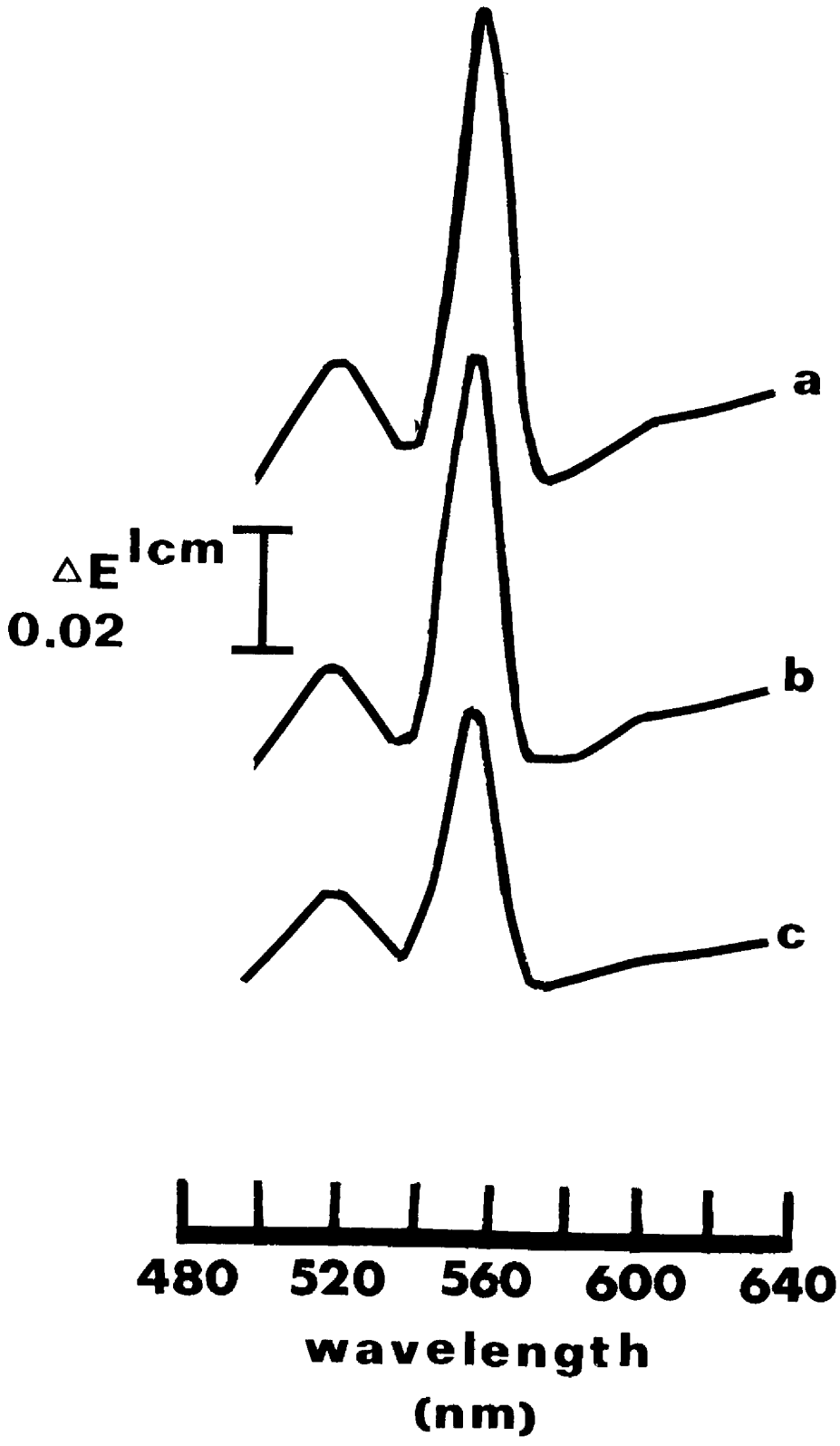

a
b
c
ΔE 1cm
0.02
480
520
560
600
640
wavelength
(nm)

Fig. 16. Comparison of infra red absorption spectra of peripheral membranes (A) and "lipid" fractions (B, C and D) from peripheral membranes of M. lysodeikticus St.O. (B) Acetone-methanol (7:2 by vol.) extract of peripheral membranes; (C) ether extract and (D) subsequent acetone-methanol (7:2 by vol.) extract of the residue (remaining after acetone-methanol extraction of peripheral membranes) following its hydrolysis to release "bound" lipid (refluxing 5% v/v HCl in methanol). Spectra of A, B, C and D have transmittance values at 3600 cm^{-1} of 72%, 81%, 92% and 62% respectively and at 1800 cm^{-1} of 94%, 97%, 95% and 81% respectively.

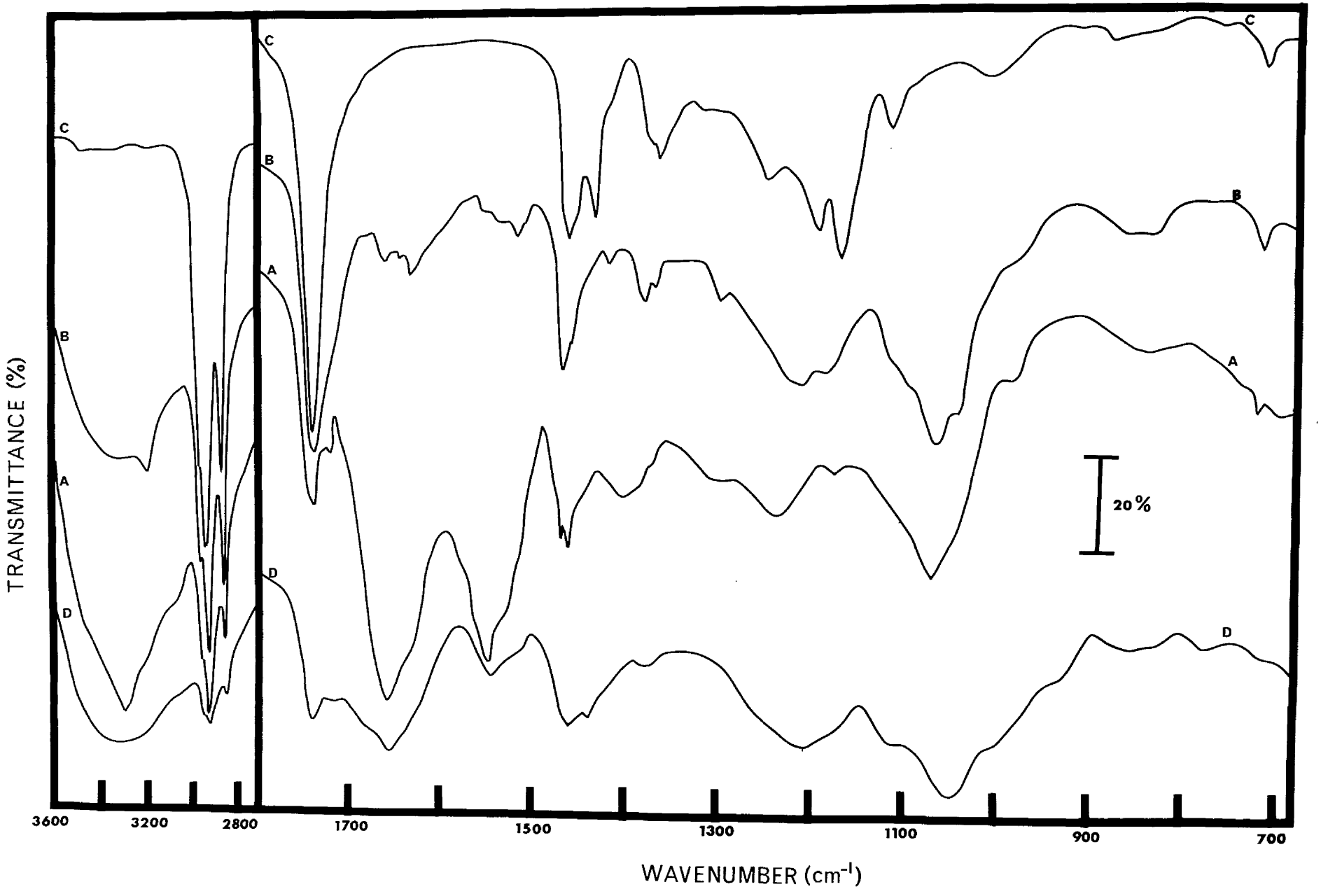

TRANSMITTANCE (%)
C
B
A
D
20%
3600
3200
2800
1700
1500
1300
1100
900
700
WAVENUMBER (cm-1)

Fig. 17. Chemical and substrate reduction of cytochromes in mesosomal membranes isolated from M. lysodeikticus St.O. Membrane preparations (17.4 mg dry wt. membrane/ml) were clarified with ultrasound and cytochromes reduced with $NADH_2$ (a - d), sodium hydrogen malate (e - i) and sodium dithionite (j - l). Difference spectra were recorded immediately following addition of reducing agent (a, e and j) after 5 min (b, f and k), after 10 min (c, g and l) after 20 min (d and h) and after 30 min (i). Note that cytochrome b_{556}, the only detectable cytochrome present in mesosome preparations, is reduced by both $NADH_2$ and malate.

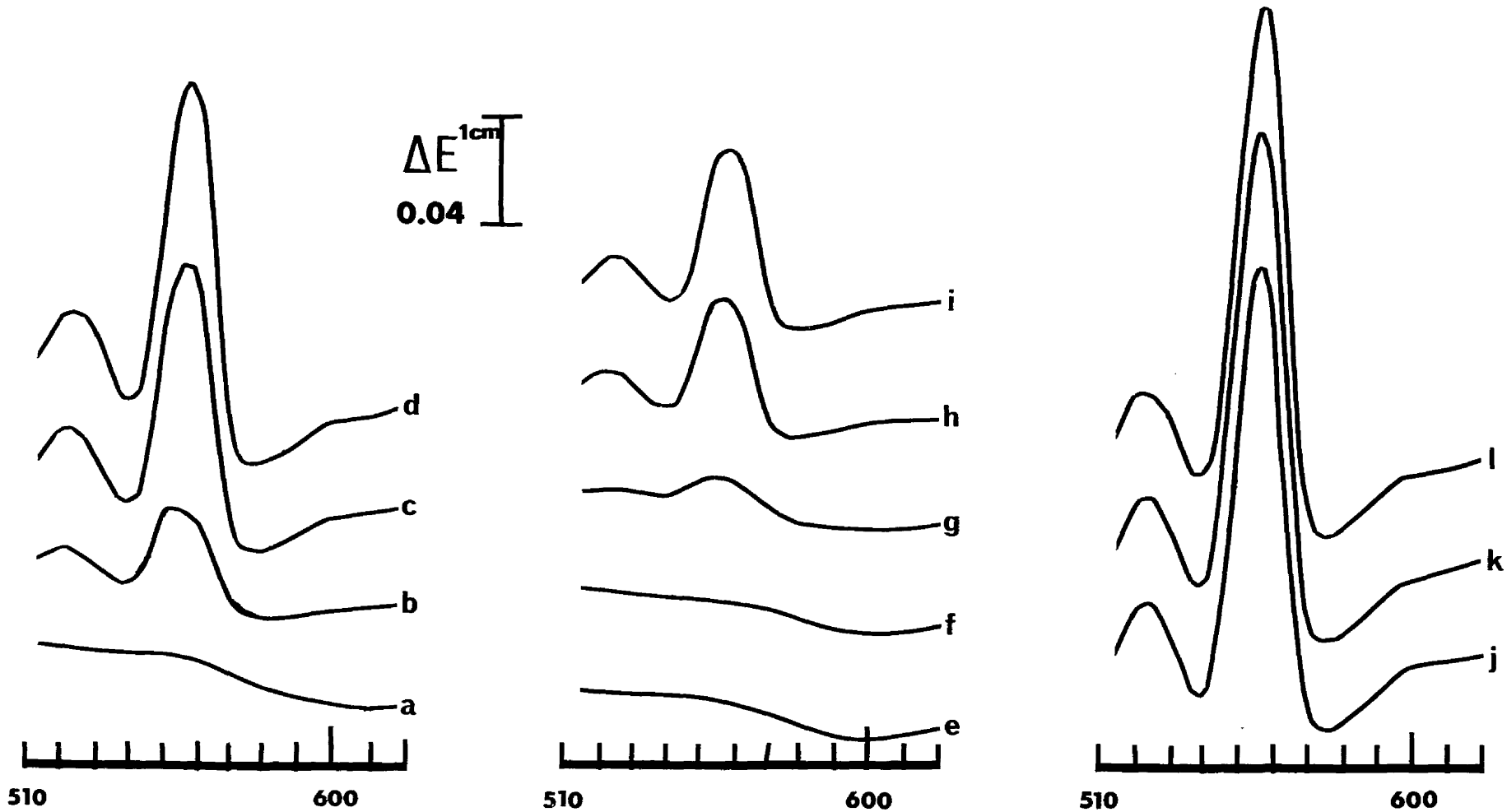

ΔE 1cm
0.04
d
c
b
a
i
h
g
f
e
l
k
j
510
600
510
600
510
600
Wavelength (nm)

Fig. 18. The effect of Triton X-100 on the kinetics of substrate reduction of cytochromes in peripheral membrane preparations from M. lysodeikticus St.0. Peripheral membrane preparations (18.4 mg dry wt. membrane/ml) were clarified by ultrasound (a - h) or by addition of Triton X-100 (i - p) and reduced by addition of $NADH_2$ (a - d and i - l) or sodium hydrogen malate (e - h and m - p). Cytochrome difference spectra were recorded immediately following addition of reducing agent (a, e, i and m) and after 30 s (b, f, i and n) 60 s (c, g, k and o) and after 5 min (d, h, l and p). Note selective reduction of cytochrome b_{556} by substrate in the presence of Triton X-100.

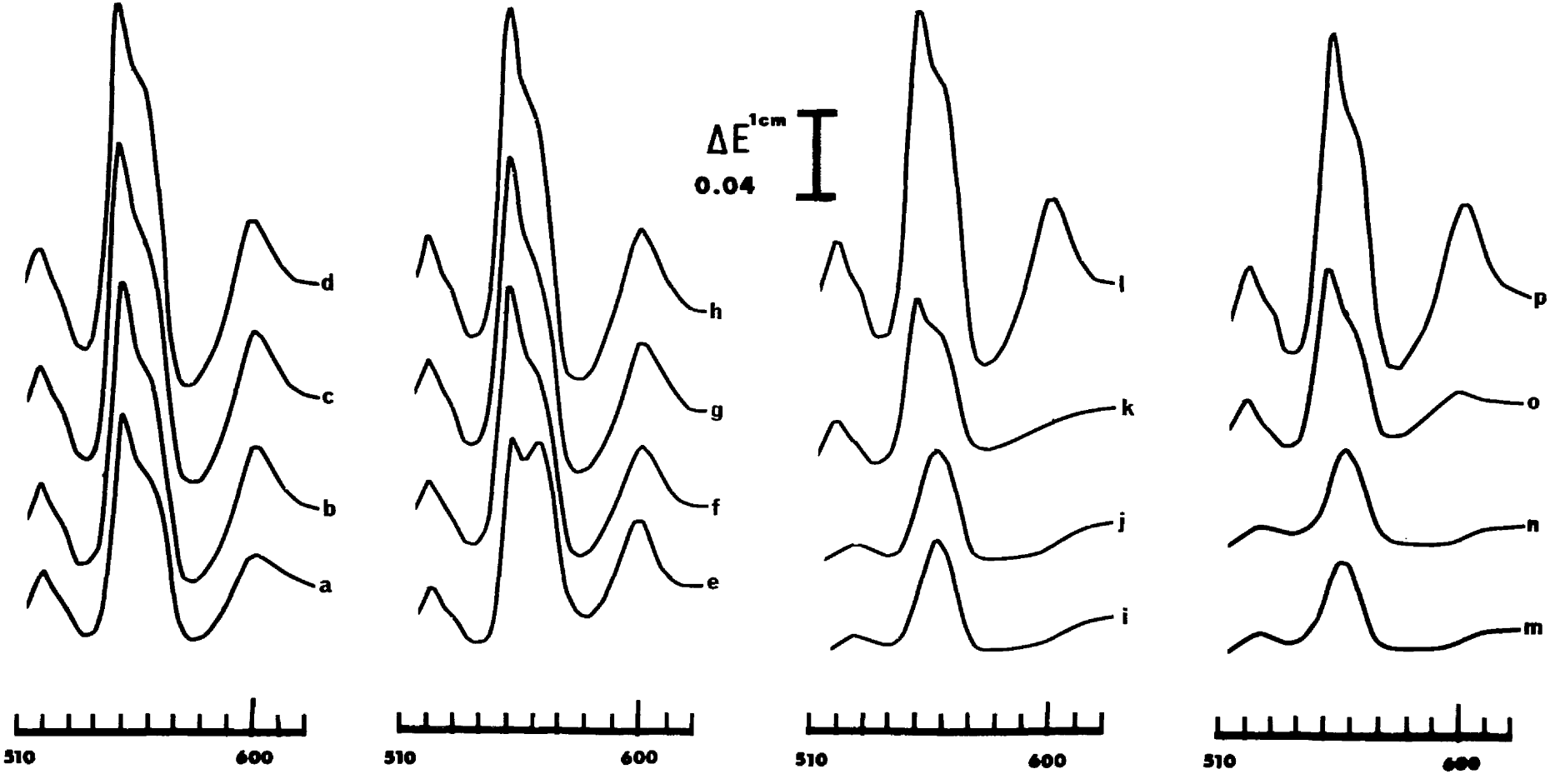

ΔE 1cm
0.04
d
c
b
a
h
g
f
e
l
k
j
i
p
o
n
m
510
600
510
600
510
600
510
600
Wavelength (nm)

Fig. 19. Effect of Vitamin K_3 on the kinetics of reduction of cytochromes in peripheral membrane preparations from M. lysodeikticus St.O. Peripheral membrane preparations (17.9 mg dry wt. membrane/ml) were clarified by addition of Triton X-100, and Vitamin K_3 added to final concentrations of 0.0 mM (a - f), 0.1 mM (g - k) and 1.0 mM (l - o). Cytochromes were reduced by addition of sodium hydrogen malate and difference spectra recorded immediately following addition (a, g and l) and after 30 s (b, h and m), 60 s (c, j and n), 90 s (d and j), 2 min (e) and 5 min (f, k and o). Note that the addition of Vitamin K_3 appears to increase the efficiency of electron transfer from cytochrome b_{556} to the other cytochromes of the respiratory chain.

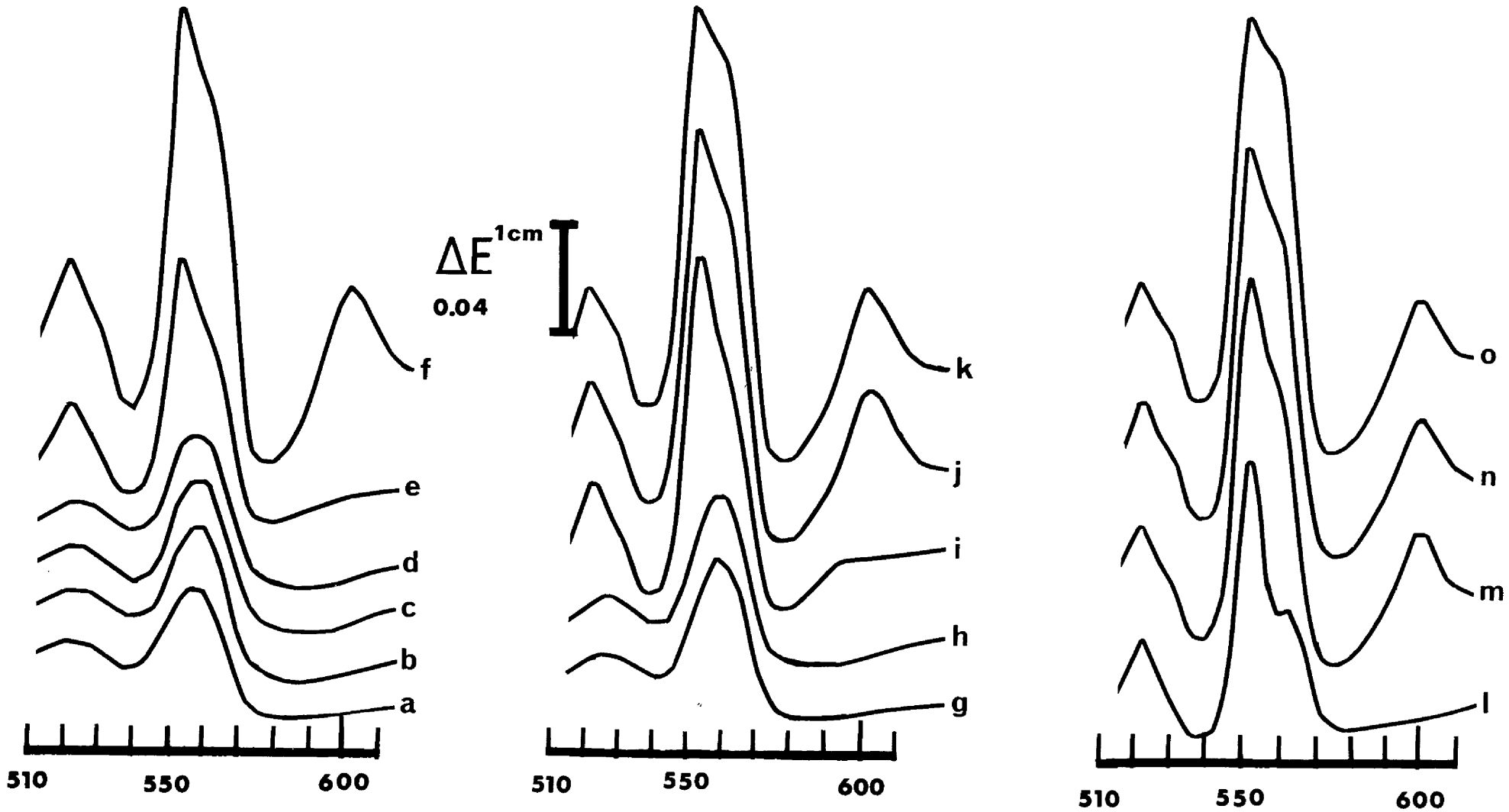

ΔE 1cm
0.04
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
510
550
600
510
550
600
510
550
600
Wavelength (nm)

Fig. 20. Cytochrome difference spectra of residues obtained after extraction of "standard" membranes of M. lysodeikticus St.0 with deoxycholate (see Methods). Residues (a) DO 1 (3.59 mg dry wt. residue/ml) (b) DO 2 (3.81 mg dry wt. residue/ml) and (c) DO 3 (0.71 mg dry wt. residue/ml) were clarified with ultrasound and suspensions reduced chemically by the addition of sodium dithionite. Difference spectra were recorded after 10 min. Unlike DO 3, both DO 1 and DO 2 indicate the presence of cytochromes a_{601}, b_{560} and c_{550}.

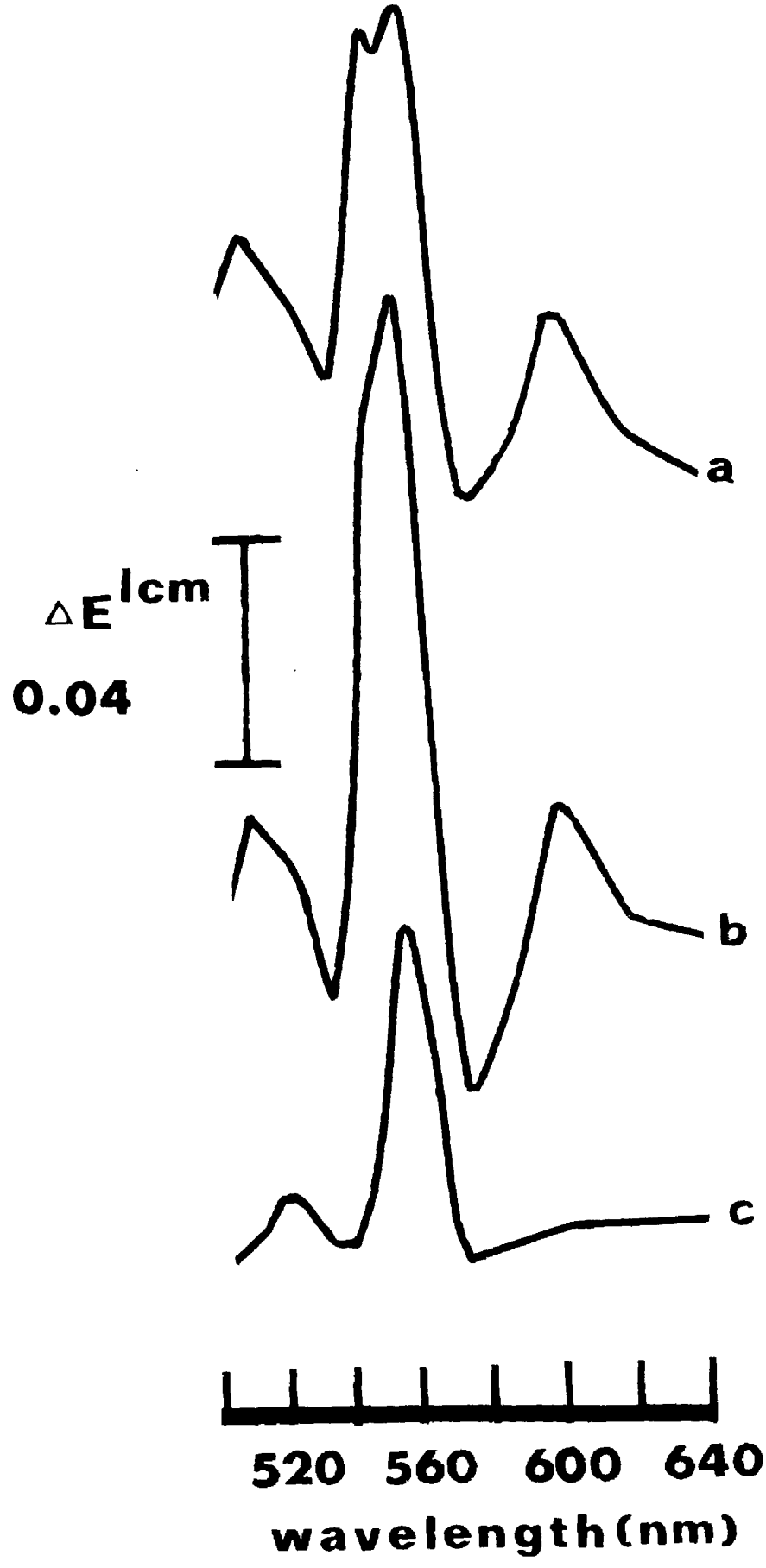
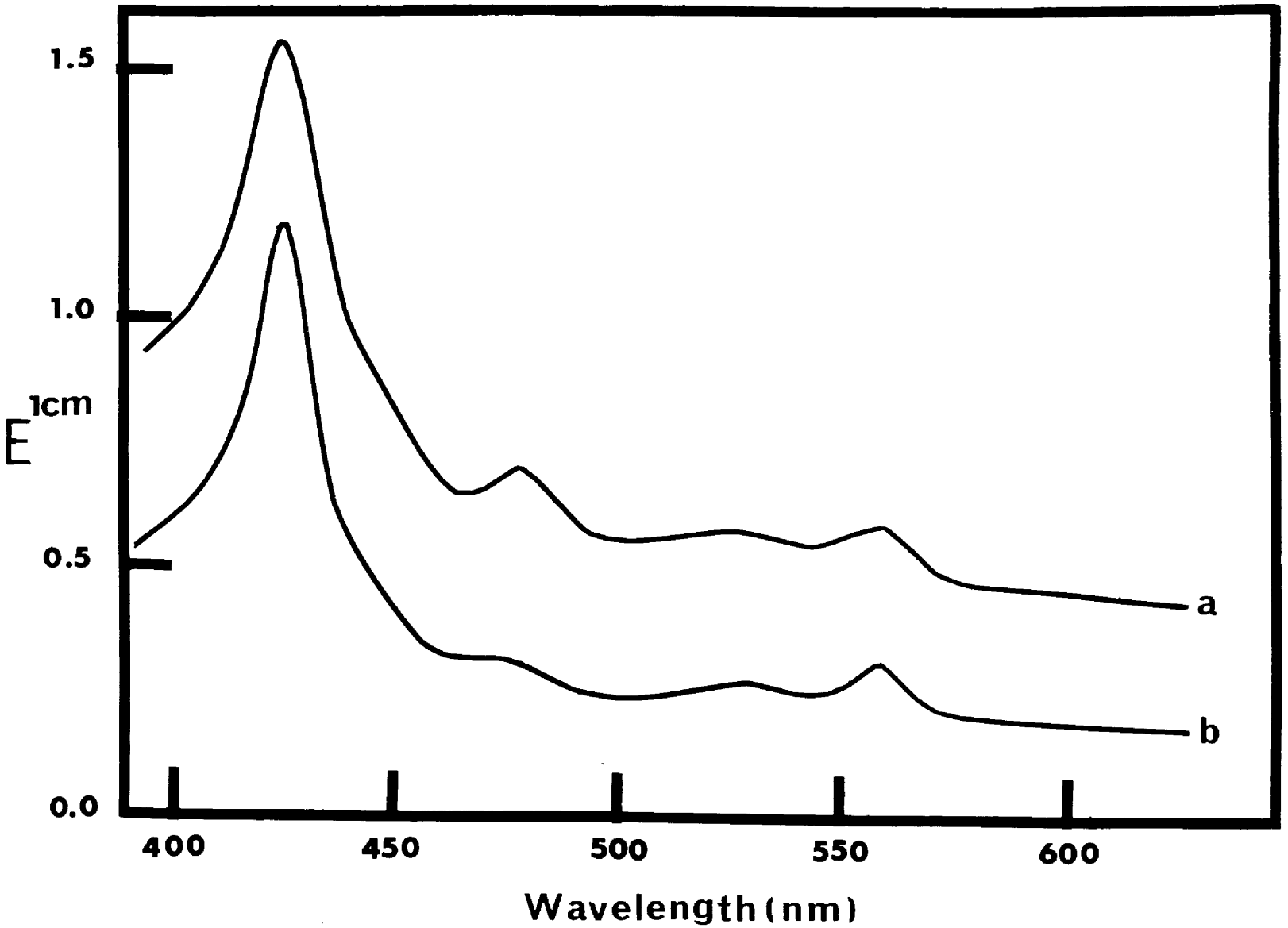

a
b
c
ΔE lcm
0.04
520 560 600 640
wavelength (nm)

Fig. 21. Reduced cytochrome spectra of residue obtained after extraction of mesosomal membranes with deoxycholate. Fresh mesosomal membrane (17.6 mg) isolated from M. lysodeikticus St.0 was extracted with 1% sodium deoxycholate (Methods) and the insoluble residue sedimented by centrifugation at 200 000 g for 7 h. The pellet was washed twice in tris buffer, made to 1 ml with tris buffer and chemically reduced with sodium dithionite after clarification of the suspension with ultrasound. The reduced spectrum was recorded after 10 min (a). The reduced spectrum of DO 3 at a concentration of 1.42 mg dry wt. residue/ml has been inserted for comparison purposes (b). Spectrum (a) has been positively displaced by 0.30 absorbance units. Note the basic similarity between the reduced cytochrome spectra of the two fractions.


1.5
1.0
0.5
0.0
E 1cm
400
450
500
550
600
Wavelength (nm)
a
b

Breakdown of Plate numbers

Plates 1 - 3 Development of a system for SDS-polyacrylamide disc gel electrophoresis of insoluble membrane proteins.

Plates 4 - 6 Comparison of polypeptide components from M. lysodeikticus St. A and St. O by SDS-polyacrylamide disc gel electrophoresis.

Plates 7 - 10 Features of thin-sectioned cell membrane and cell wall of M. lysodeikticus St. A and St. O.

Plates 11 - 24 Features of negatively stained mesosomal membrane prepared at different Mg^{2+} concn.

Plates 25 - 29 Features of negatively stained peripheral membrane preparations.

Plate 30 Comparison of polypeptide components from mesosomal and peripheral membrane.

Plates 31 - 36 Comparison of deoxycholate-insoluble membrane residue from M. lysodeikticus.

Plates 37 - 45 Study of the division process in M. lysodeikticus.

Plates 46 - 60 Features of freeze-fractured whole cells of M. lysodeikticus St. A and St. O.

Plates 61 - 67 Features of freeze-fractured isolated membrane from M. lysodeikticus St. A and St. O.

Plates 68 - 73 Features of freeze-fractured and etched fixed protoplast of M. lysodeikticus St. O.

Plates 74 - 82 Features of freeze-fractured and etched isolated membrane from *M. lysodeikticus* St. A and St. O.

Plates 84 & 85 Features of negatively stained "shocked" membranes from *M. lysodeikticus* St. O.

Plates 86 - 89 Features of freeze-fractured mesosomal membrane from *M. lysodeikticus* St. O.

Plates 90 - 96 Features of freeze-fractured and etched mesosomal membrane from *M. lysodeikticus* St. O.

Plate 97 Features of freeze-fractured deoxycholate-insoluble membrane residue from *M. lysodeikticus* St. O.

ABBREVIATIONS USED ON PLATES

a	-	areas devoid of intramembrane particles occuring on convex fracture faces of protoplast peripheral membrane
b	-	bridges connecting membrane and wall as observed in thin sections
c	-	craters
cpm	-	cross-fractured peripheral membrane
ctm	-	cross-fractured membrane of mesosomal tubules
cy	-	cytoplasm
d	-	depressions observed in fractured mesosomal tubules
dm	-	double membrane
e	-	edge of etched surface
icp	-	intracytoplasmic particles
imp	-	intramembrane particles
iom	-	inner surface of outer half of peripheral membrane
iov	-	inner surface of outer half of mesosomal vesicle membrane
itv	-	intratubular vesicle
iwp	-	intrawall particle
l	-	linear depressions
M	-	Mesosome
n	-	neck of septum
oim	-	outer surface of inner half of peripheral membrane
oiv	-	outer surface of inner half of mesosomal vesicle membrane
oom	-	outer surface of outer half of peripheral membrane
oot	-	outer surface of outer half of mesosomal tubular membrane
oov	-	outer surface of outer half of mesosomal vesicular membrane

p - protuberances

pm - peripheral membrane

s - septum

- concealed septum

st - stalk

t - mesosomal membrane tubules

ucw - unruptured cell wall

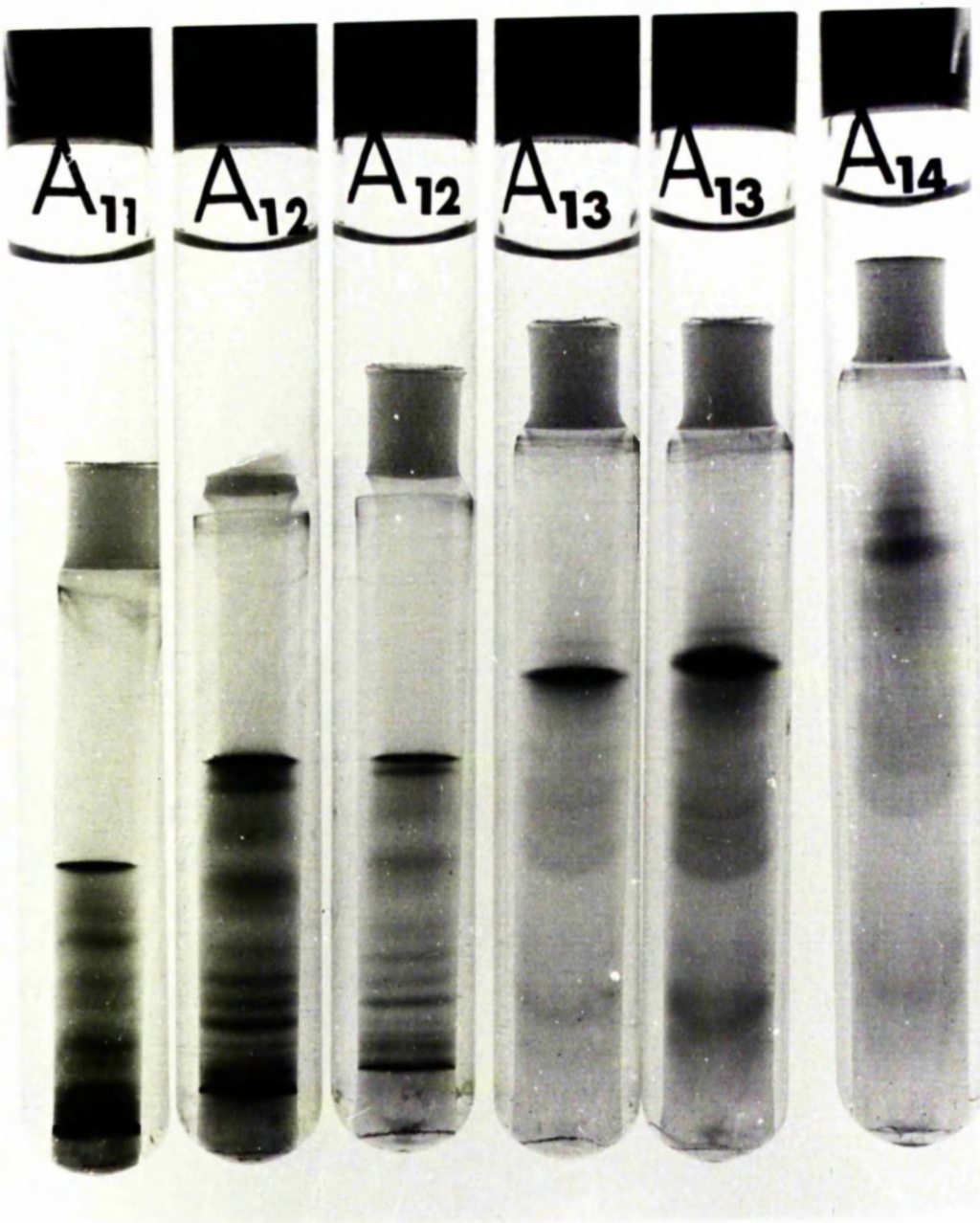
v - mesosomal membrane vesicle (v)

w - wall

 - direction of shadow

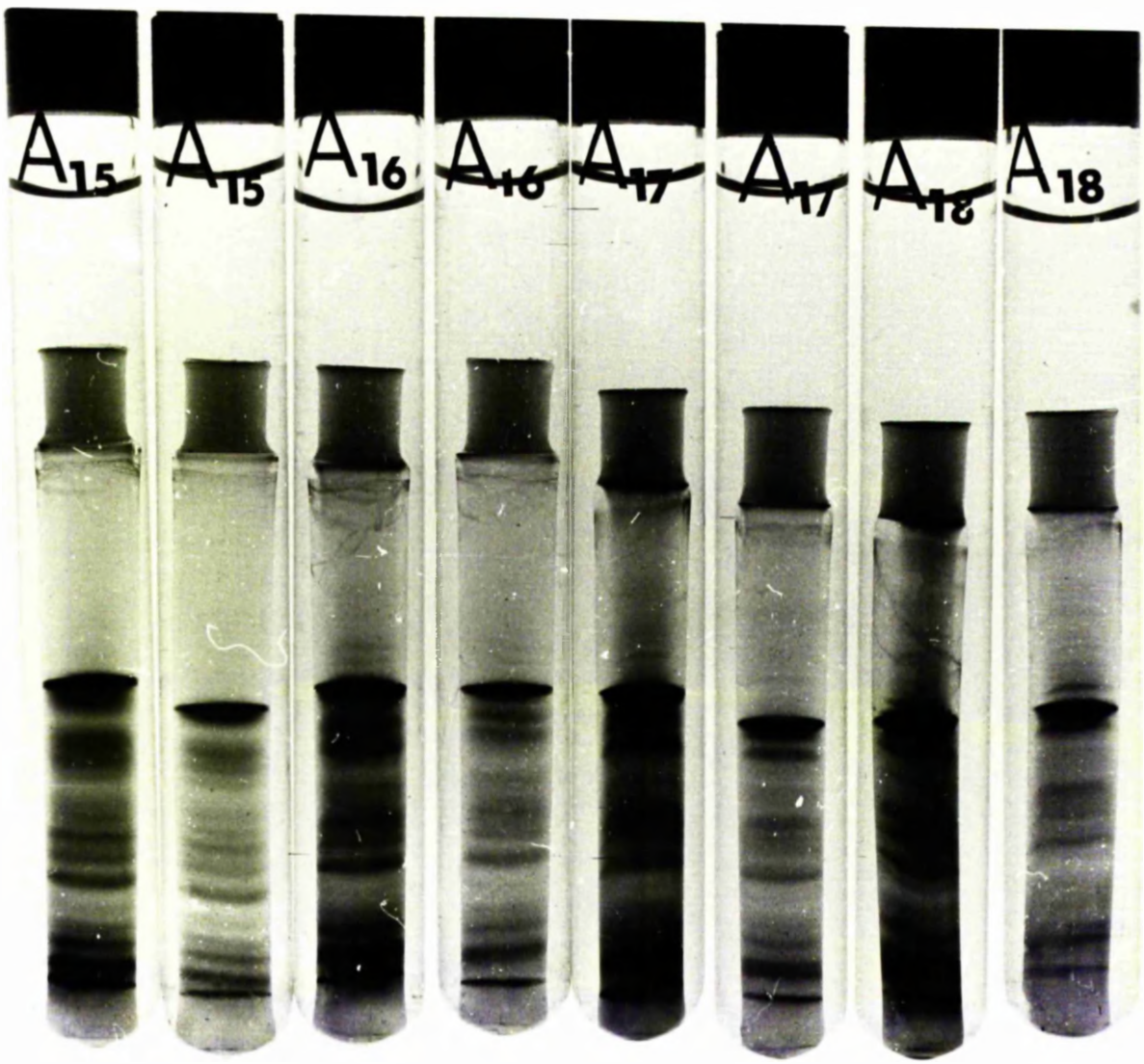
EXPLANATION OF PLATE 1

Membrane proteins from M. lysodeikticus St.0 subjected to SDS-polyacrylamide gel electrophoresis in separating gels of varying constitutions. A_{11} : approximately 200 µg membrane protein separated on gels containing 7% w/v acrylamide and 0.184% NN'-methylenebisacrylamide (bis). A_{12} : approximately 200 µg and 100 µg membrane protein separated on gels containing 11.7% w/v acrylamide and 0.153% bis. A_{13} : approximately 100 µg and 200 µg of membrane protein separated on gels containing 16.3% w/v acrylamide and 0.121% bis. A_{14} : approximately 200 µg membrane protein separated on gels containing 21% w/v acrylamide and 0.092% bis. Optimum resolution of faster migrating components appears to occur in gels containing approx. 12% w/v acrylamide.


A11
A12
A12
A13
A13
A14

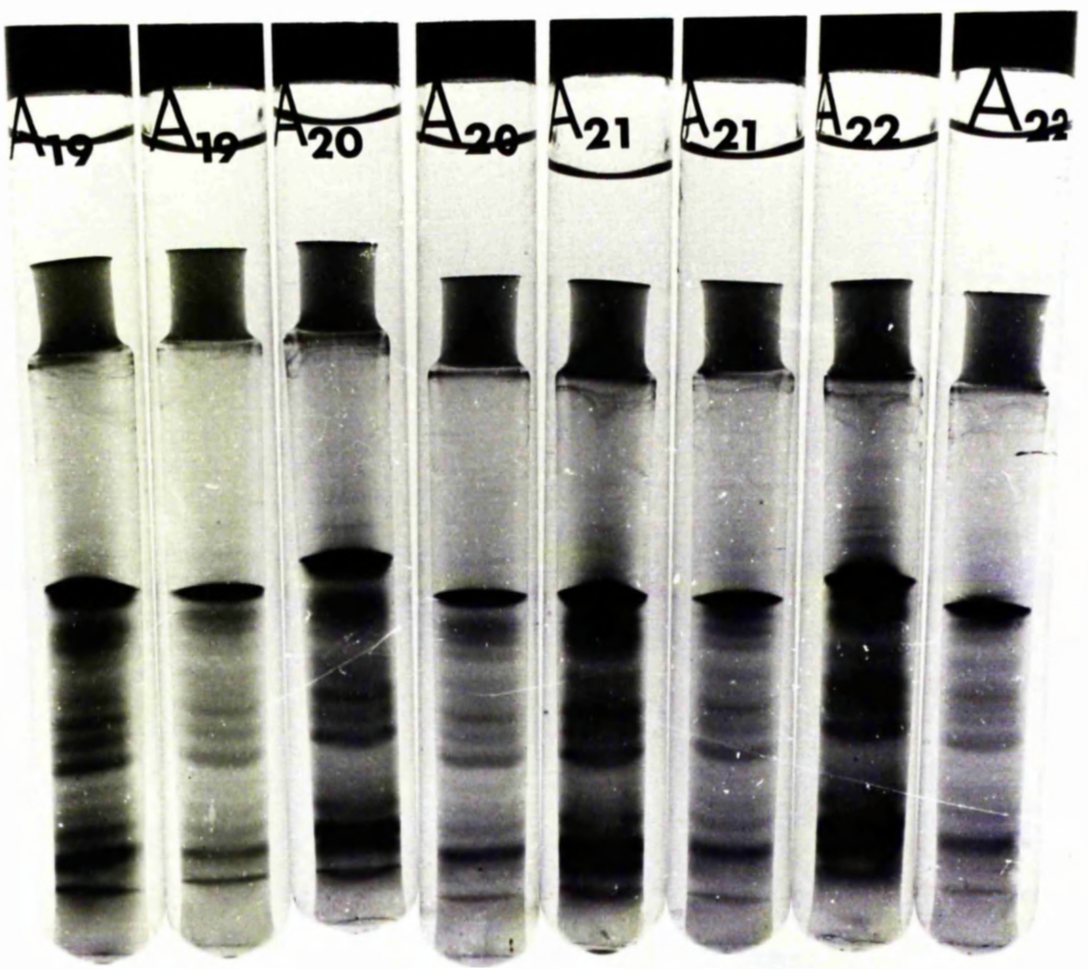
EXPLANATION OF PLATE 2

Membrane proteins of M. lysodeikticus St.O subjected to SDS-polyacrylamide gel electrophoresis in separating gels of varying bis concentrations. 200 μg (first gel of each series) and 100 μg (second gel of each series) of membrane protein were electrophoresed in separating gels containing 11.7% w/v acrylamide and made 0.153% (A_{15}), 0.210% (A_{16}), 0.274% (A_{17}) and 0.347% (A_{18}) with respect to bis. Note the distortion of protein bands which occurs in gels containing concentrations of bis above 0.21% w/v (i.e. in gels A_{17} and A_{18}). Gels A_{15} appear to give least distortion of bands after electrophoresis of 200 μg of membrane protein.


A15
A15
A16
A16
A17
A17
A18
A18

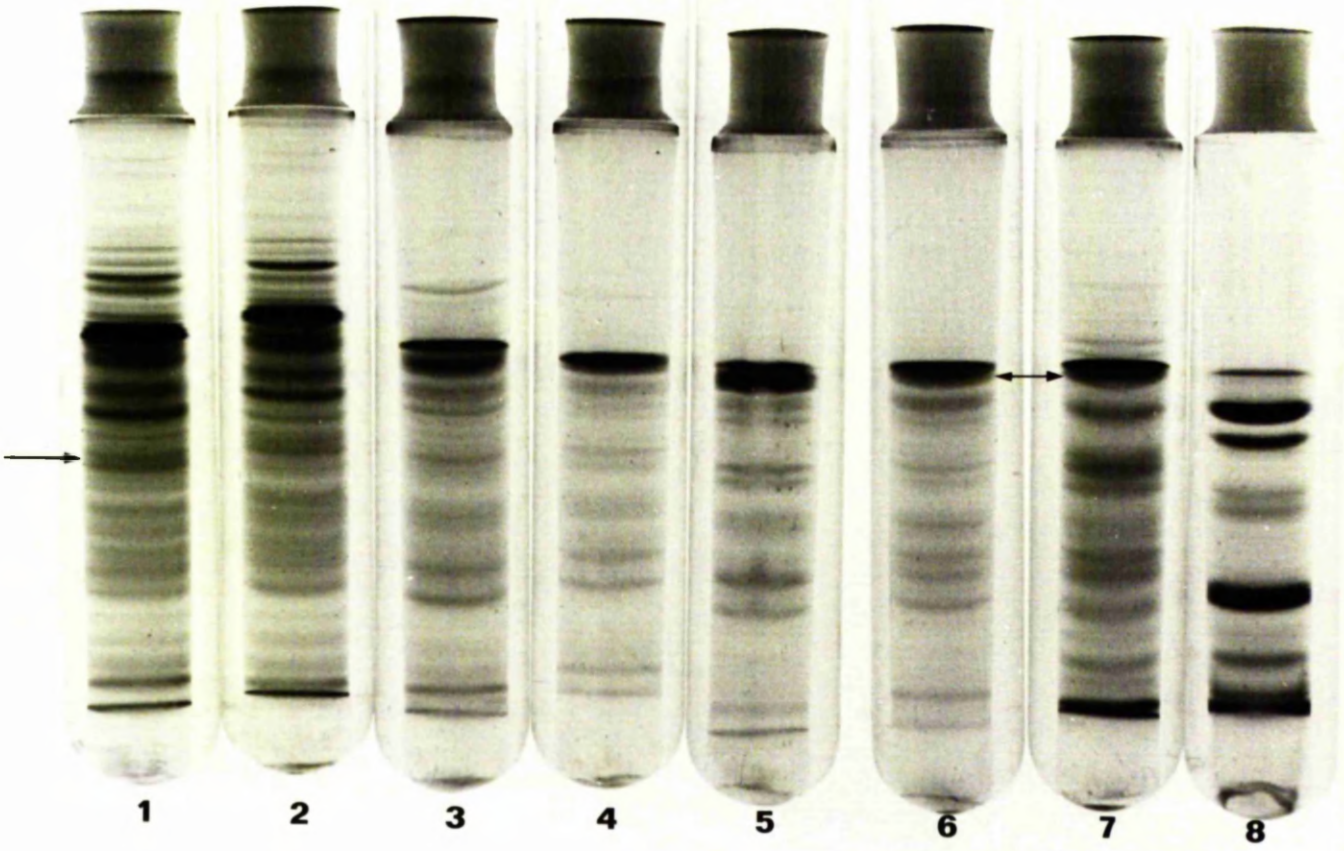
EXPLANATION OF PLATE 3

Membrane proteins of M. lysodeikticus St.0 subjected to SDS-polyacrylamide gel electrophoresis in separating gels of varying bis concentrations. 200 μg (first gel of each series) and 100 μg (second gel of each series) of membrane protein were electrophoresed in separating gels containing 13% w/v acrylamide and made 0.100% (A_{19}), 0.128% (A_{20}), 0.159% (A_{21}) and 0.220% (A_{22}) with respect to bis. Note that none of these gels show the lack of band distortion and/or the resolution offaster migrating components obtained with gels containing 11.7% w/v acrylamide and 0.153% bis (Plate 2, A_{15} or Plate 1, A_{12}).


A19
A19
A20
A20
A21
A21
A22
A22

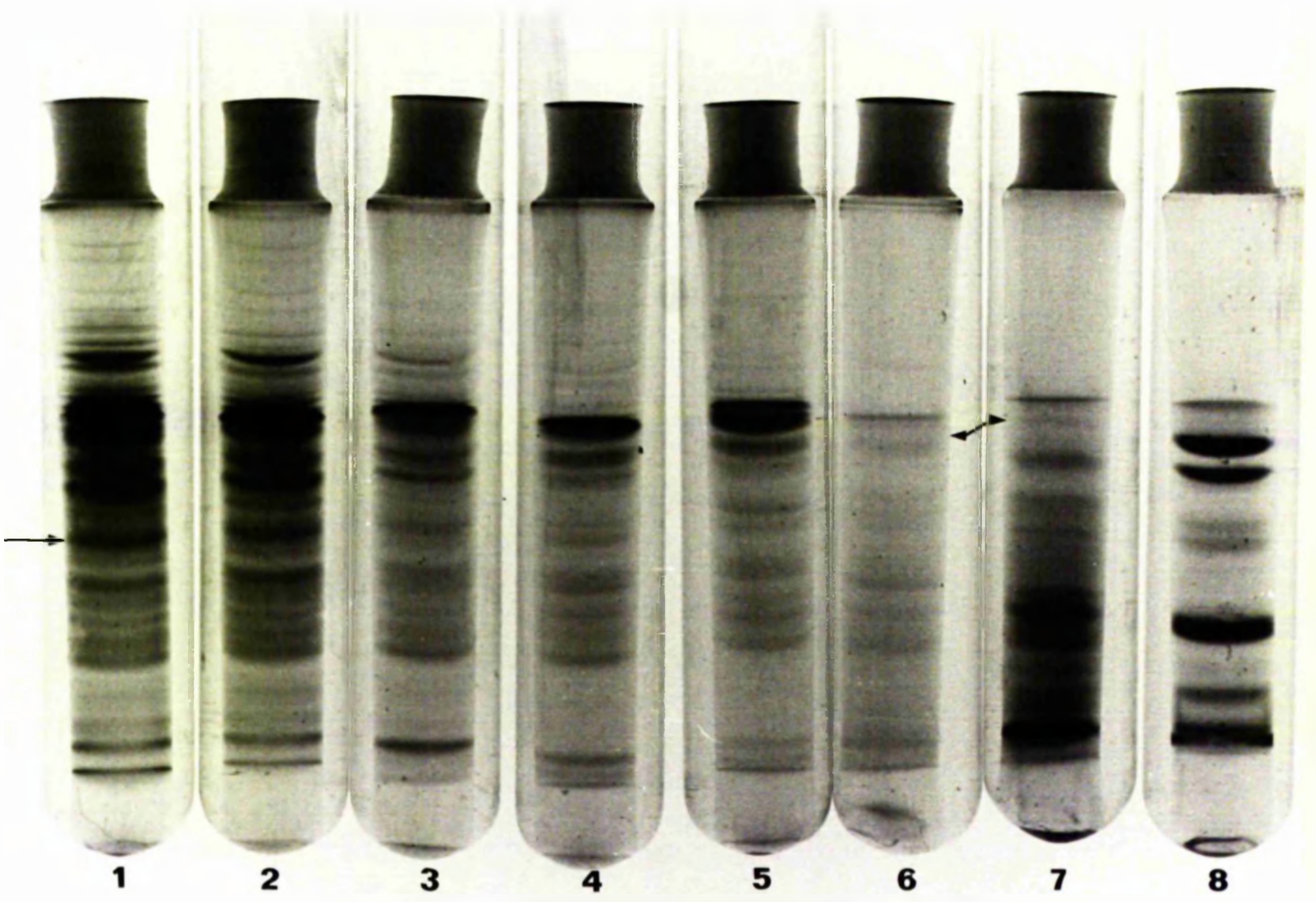
EXPLANATION OF PLATE 4

Components from supernatant washing fluids of "standard" membranes of M. lysodeikticus St.0 subjected to SDS-polyacrylamide disc gel electrophoresis. Supernatant washing fluids were dialysed against distilled water, freeze dried and 200 µg applied to 11.7% acrylamide gels. Tube 1 - cytoplasmic fraction; Tubes 2 - 6 - supernatant washing 1 to 5 respectively; Tube 7 - 200 µg lyophilized "standard" membrane; Tube 8 - standard proteins as per Plate 30. Note the great similarity with corresponding fractions isolated from St.A (Plate 6). Arrows point to the few observable differences in polypeptide spectrum of fractions prepared from the two strains.


1
2
3
4
5
6
7
8

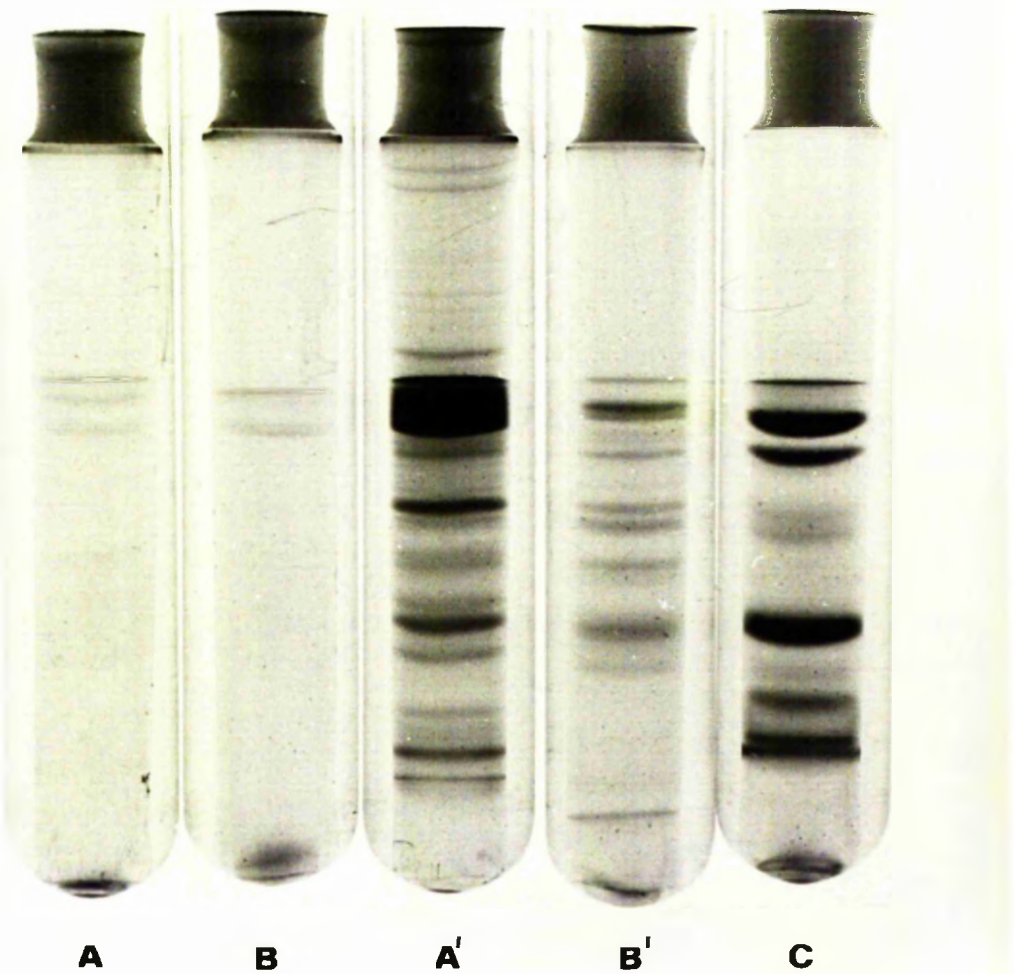
EXPLANATION OF PLATE 5

Components from supernatant washing fluids of "standard" membranes of M. lysodeikticus St.A subjected to SDS-polyacrylamide disc gel electrophoresis. Supernatant washing fluids were dialysed against distilled water, freeze dried and approx. 200 µg applied to 11.7% acrylamide gels. Tube 1 - cytoplasmic fraction; Tubes 2 - 6 - supernatant washing 1 to 5 respectively; Tube 7 - 200 µg lyophilized "standard" membrane; Tube 8 - standard proteins as per Plate 30. Note the great similarity with corresponding fractions isolated from St.O (Plate 5). Arrows point to the few observable differences in the polypeptide spectrum of fractions prepared from the two strains.


1
2
3
4
5
6
7
8

EXPLANATION OF PLATE 6

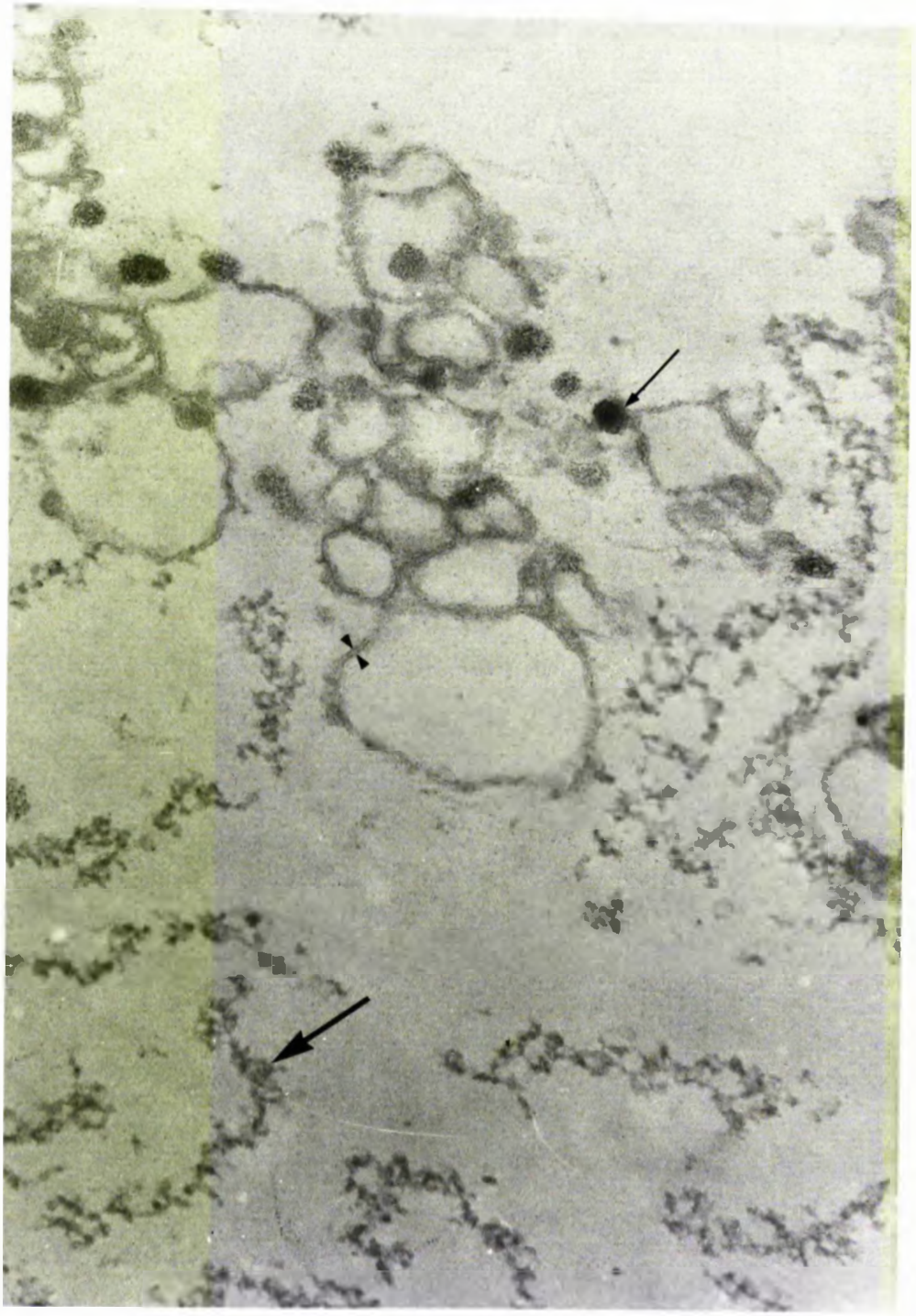
Membrane polypeptides released from "standard" membranes of M. lysodeikticus St.O and St.A by treatment with EDTA and by ionic shock and subjected to SDS-polyacrylamide disc gel electrophoresis. "Standard" membrane suspensions from St.O and St.A were subjected to ionic shock or to treatment with EDTA as detailed in Methods. The membrane supernatant fluids were dialysed and freeze dried and approx. 200 μg dry wt. applied to acrylamide gels (containing 11.7% acrylamide). A and A' - ionic shock supernatant from St.A and St.O respectively. B and B' - EDTA extracted components from St.A and St.O respectively. C - Marker proteins (for identification see Plate 30). It should be noted that membrane supernatant fractions obtained from St.A contained a large amount of nonstaining material, probably residual peptidoglycancomponent. Hence the amount of staining material in the gels per 200 μg of sample added was very much lower for fractions isolated from St.A than for corresponding fractions from St.O. Although not immediately apparent from the Plate, due to inherent reproduction problems, the pattern of staining bands in gels A and A' and in gels B and B' were closely similar.


A
B
A'
B'
C

EXPLANATION OF PLATE 7

Thin section of "standard" membrane preparation isolated from M. lysodeikticus St.A. The "triple-track" profile characteristic of the membrane system is apparent (opposing arrows). Note also the presence of amorphous material (large arrow) in the preparation. The densely staining areas, apparently continuous with the membrane system (small arrow) are frequently visualised in thin sections of the mesosome in situ in this organism. (see Plate 40).

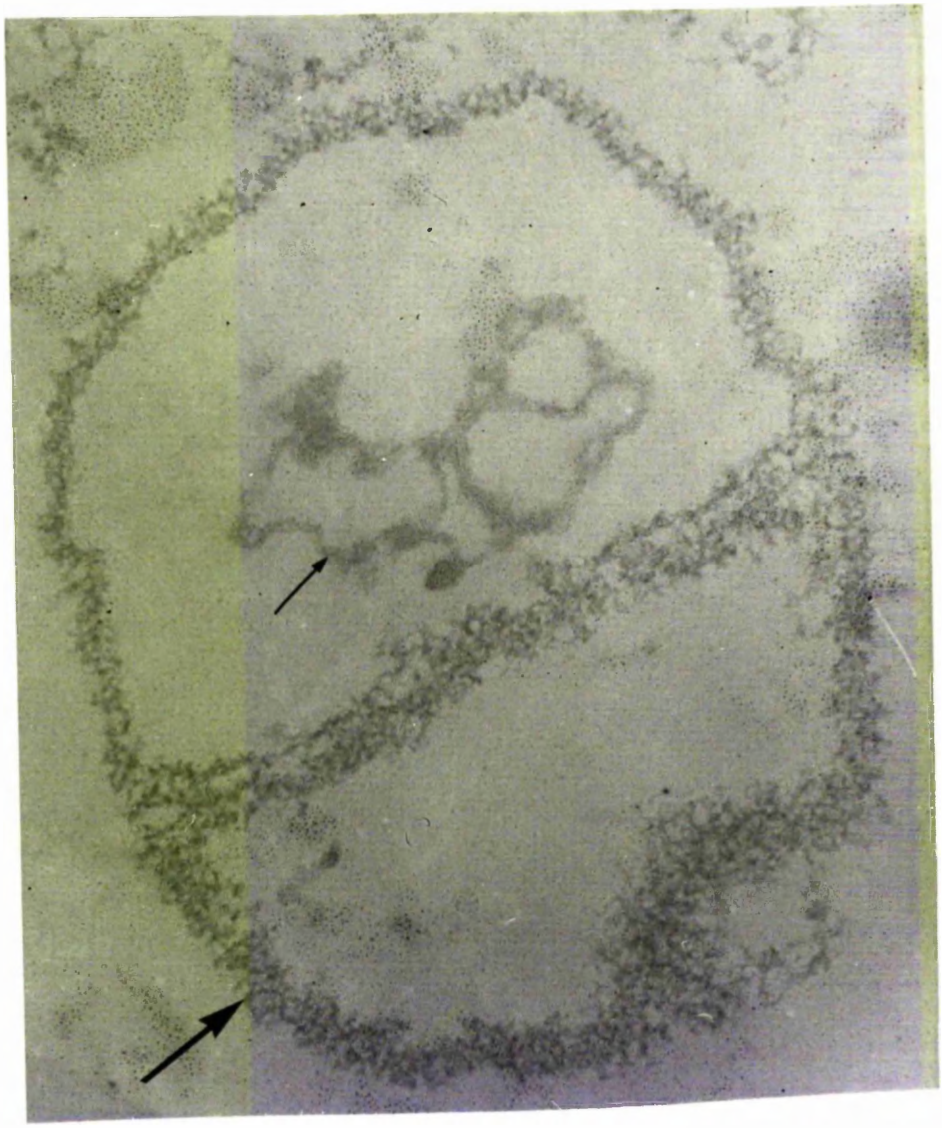
Magnification X 90 900



EXPLANATION OF PLATE 8

Thin section of "standard" membrane preparation isolated from M. lysodeikticus St.A. A membrane system (small arrow) is enclosed by a structure possessing the cell wall profile of a bacterium which has undergone division (large arrow).

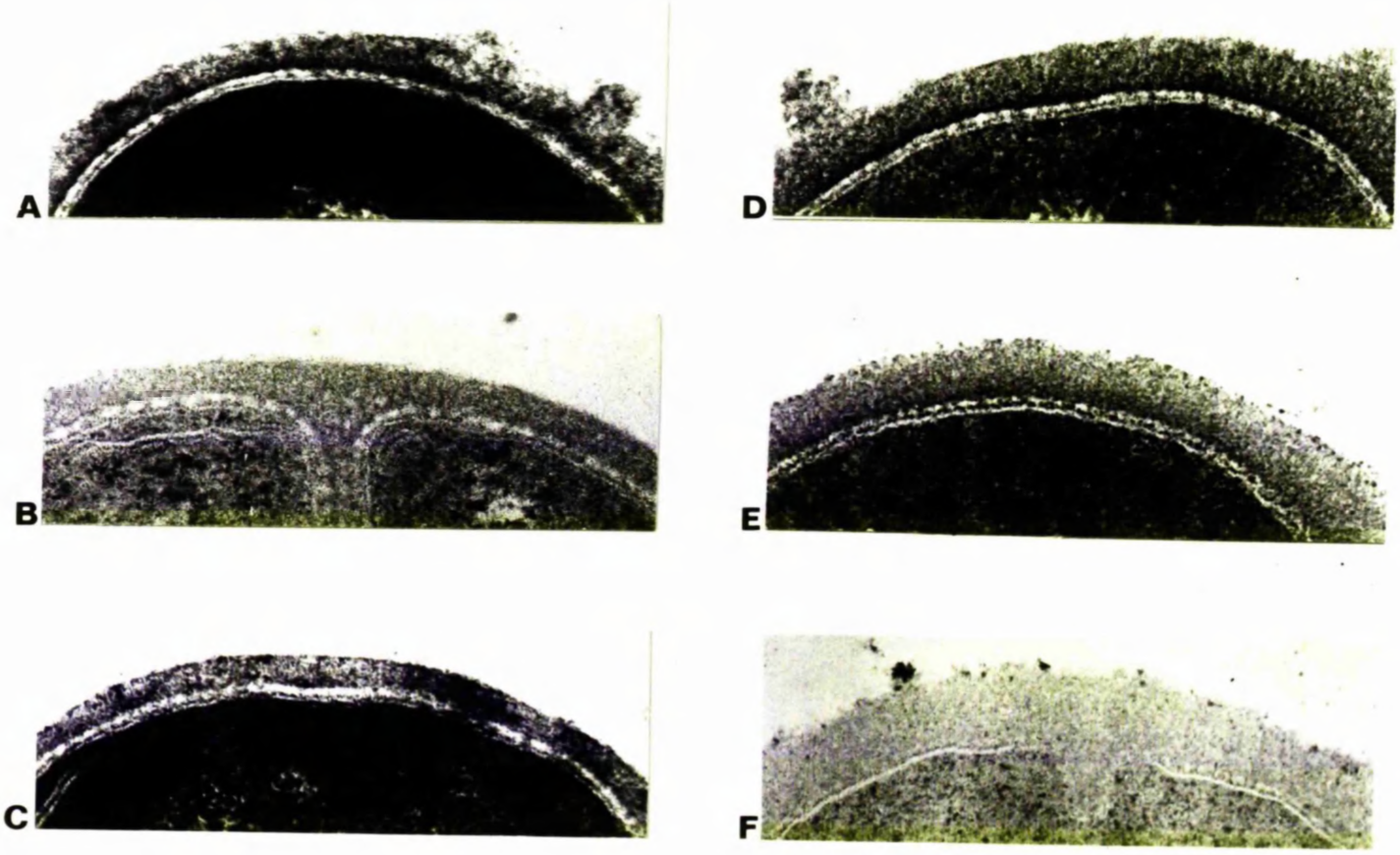
Magnification X 90 900



EXPLANATION OF PLATE 9

Thin sections of cells of M. lysodeikticus St.0 and St.A revealing alteration in wall thickness with growth. Cells of M. lysodeikticus St.0 (A - C) and St.A (D - F) were fixed after 10 h (A & D), 18 h (B & E) and 36 h (C & F) of growth and prepared for electron microscopy as described in Methods. Cells chosen for illustration were representative of the total population in regard to their wall thickness. Cells in the process of, or obviously having recently undergone, cleavage were avoided. Note that whereas the cell wall of St.0 remains of approx. constant thickness (45 nm) the cell wall of St.A increases from approx. 50 nm in late logarithmic phase to approx. 85 nm in stationary phase (36 h) culture.

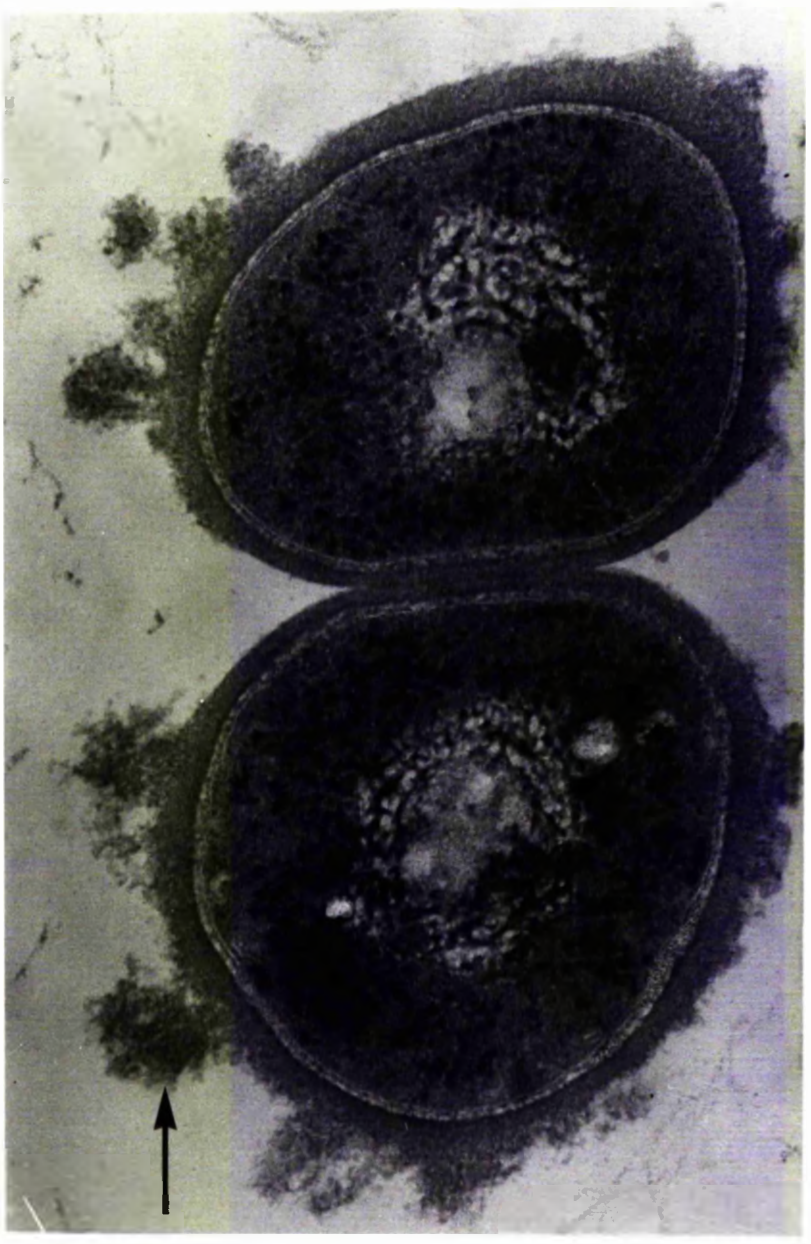
Magnification X 117 000


A
D
B
E
C
F

EXPLANATION OF PLATE 10

Thin section of a dividing cell of M. lysodeikticus St.A after 10 h growth. Extensive sloughing of wall material is apparent (arrow). Note also the absence of a discernable electron transparent zone down the undivided septum.

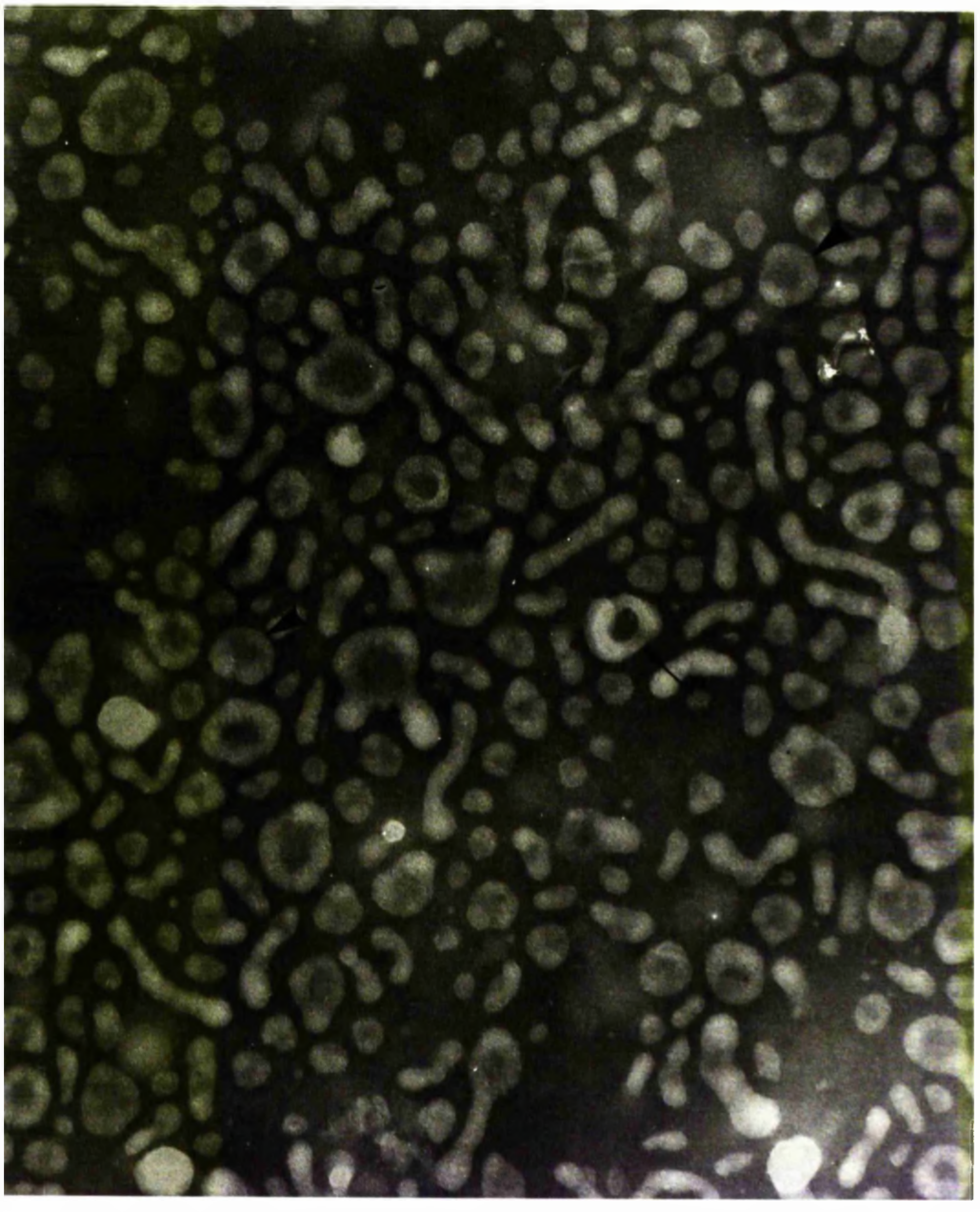
Magnification X 90 900



EXPLANATION OF PLATE 11

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.O. The Mg^{2+} concn. throughout the isolation procedure was 10 mM and membranes were washed 3 times in tris buffer prior to examination. Arrow points to "doughnut" shaped mesosomal vesicle, and arrow heads to structures comparable to indented spherical vesicles.

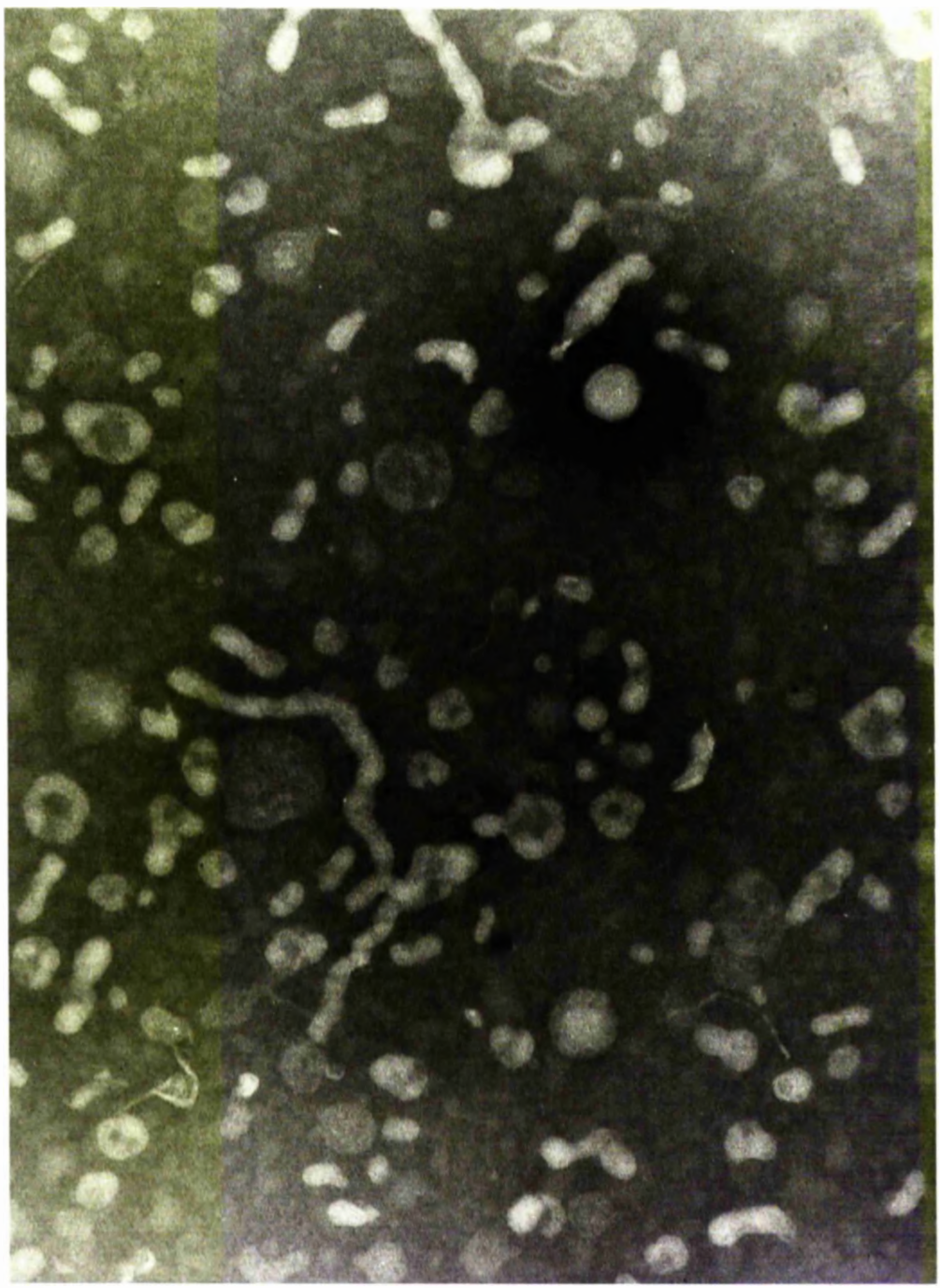
Magnification X 100 800



EXPLANATION OF PLATE 12

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.0. The Mg^{2+} concn. throughout the isolation procedure was 40 mM and membranes were washed 3 times in tris buffer before examination.

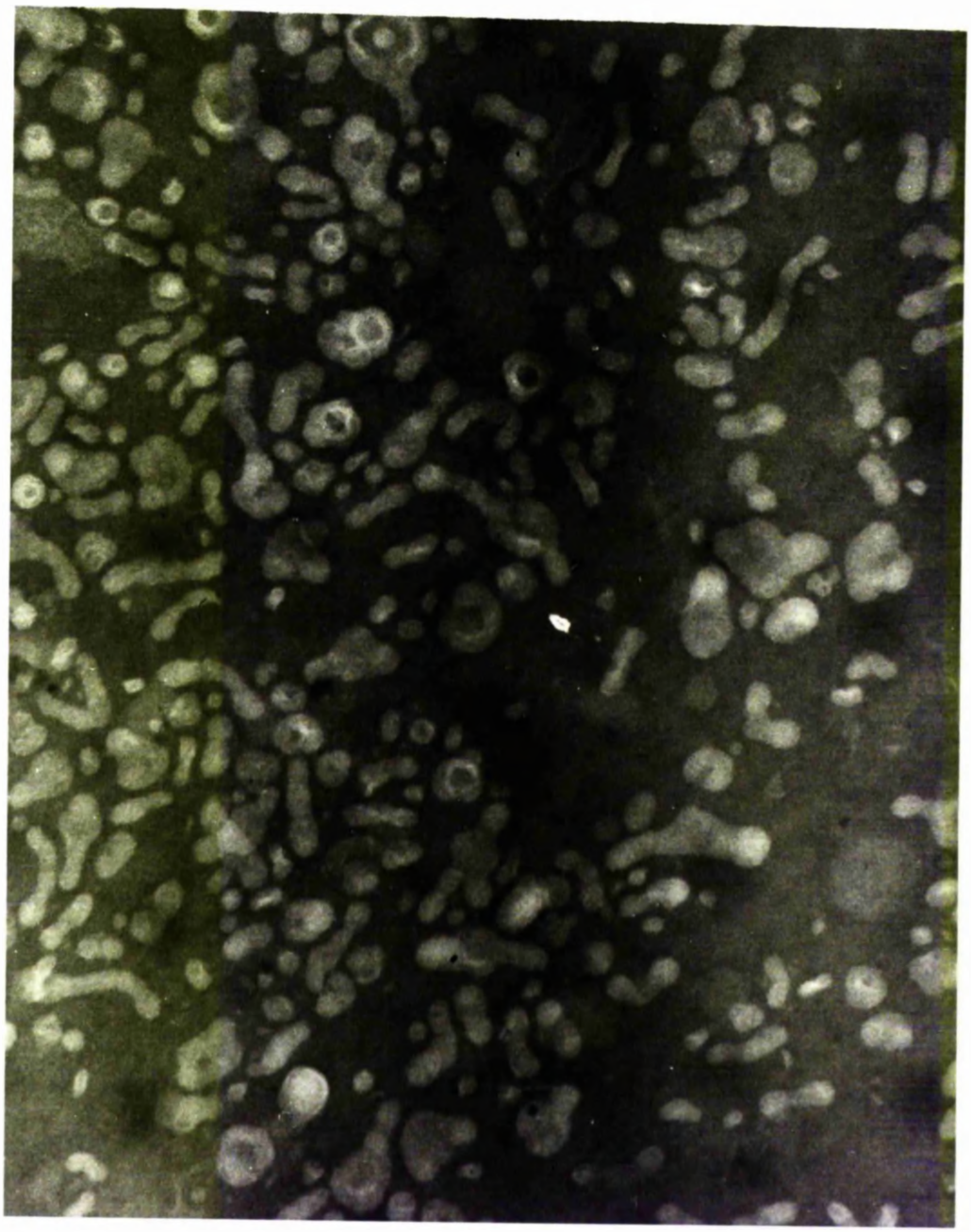
Magnification X 100 800



EXPLANATION OF PLATE 13

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.0. The Mg^{2+} concn. throughout the isolation procedure was 30 mM and membranes were washed 3 times in tris buffer prior to examination.

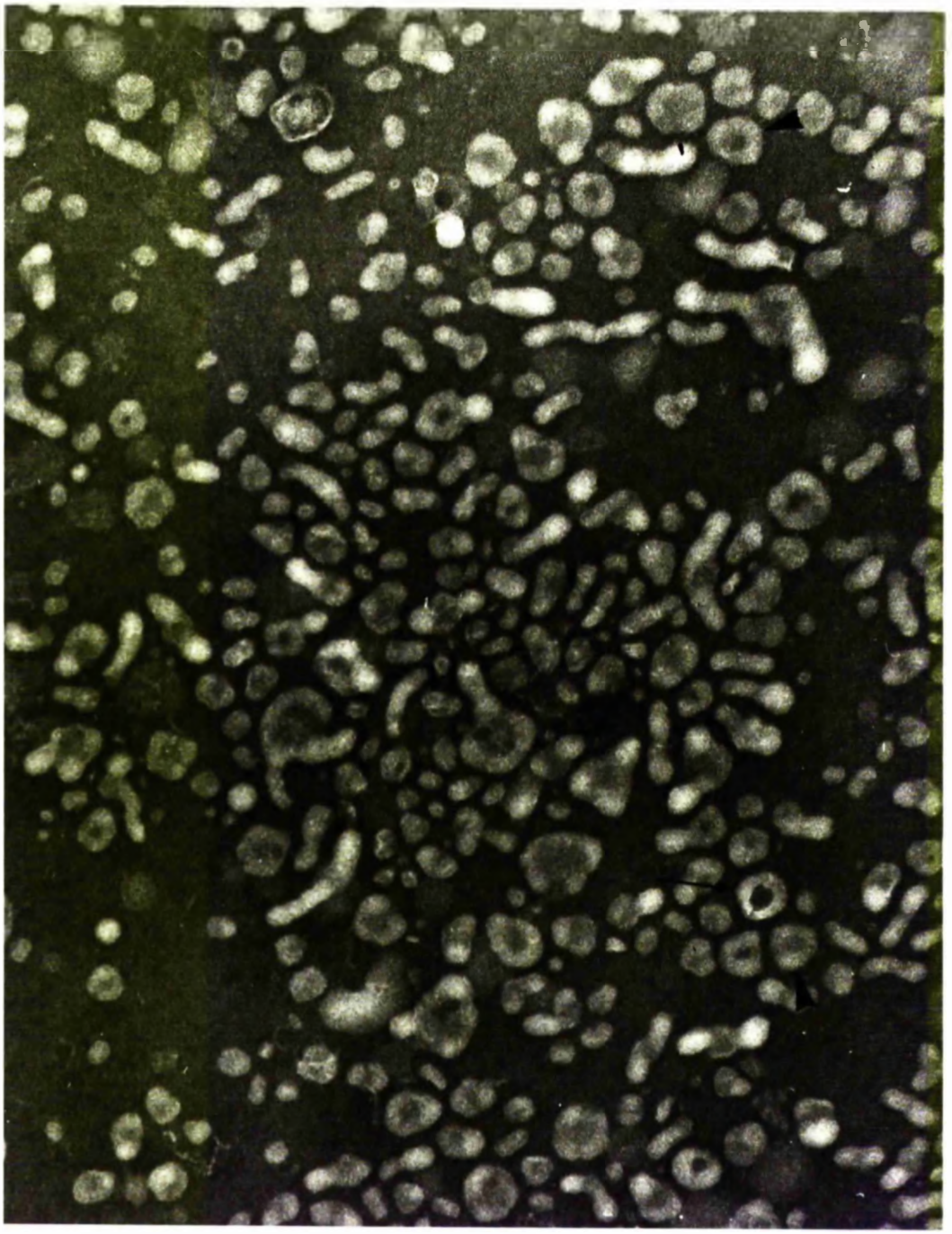
Magnification X 100 800



EXPLANATION OF PLATE 14

Negatively stained mesosomal membrane preparation isolated from M. lysodeikticus St.O. The Mg^{2+} concn. throughout the isolation procedure was 25 mM and membranes were washed 3 times in tris buffer prior to examination. Arrows point to "doughnut" shaped mesosomal vesicles and arrow head to structures comparable to indented spherical vesicles.

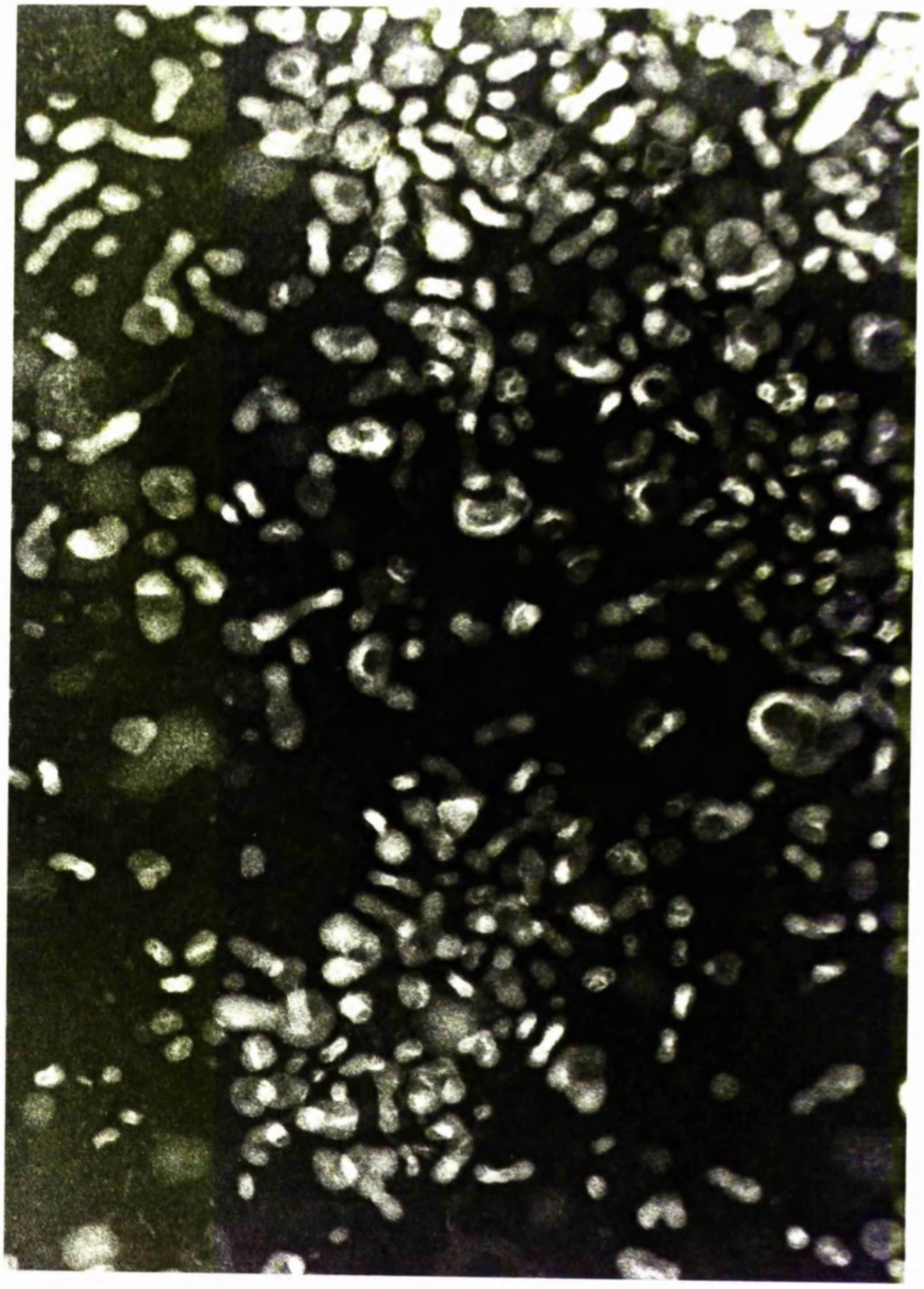
Magnification X 100 800



EXPLANATION OF PLATE 15

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.O. The Mg^{2+} concn. throughout the isolation procedure was 20 mM and membranes were washed 3 times in tris buffer prior to examination.

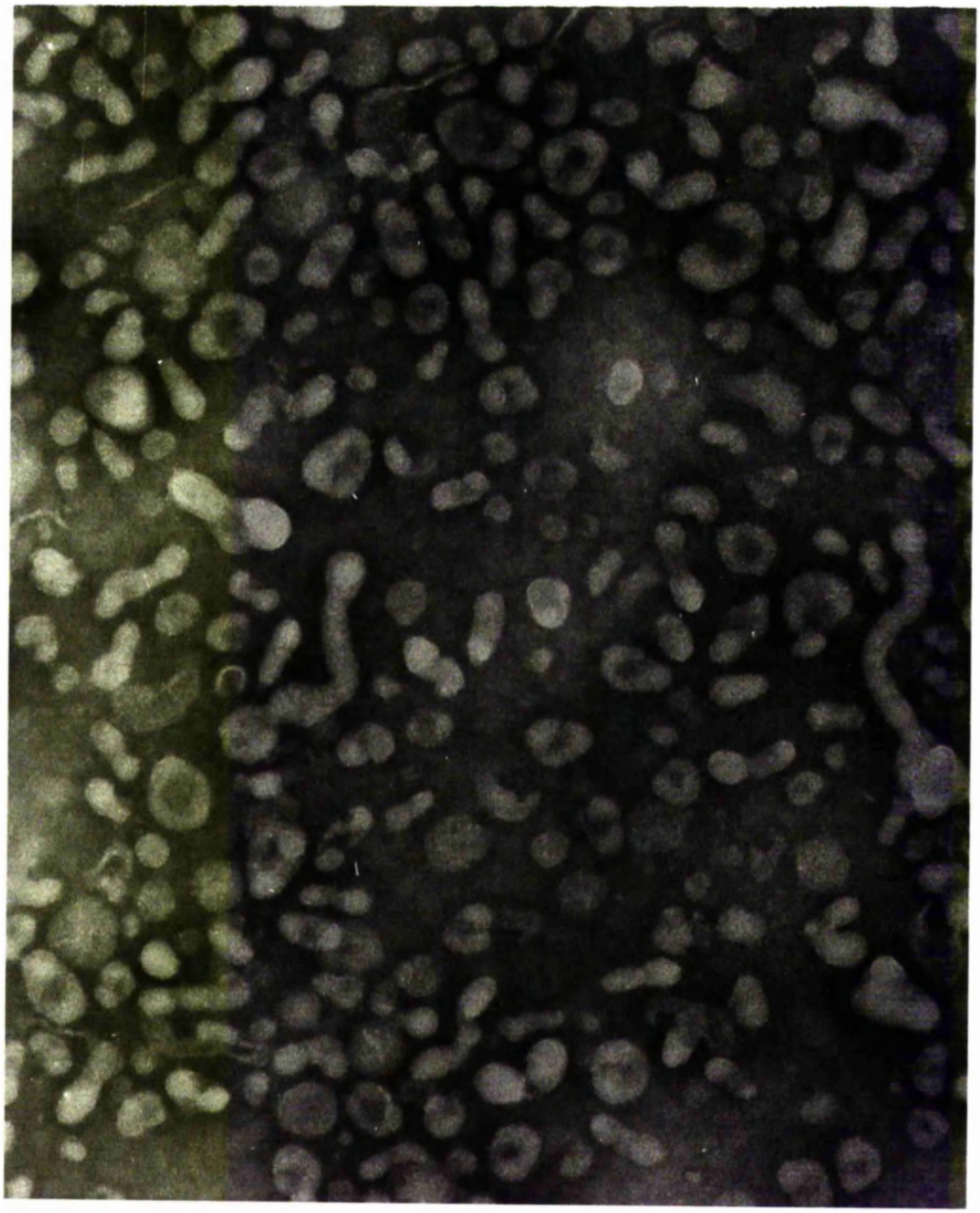
Magnification X 100 800



EXPLANATION OF PLATE 16

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.O. The Mg^{2+} concn. throughout the isolation procedure was 15 mM and membranes were washed 3 times in tris buffer prior to examination.

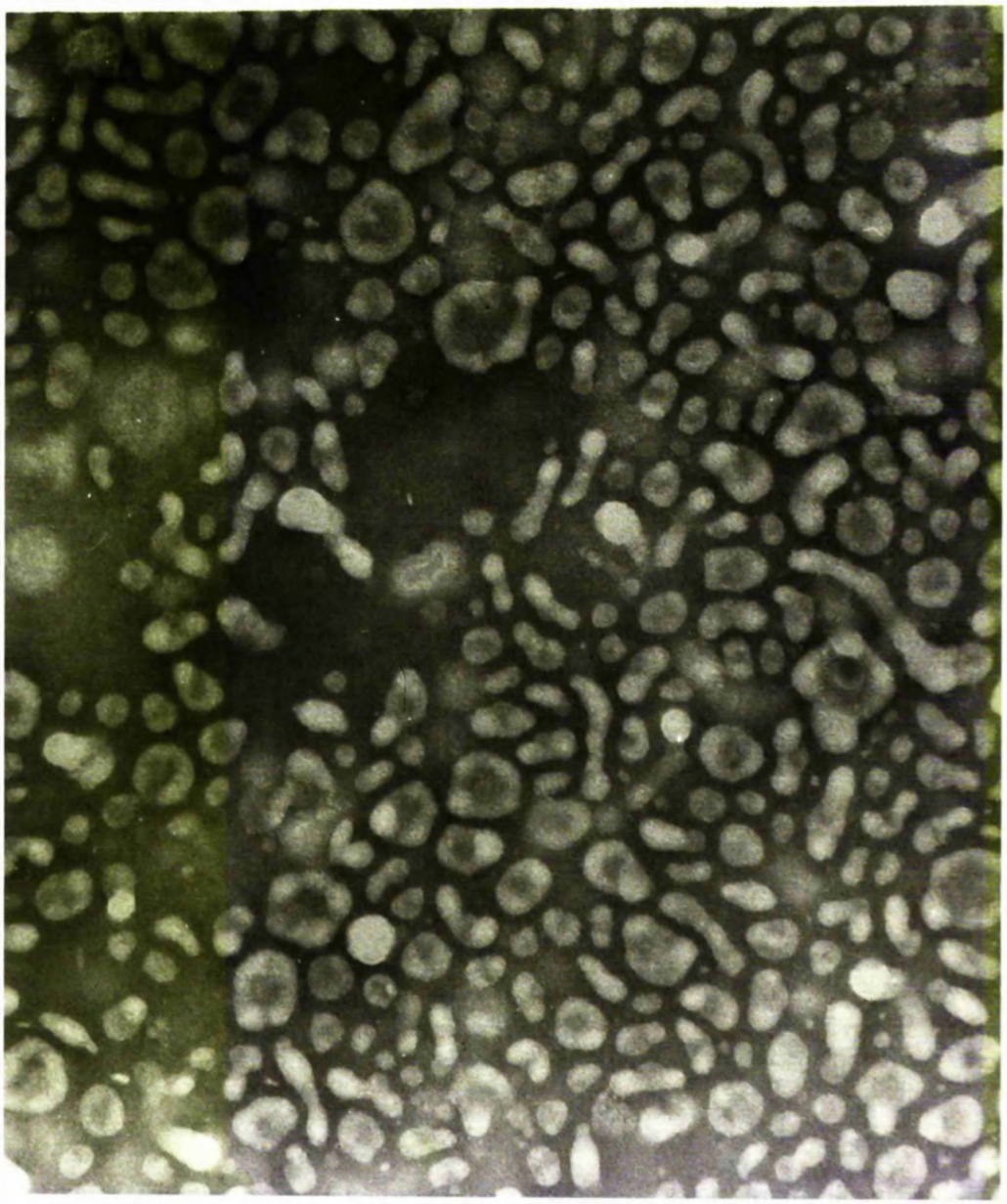
Magnification X 100 800



EXPLANATION OF PLATE 17

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.0. The Mg^{2+} concn. throughout the isolation procedure was 10 mM and membranes were washed 3 times in tris buffer prior to examination.

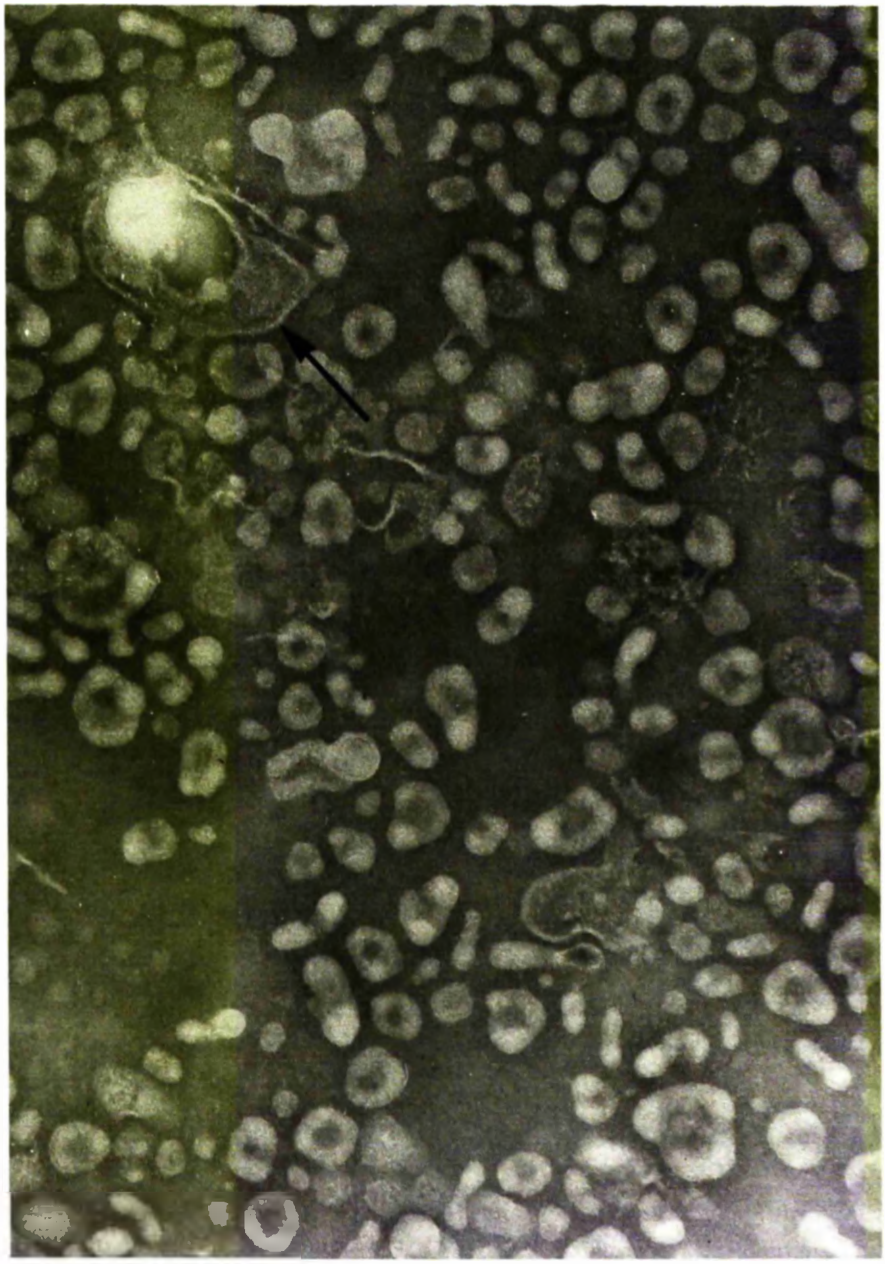
Magnification X 100 800



EXPLANATION OF PLATE 18

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.O. The Mg^{2+} concn. throughout the isolation procedure was 7.5 mM and membranes were washed 3 times in tris buffer prior to examination. Arrow points to contaminating peripheral membrane fragments.

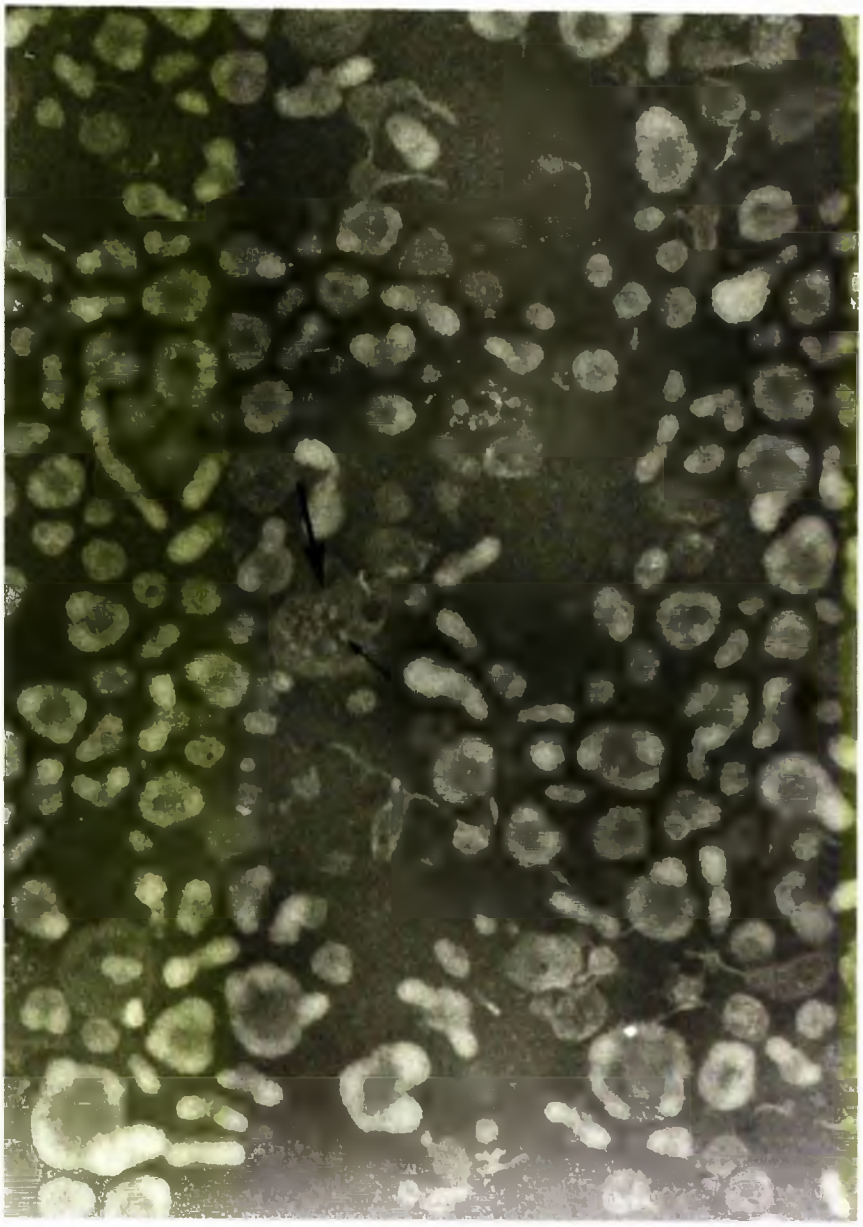
Magnification X 100 800



EXPLANATION OF PLATE 19

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.0. The Mg^{2+} concn. throughout the isolation procedure was 7.5 mM and membranes were washed 3 times in tris buffer prior to examination. Large arrow points to contaminating peripheral membrane fragments and small arrow to enzyme complexes present on the peripheral membrane fragments.

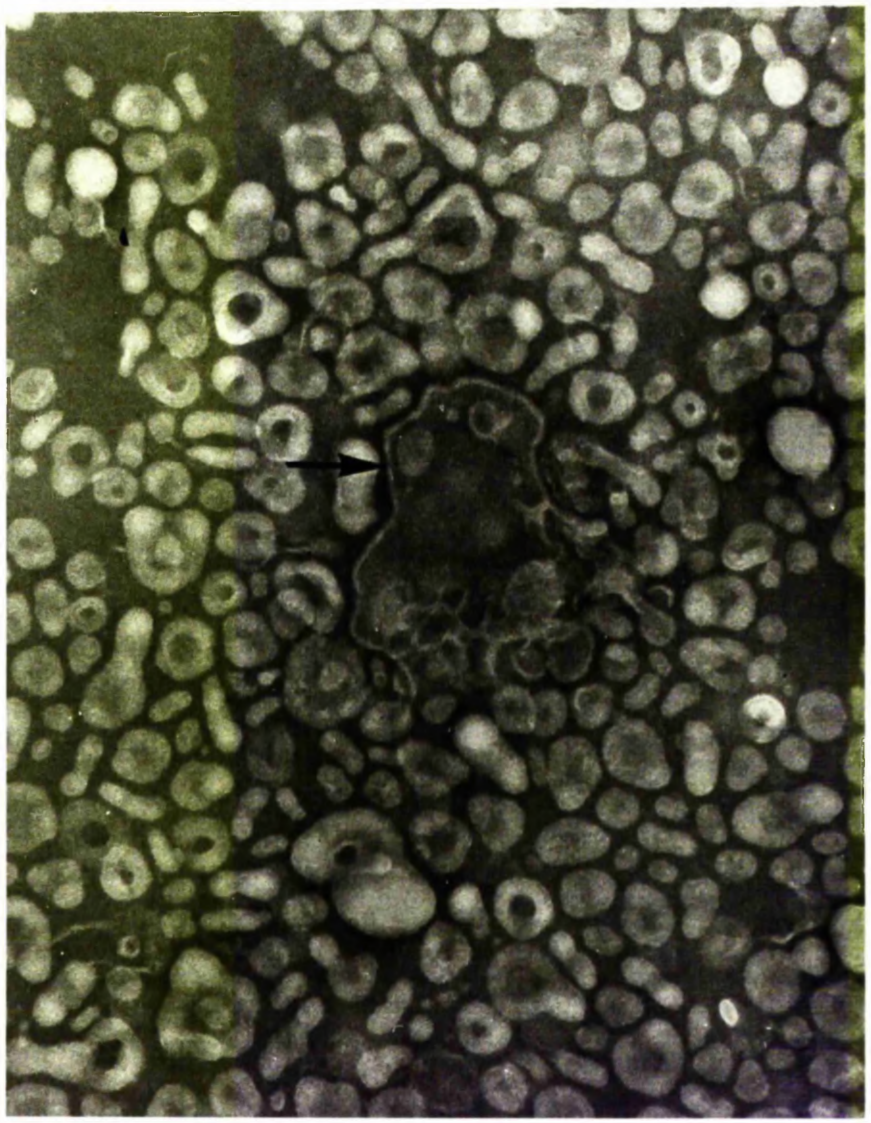
Magnification X 100 800



EXPLANATION OF PLATE 20

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.0. The Mg^{2+} concn. throughout the isolation procedure was 5.0 mM and membranes were washed 3 times in tris buffer prior to examination. Arrow points to contaminating peripheral membrane fragments.

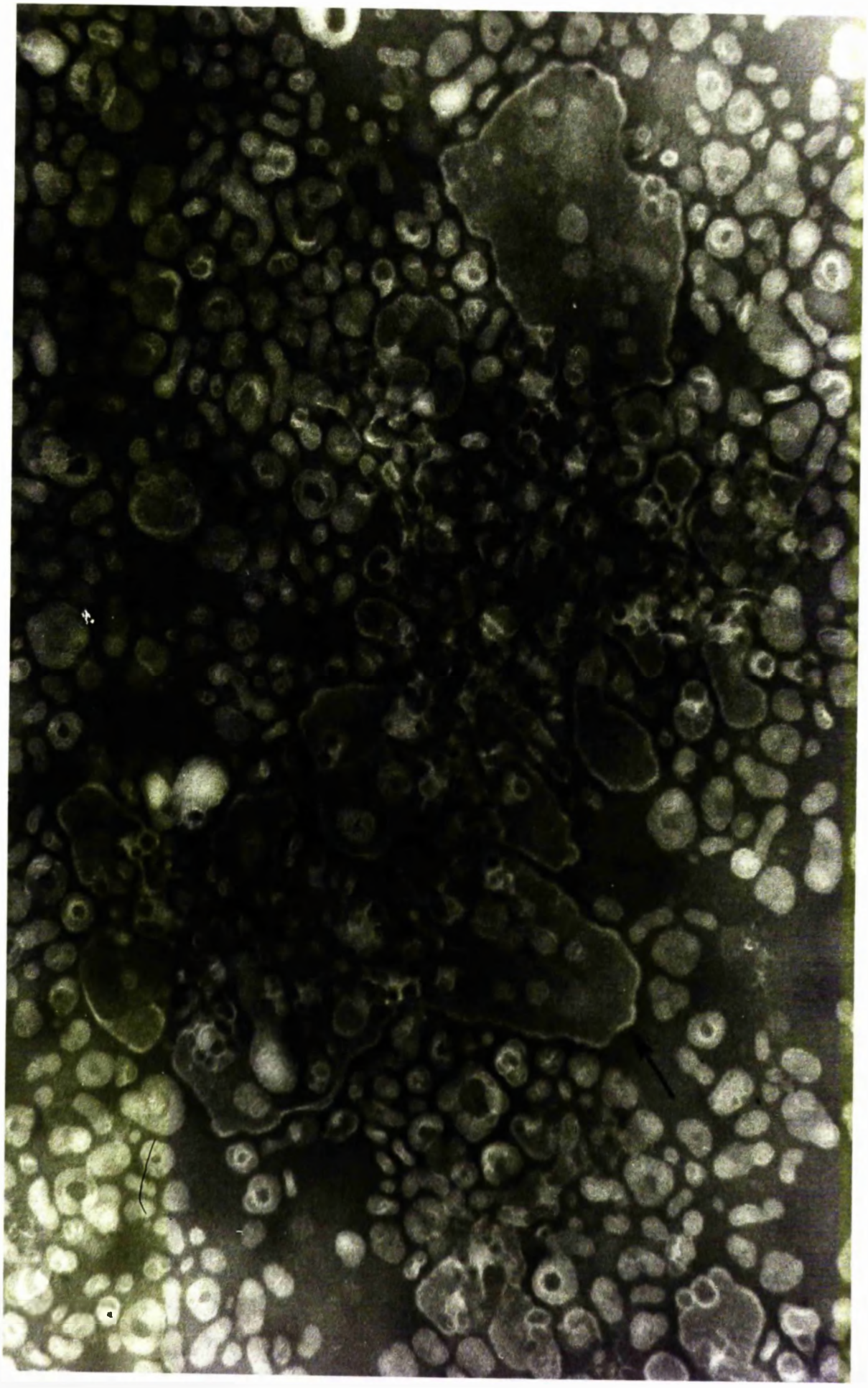
Magnification X 100 800



EXPLANATION OF PLATE 21

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.O. The Mg^{2+} concn. throughout the isolation procedure was 5 mM and membranes were washed 3 times in tris buffer prior to examination. Note the extensive sheets of membrane (arrow) probably representing part of the peripheral membrane of a disrupted protoplast.

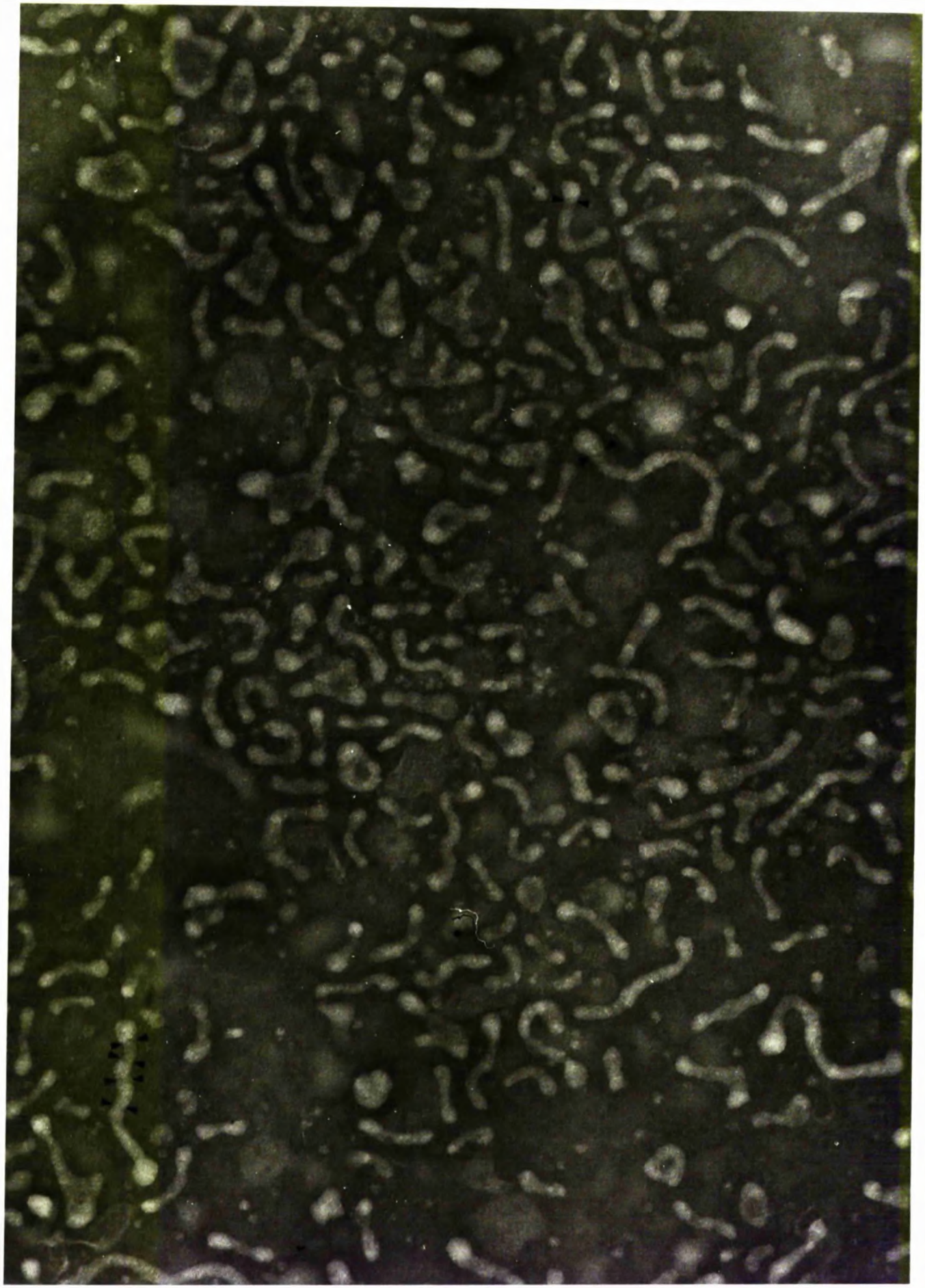
Magnification X 100 800



EXPLANATION OF PLATE 22

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.O. The Mg^{2+} concn. throughout the isolation procedure was 10 mM and sedimented unwashed membranes were resuspended in tris buffer prior to examination. Arrows point to constrictions along length of vesicle.

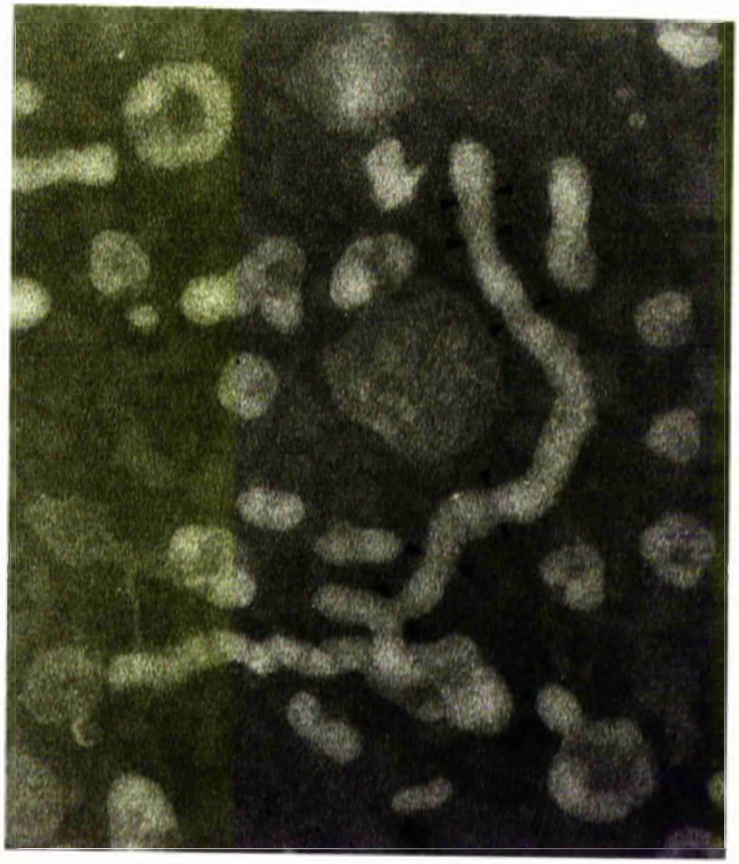
Magnification X 100 800



EXPLANATION OF PLATE 23

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.O. The Mg^{2+} concn. throughout the isolation procedure was 40 mM and membranes were washed 3 times in tris buffer prior to examination. Arrows point to constrictions along length of mesosomal tubule.

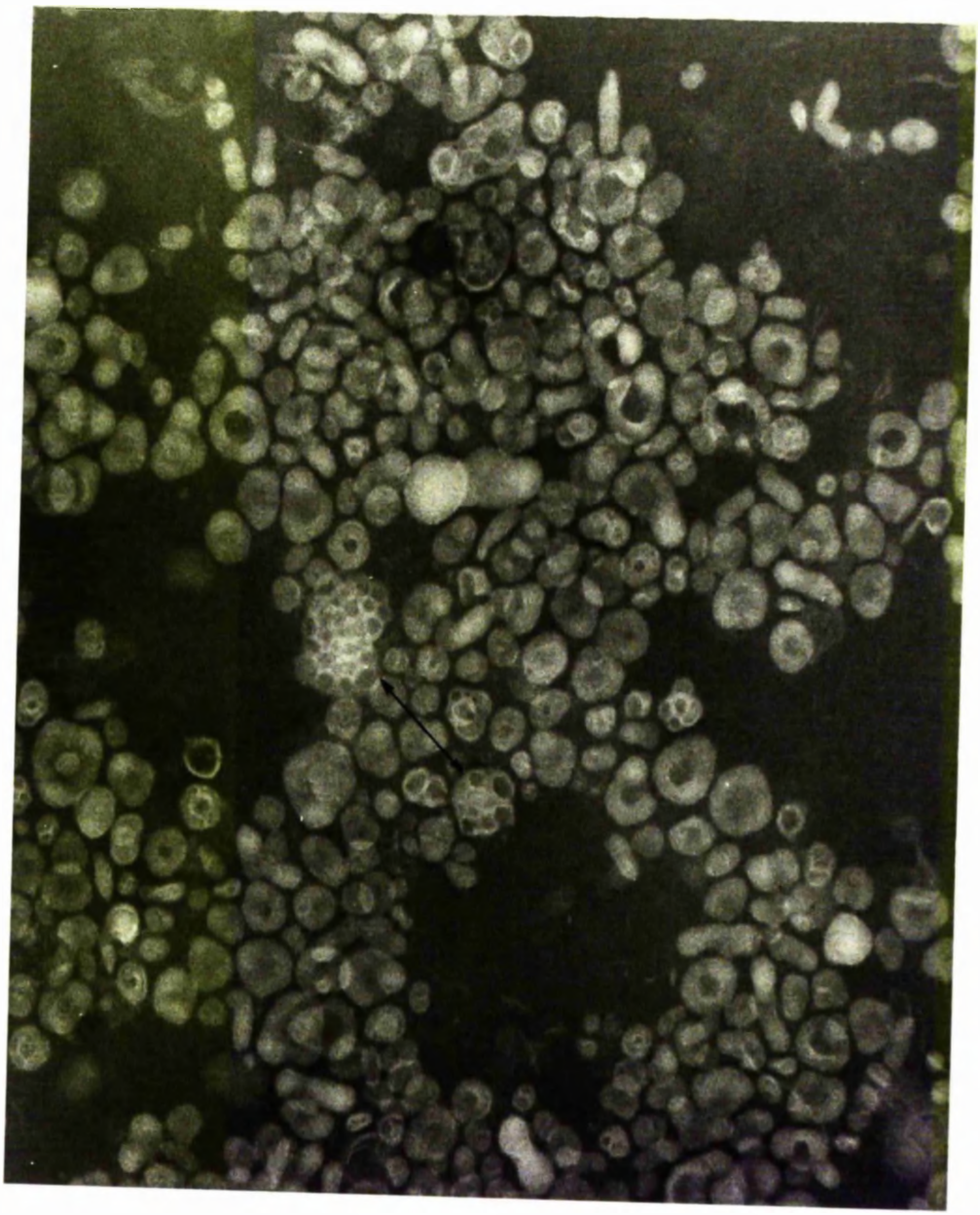
Magnification X 151 200



EXPLANATION OF PLATE 24

Negatively stained preparation of mesosomal membrane isolated from M. lysodeikticus St.O. The Mg^{2+} concn. throughout the isolation procedure was 5 mM and membranes were washed 3 times prior to examination. Arrows point to "honeycombed" structures.

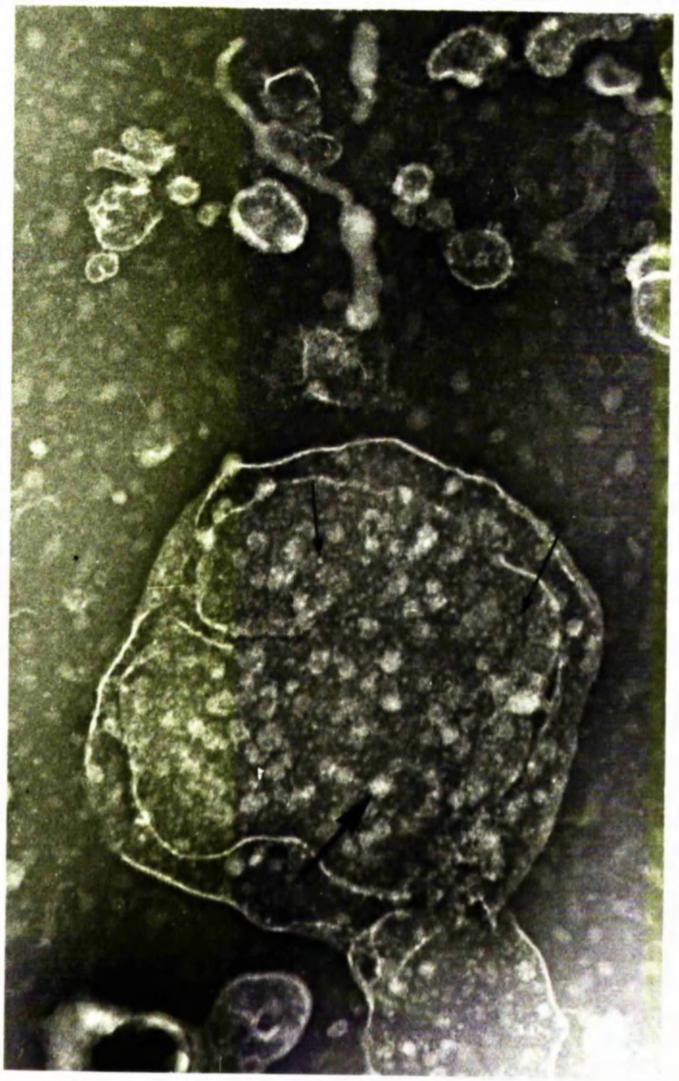
Magnification X 100 800



EXPLANATION OF PLATE 25

Negatively stained peripheral membrane isolated from M. lysodeikticus St.O. Peripheral membrane fragments are covered with particles approx. 10.5mmin diameter (small arrow) and also larger structures approx. 35 nm in diameter (large arrow). A contaminating mesosomal tubule can be seen in the top centre of the micrograph.

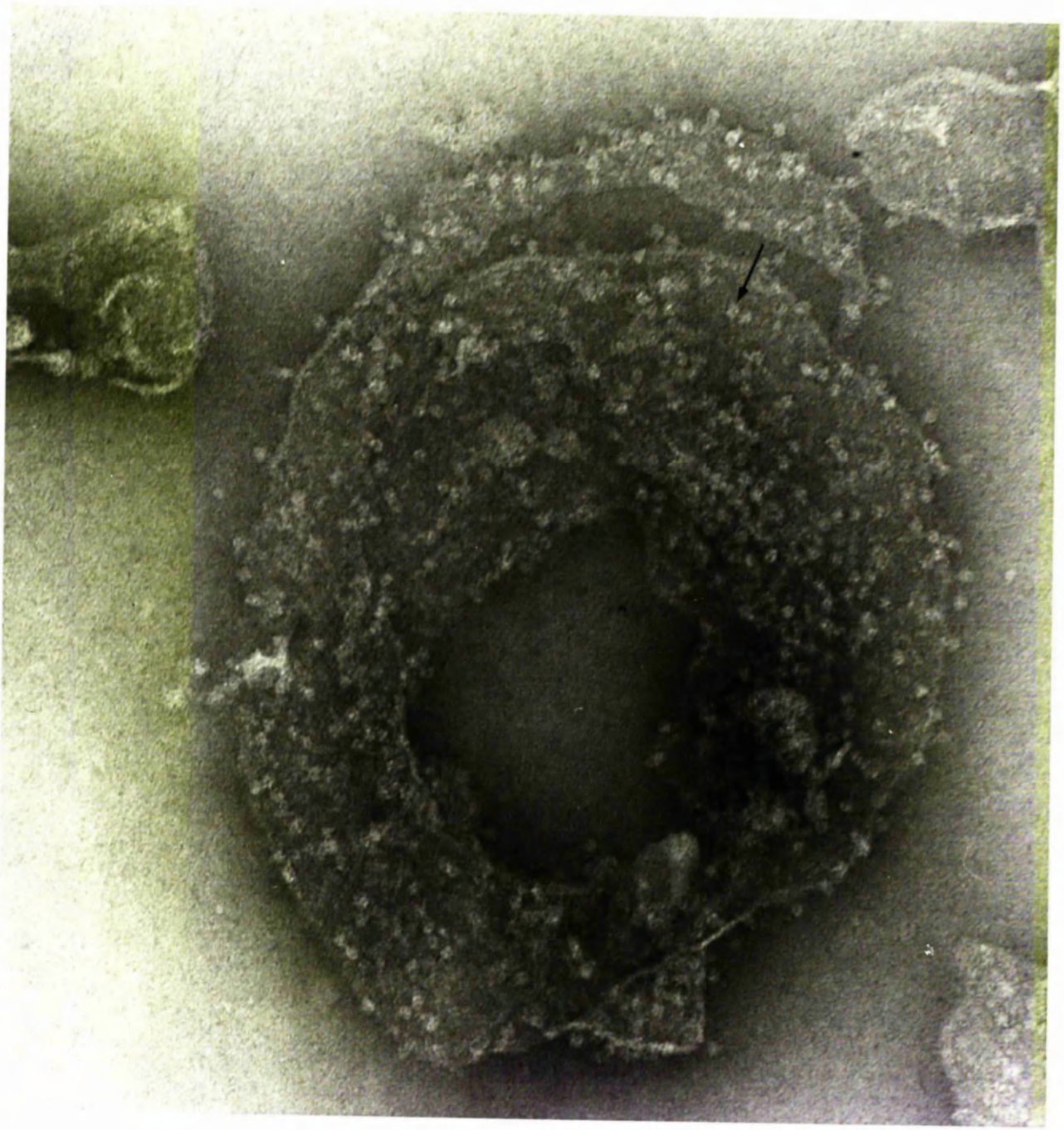
Magnification X 72 600



EXPLANATION OF PLATE 26

Negatively stained preparation of "standard" membranes isolated from M. lysodeikticus St.O. Membrane fragment shows the presence of surface particles approx. 10 nm in diameter (arrow). Note the absence of larger surface structures.

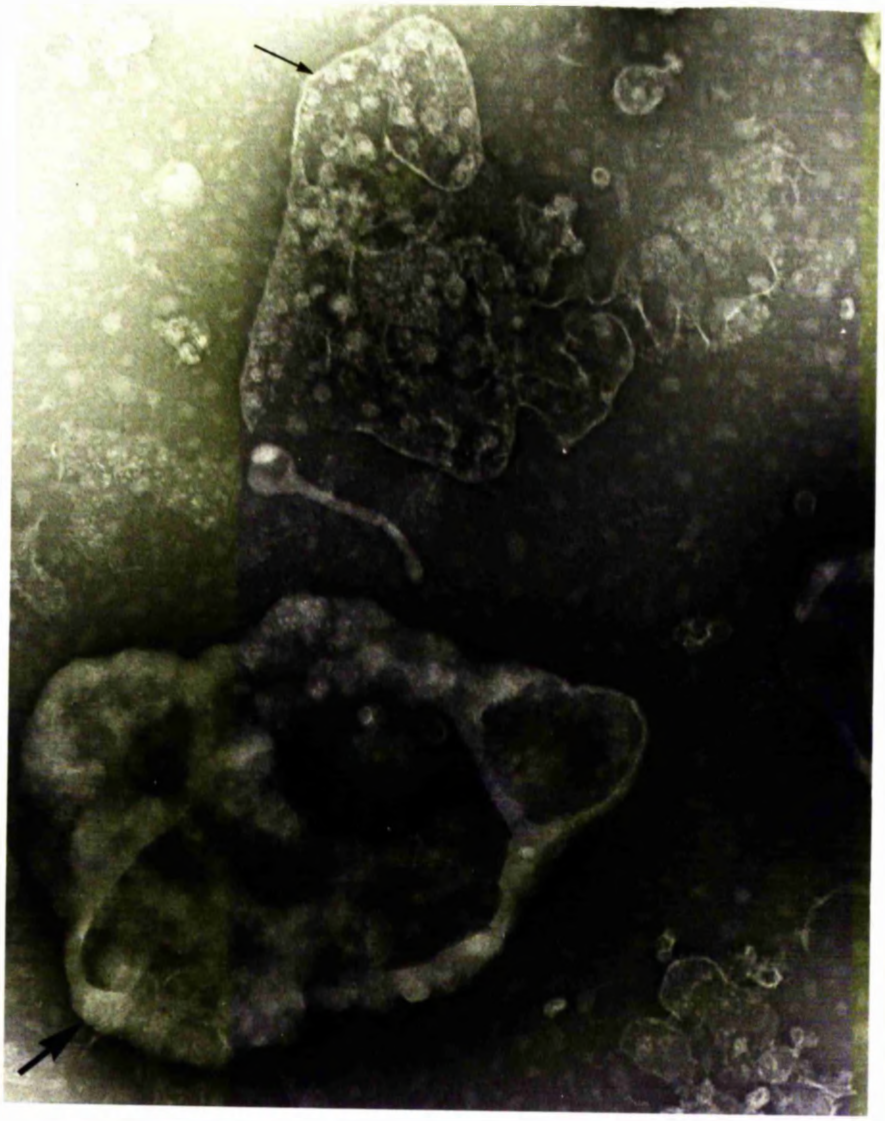
Magnification X 258 800



EXPLANATION OF PLATE 27

Negatively stained peripheral membrane preparation from M. lysodeikticus St.O. Note the large smooth-surfaced structure (large arrow) which appears less penetrable to the stain than the particle studded fragments (small arrow). A contaminating mesosomal tubule can be observed in the centre of the micrograph.

Magnification X 72 600



EXPLANATION OF PLATE 28

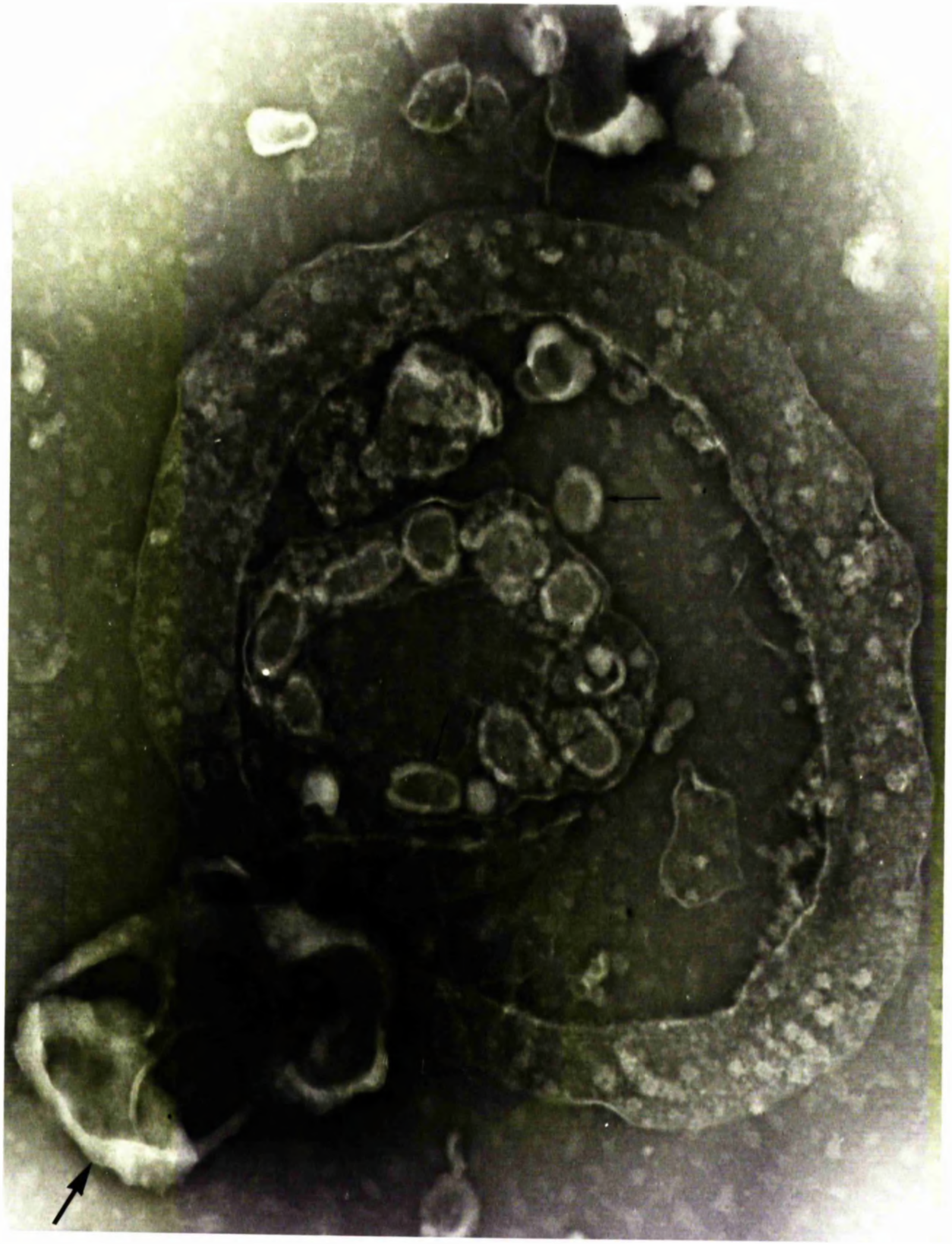
Negatively stained preparation of peripheral membrane isolated from M. lysodeikticus St.O. Although slightly out of focus this micrograph conveys the bag-shaped morphology frequently observed for the large smooth-surfacedstructures in peripheral membrane preparations.

Magnification X 153 900

EXPLANATION OF PLATE 29

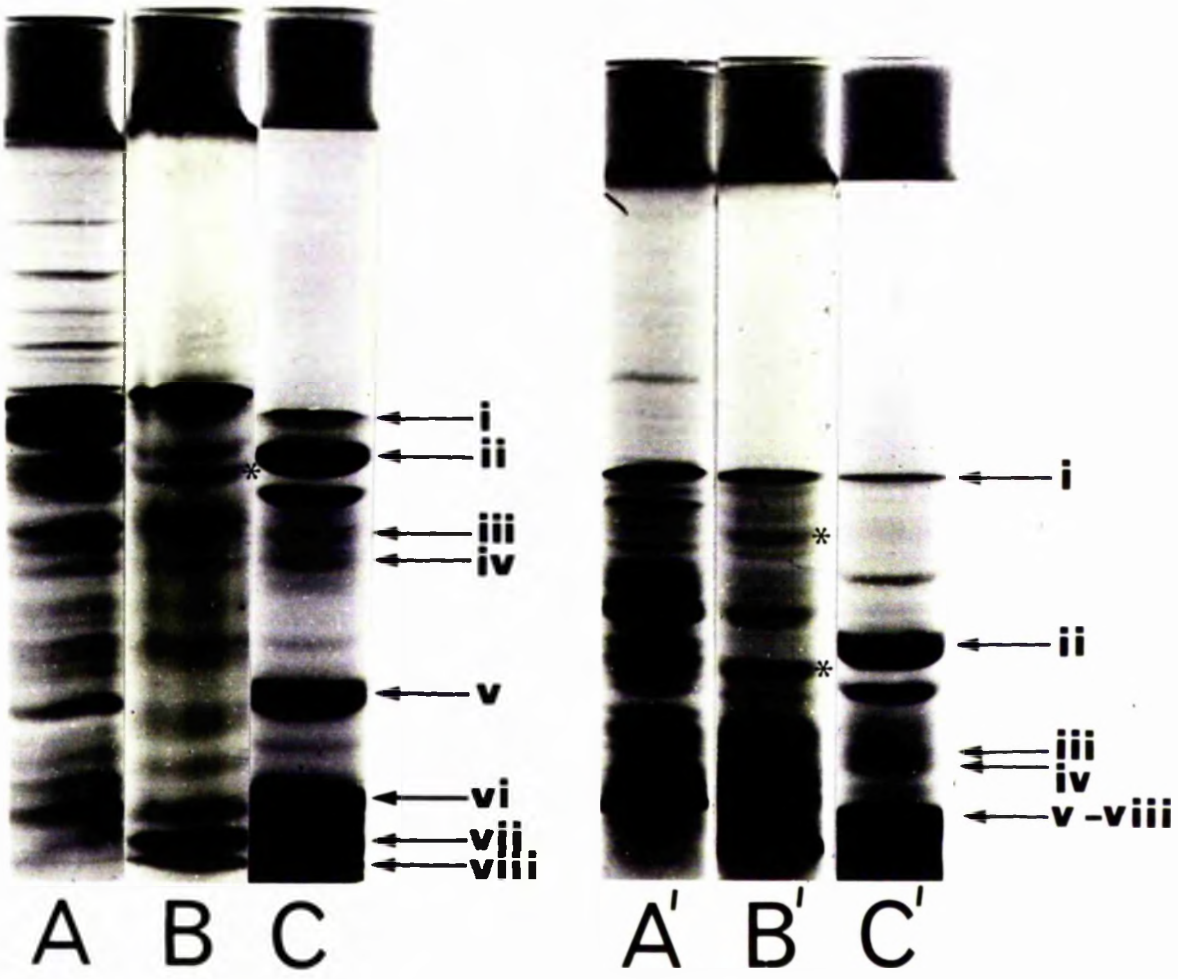
Negatively stained peripheral membrane preparation from M. lysodeikticus St.O. Membranous vesicles, often displaying a morphology similar to a biconcave disc (small arrows) appear entrapped within sheets of peripheral membrane. These vesicles may result from disruption of larger smooth-surfaced structures (large arrow).

Magnification X 90 900



EXPLANATION OF PLATE 30

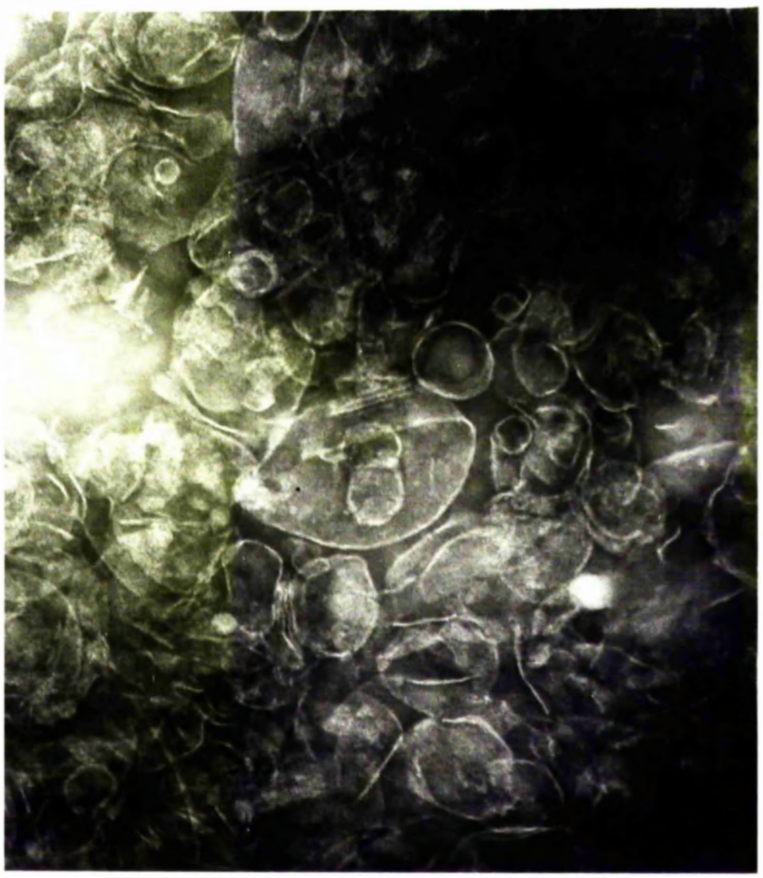
Mesosomal and peripheral membrane proteins from M. lysoedikticus St.O. subjected to SDS-polyacrylamide gel electrophoresis. Membrane preparations were obtained from plasmolysed cells, the Mg^{2+} concentration being held at 10 mM throughout the isolation procedure. Separating gels A, B and C contain 11.7% (w/v) acrylamide and 0.153% NN' methylene bisacrylamide whereas gels A', B' and C' contain 7% (w/v) acrylamide and 0.184% NN' methylene bisacrylamide. A and A', peripheral membrane (140 µg dry wt. membrane); B and B', mesosomal membrane (250 µg dry wt. membrane); C and C', marker proteins - (i) artifact band; (ii), bovine serum albumin (mol. wt. 67 000); (iii), ovalbumin (mol. wt. 45 000); (iv) hexokinase (mol. wt. 45 000) (v), chymotrypsinogen (mol. wt. 25 000); (vi), myoglobin (mol. wt. 17 800); (vii) cytochrome-c (mol. wt. 12 400); (viii), bromophenol blue. Mechanically strengthened stacking gels (McNiven et al., 1972) are opaque and contain a negligible percentage of the total staining material. Note that peripheral membrane displays a larger spectrum of polypeptides than does mesosomal membrane fractions. Whilst there are many polypeptides exclusive to the peripheral membrane, or predominantly localised in them, there appears to be only two discernable polypeptides (*) predominantly localized in the mesosomal membrane.


i
ii
iii
iv
v
vi
vii
viii
A
B
C
i
ii
iii
iv
v -viii
A′
B′
C′

EXPLANATION OF PLATE 31

Negatively stained preparation of the deoxycholate-insoluble membrane residue (DO 1) isolated from "standard" membranes of M. lysodeikticus St.O. These smooth-surfaced membranous sheets display few, if any, ATP-ase particles (Munoz, Freer, Ellar and Salton, 1968).

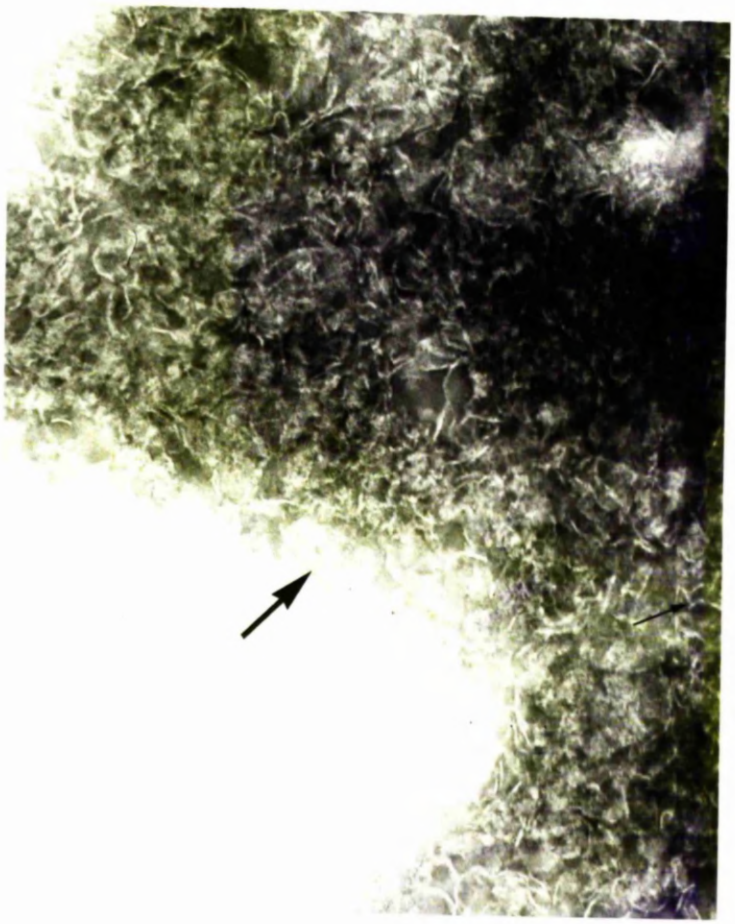
Magnification X 75 600



EXPLANATION OF PLATE 32

Negatively stained membrane residue DO 2 obtained from deoxycholate extraction of "standard" membranes isolated from M. lysodeikticus. Note the presence of both sheets of membrane (large arrow) and smaller lengths of membrane (small arrow).

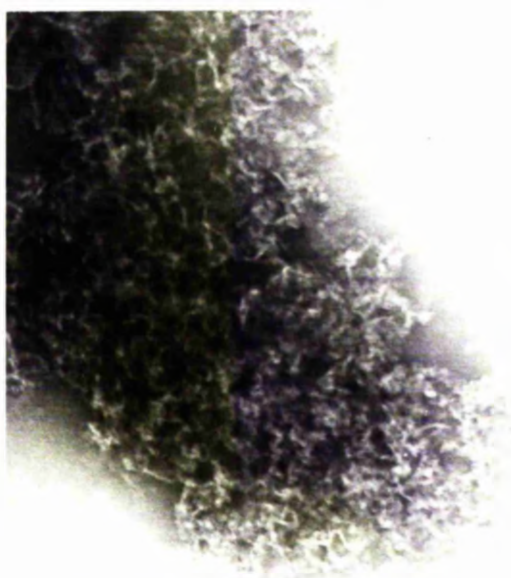
Magnification X 75 600



EXPLANATION OF PLATE 33

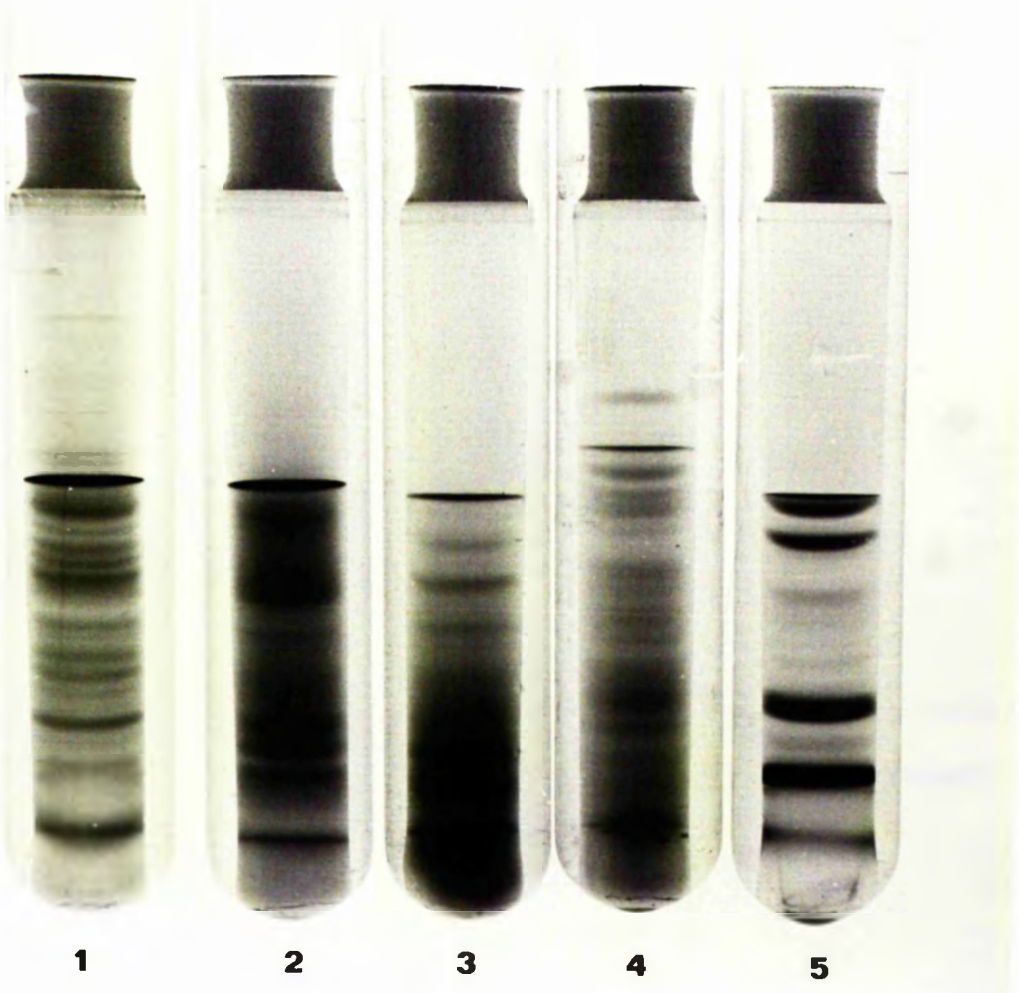
Negatively stained membranous residue DO 3 obtained from deoxycholate extraction of standard membranes from M. lysodeikticus St.O. Note that this fraction consists almost entirely of loosely aggregated short lengths of membranous material.

Magnification X 75 600



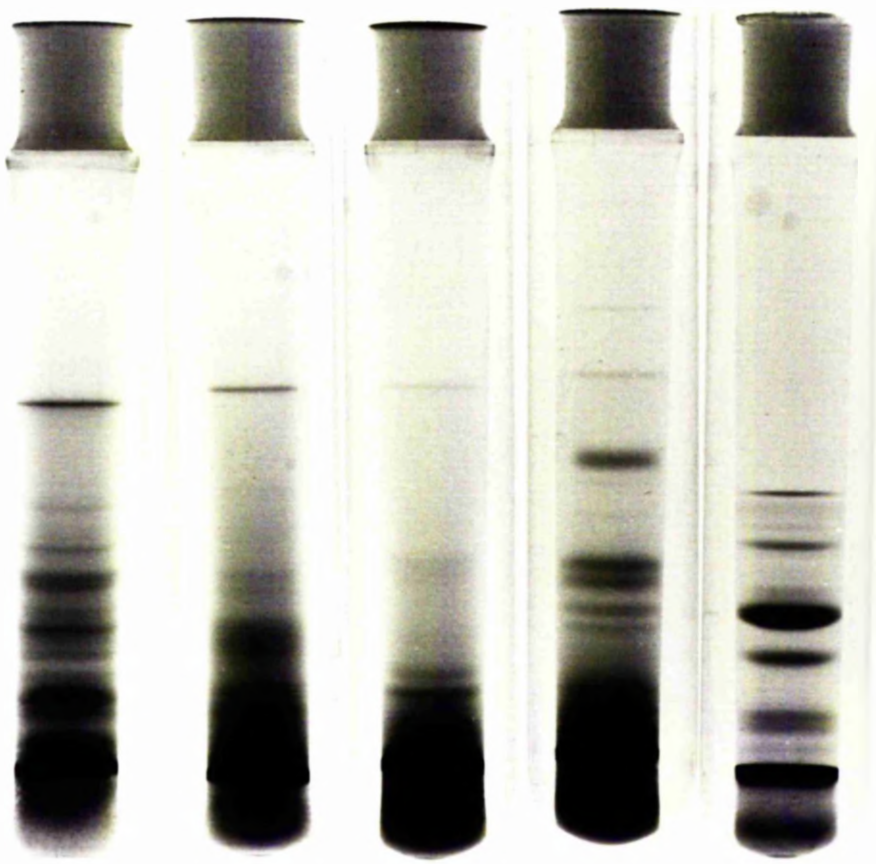
EXPLANATION OF PLATE 34

Membrane proteins from "standard" membranes and deoxycholate-insoluble residues of total membranes subjected to SDS-polyacrylamide disc gel electrophoresis (11.7% w/v acrylamide gels). Lyophilised samples of "standard" membranes (150 µg dry wt.) - Tube 1; DO 1 (200 µg dry wt.) - Tube 2; DO 2 (200 µg dry wt.) - Tube 3; and DO 3 (100 µg dry wt.) - Tube 4 were subjected to SDS-acrylamide gel electrophoresis. Known "marker" proteins (Tube 5) were collectively electrophoresed under identical conditions. (see Plate 30 for identification).


1
2
3
4
5

EXPLANATION OF PLATE 35

7.0% (w/v) polyacrylamide-SDS gels of total membranes and deoxycholate-insoluble residues of total membranes. "Standard" membranes and deoxycholate-insoluble residues DO 1, DO 2 and DO 3 were prepared from M. lysodeikticus St.O as described in Methods. Lyophilised samples of "standard" membranes (300 µg dry wt.) - Tube 1, DO 1 (300 µg dry wt.) - Tube 2; DO 2 (200 µg dry wt.) - Tube 3; and DO 3 (400 µg dry wt.) - Tube 4, were subjected to SDS-polyacrylamide disc gel electrophoresis in separating gels containing 7% (w/v) acrylamide. Known marker proteins (15 µg dry wt. each) were collectively run under identical conditions. (see Plate 30 for identification).


1
2
3
4
5

EXPLANATION OF PLATE 36

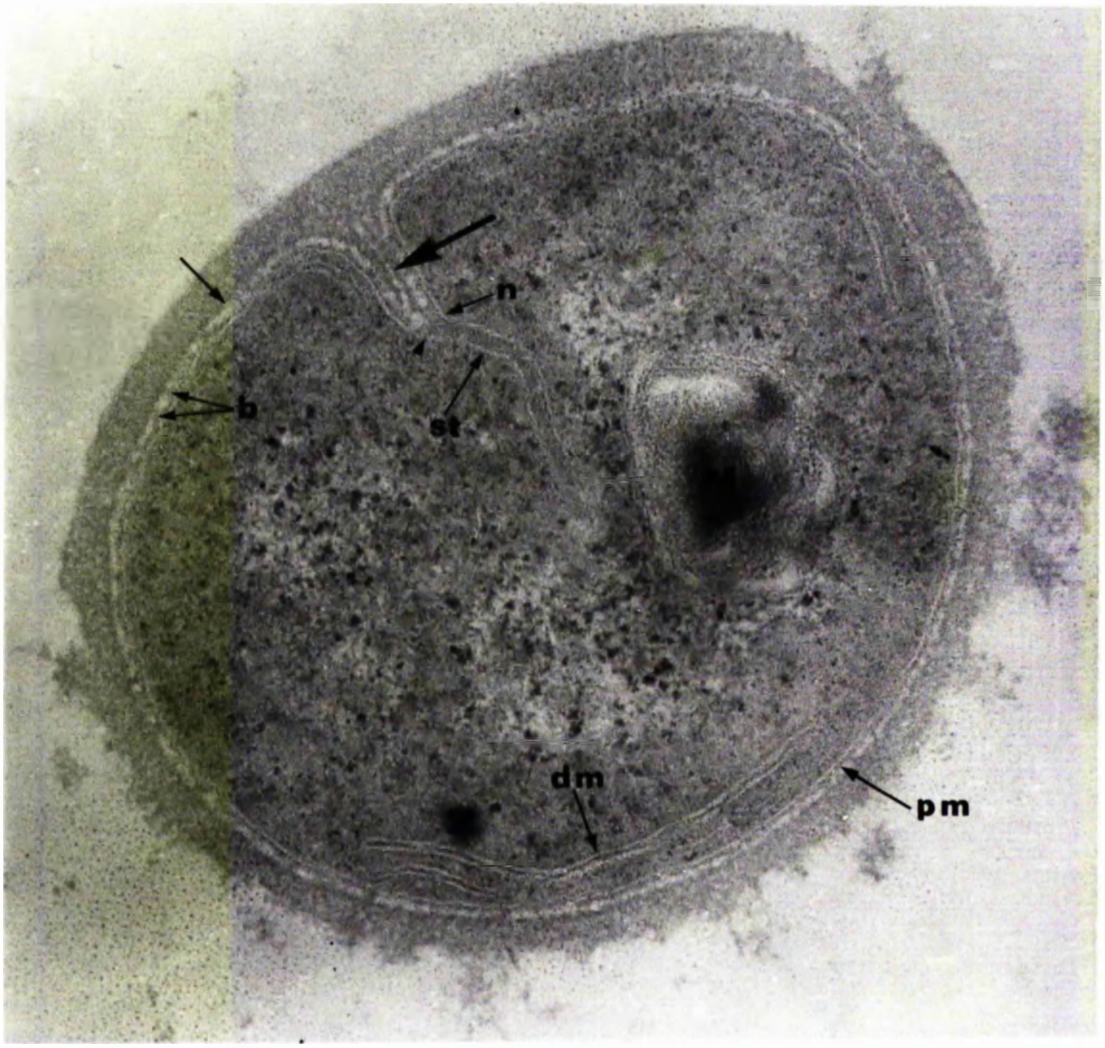
Negatively stained membrane residue obtained after extraction of a mesosomal membrane preparation from M. lysodeikticus St.0 with deoxycholate. The short length of membrane residue (arrow) has a similar morphology and dimensions to the insoluble residue DO 3 obtained from "standard" membranes of the same organism. (compare with Plate 33).

Magnification X 72 600

EXPLANATION OF PLATE 37

Thin section of a cell of M. lysodeikticus St.0 at the early stages of septum development. The developing septum is linked via a stalk (st) to a mesosome (M). Note the central electron-transparent zone bisecting the septum (large arrow) and a similar zone between the peripheral membrane and wall (small arrow). This latter region is often crossed with bridges (b). Both zones terminate at the neck (n) of the septum. Note also the lengths of double membrane (dm) and the poor resolution of the cytoplasmic leaflet of the peripheral membrane (pm).

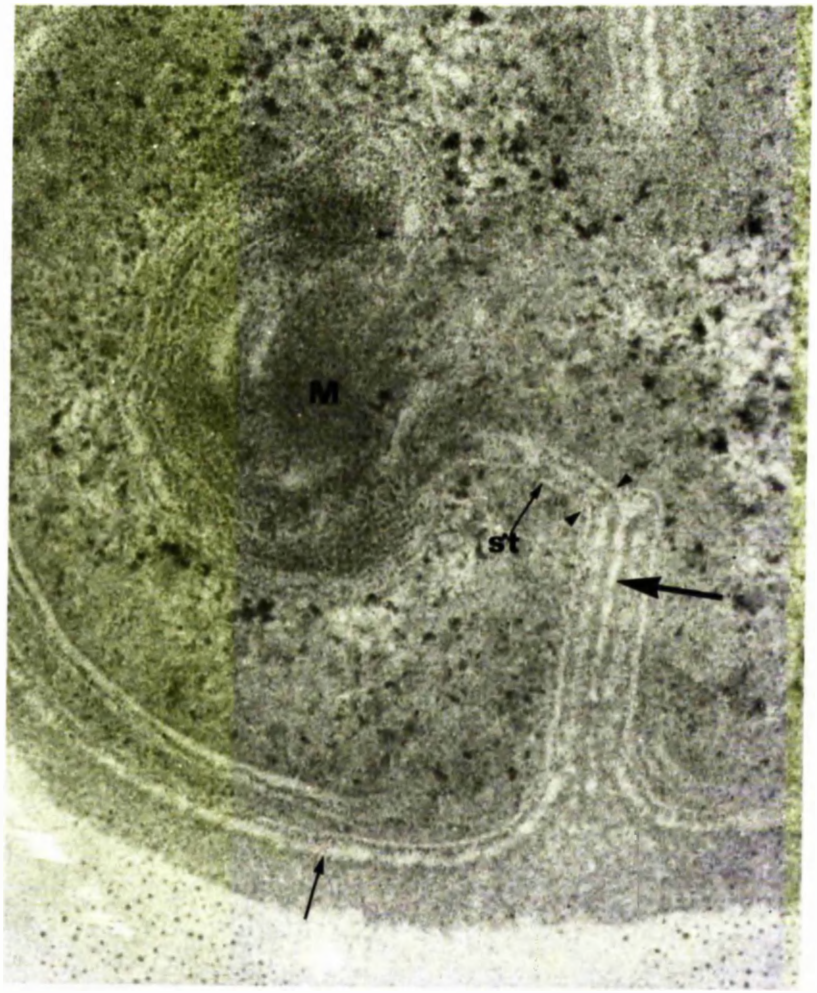
Magnification X 117 300


n
b
st
dm
pm

EXPLANATION OF PLATE 38

Thin section of part of a dividing cell of M. lysodeikticus St.O. A lamellar septal mesosome (M) is linked via a "stalk" (st) to the developing septum. The neck of the septum is well defined (opposing arrows) and both the central electron-transparent zone (large arrow) and also the zone of similar staining properties between membrane and wall (small arrow) appear to originate there.

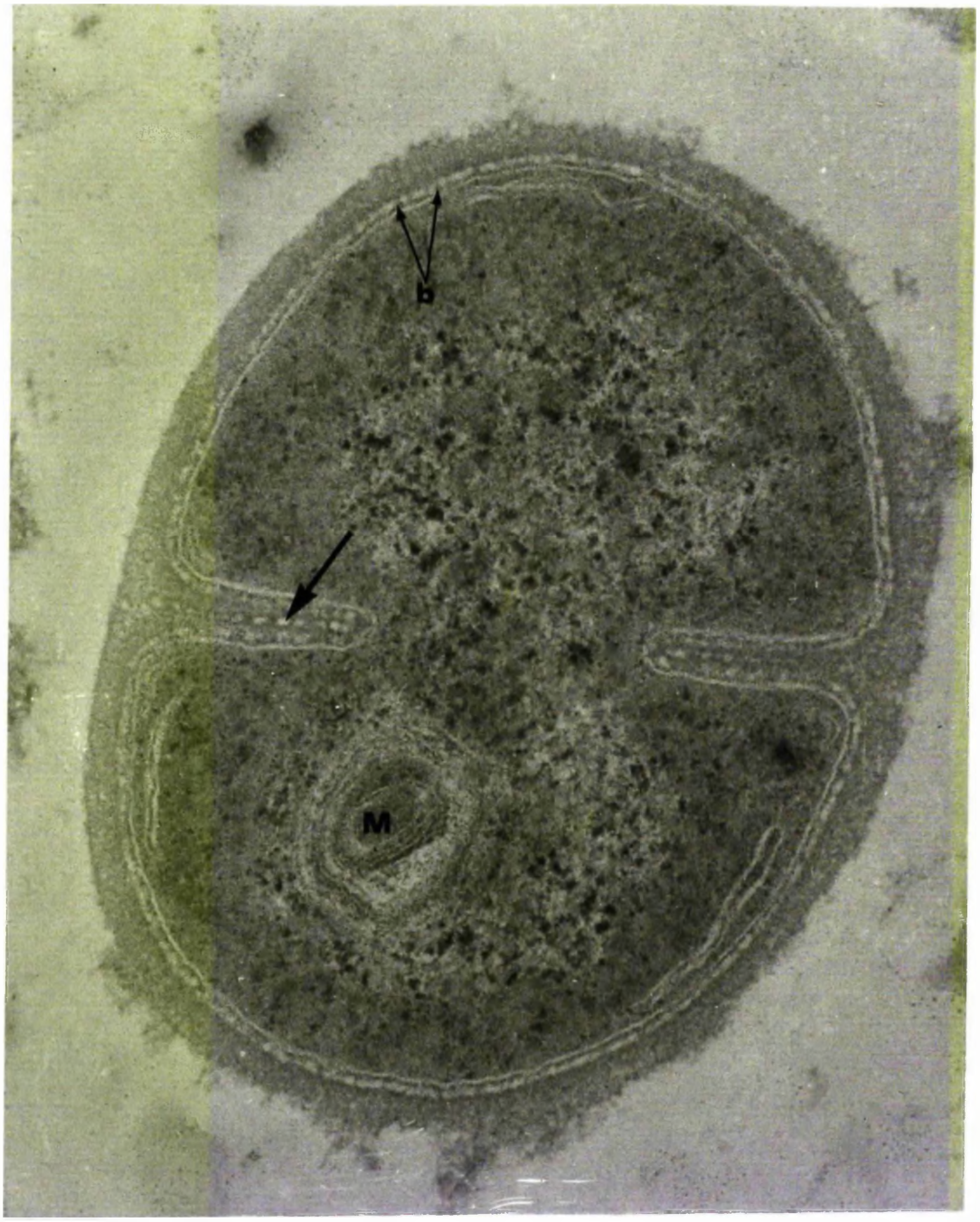
Magnification X 181 800


M
st

EXPLANATION OF PLATE 39

Thin section of a dividing cell of M. lysodeikticus St.O. Both developing septa are bisected by a central electron-transparent zone (large arrow). Note the lamellar mesosome (M) and the "bridges" (b) connecting the peripheral membrane to the cell wall.

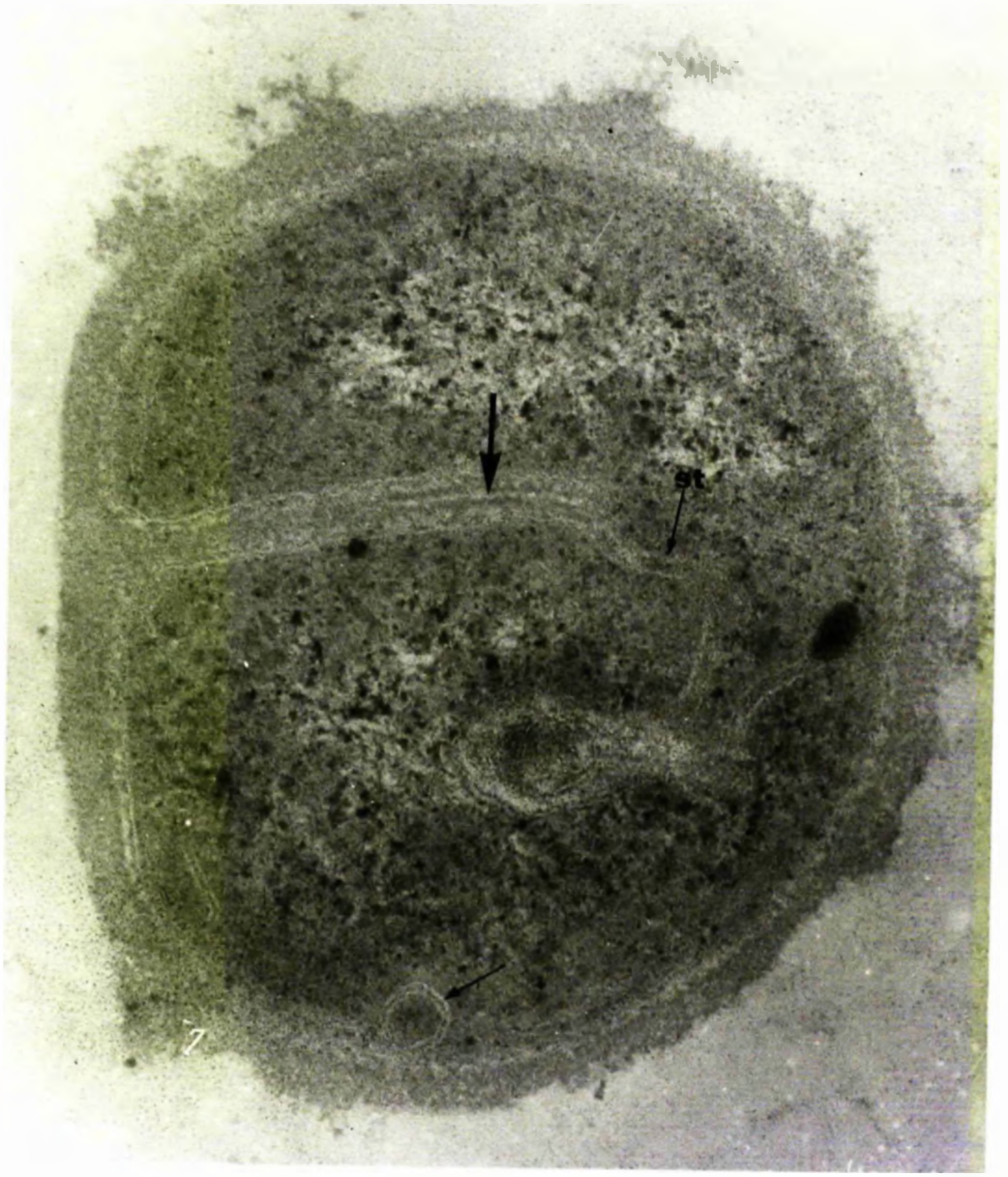
Magnification X 117 300


M

EXPLANATION OF PLATE 40

Thin section of a cell of M. lysodeikticus St.O in the later stages of septum formation. The stalk (st) connecting septum and mesosome is clearly visible as is the electron-transparent zone bisecting part of the septum (large arrow). Note also the circular extra membrane (small arrow) at the cell periphery.

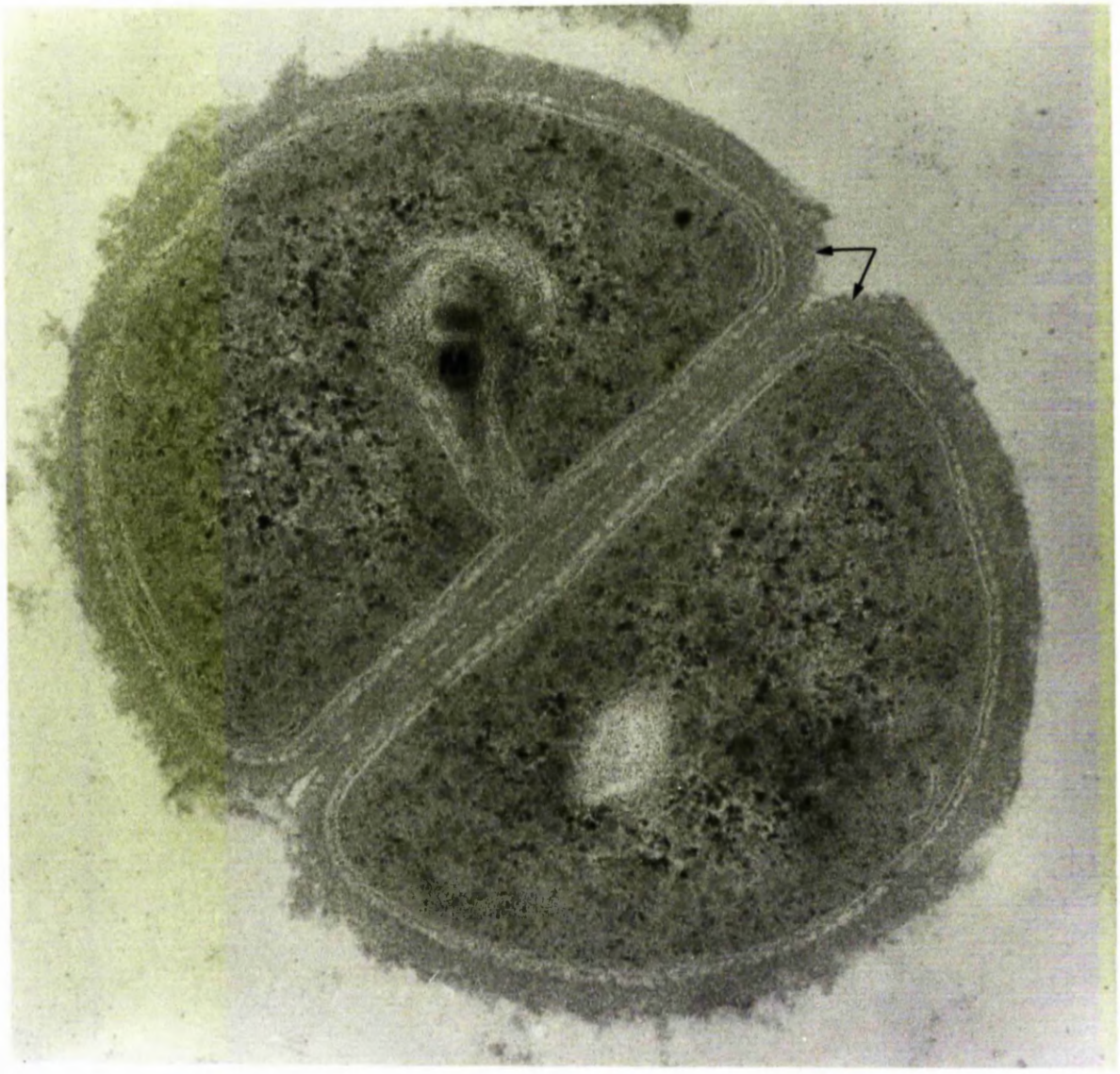
Magnification X 117 300


St
7

EXPLANATION OF PLATE 41

Thin section of a divided cell of M. lysodeikticus St.O at the initial stages of cell-cell separation. A mesosome (M) is in connection with the fully developed septum which is completely bisected by an electron-transparent zone. Note the rugged appearance of the initial cleavage lesion (arrows).

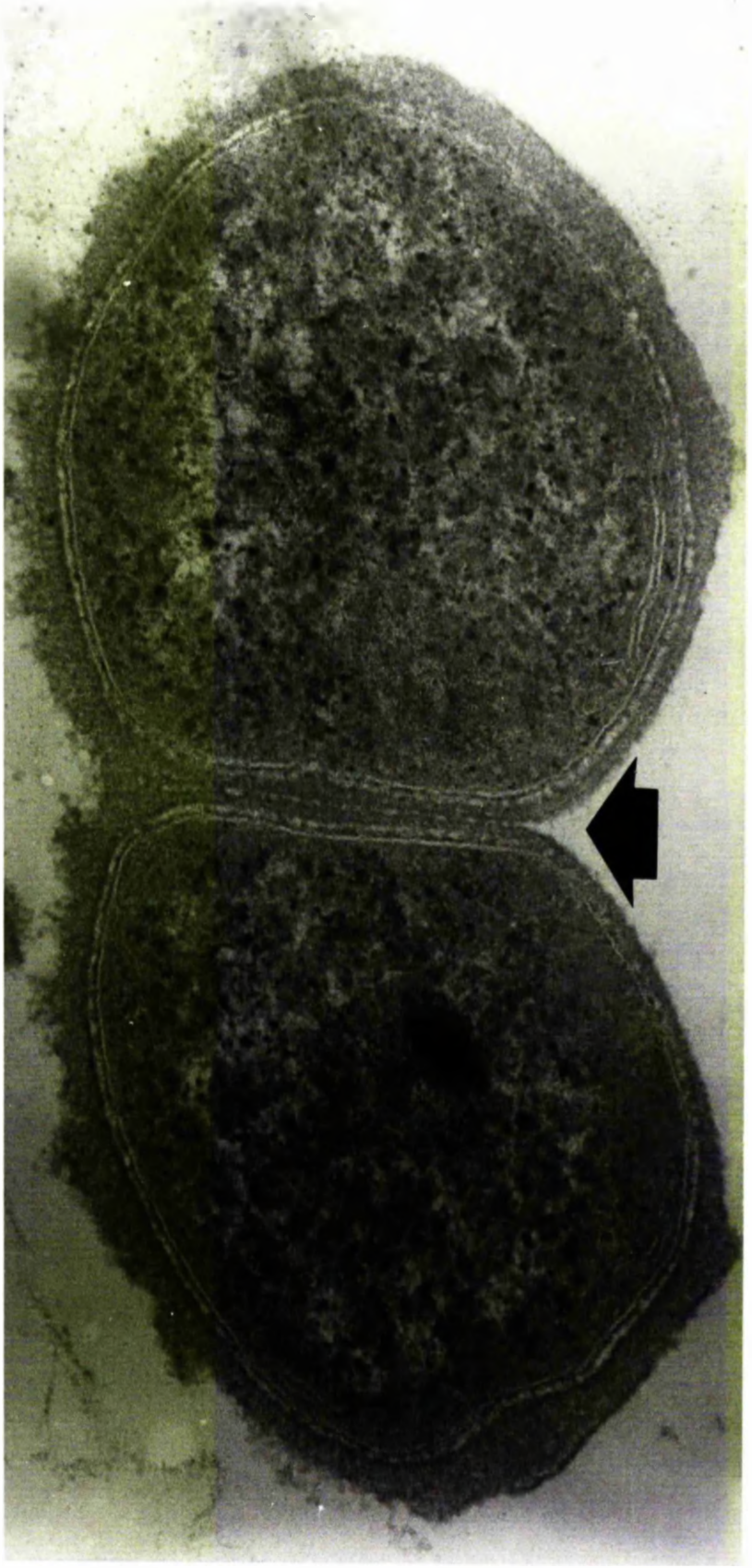
Magnification X 117 300



EXPLANATION OF PLATE 42

Thin section of a divided cell of M. lysodeikticus St.0 in the process of cell-cell separation. Cleavage (large arrow) is proceeding along the central electron-transparent zone bisecting the developed septum.

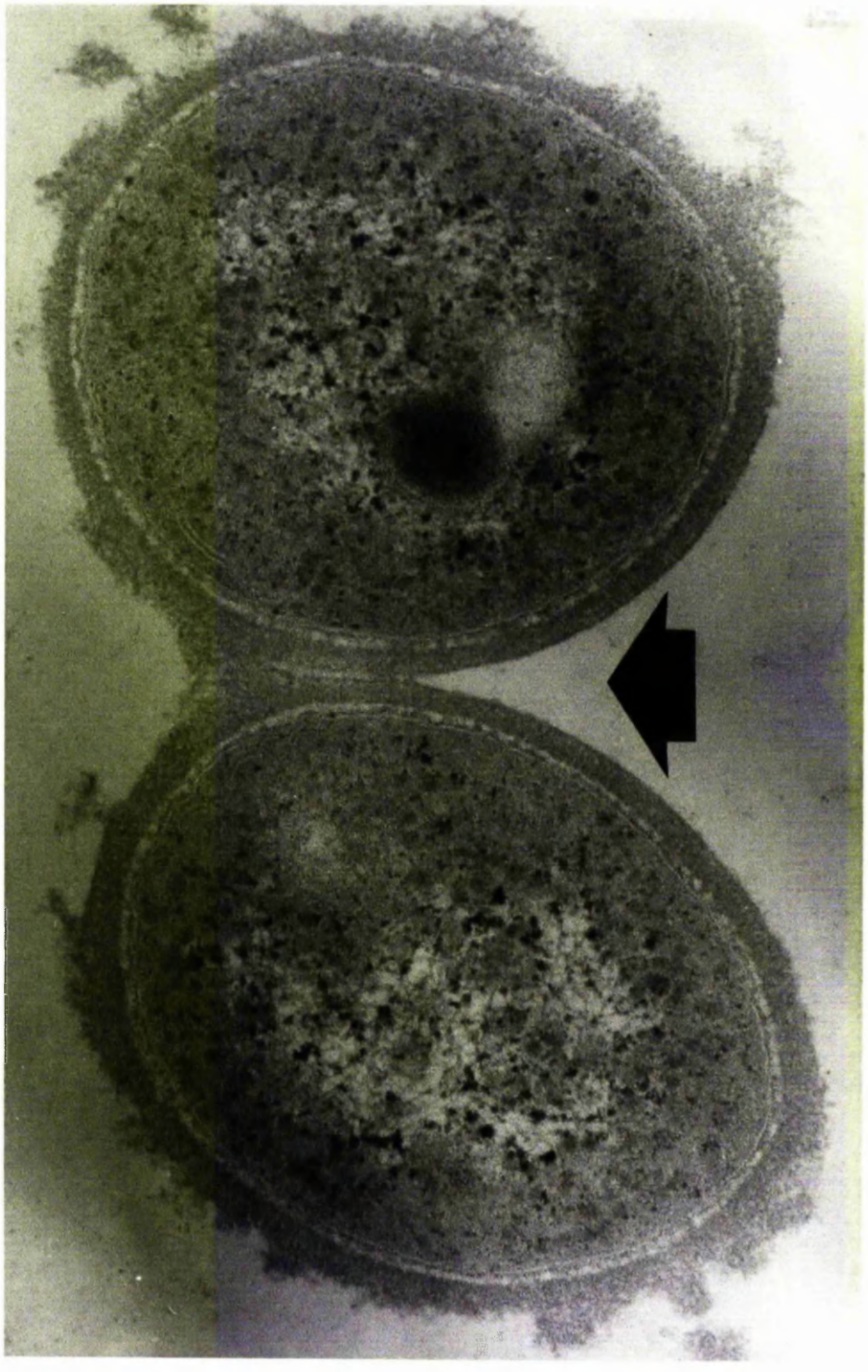
Magnification X 117 300



EXPLANATION OF PLATE 43

Thin section of cells of M. lysodeikticus St.0 in the later stages of cell-cell separation. Cleavage (large arrow) along the electron-transparent zone is almost complete. Note the smooth profile of the cleaved wall in comparison with that around the rest of the cell periphery.

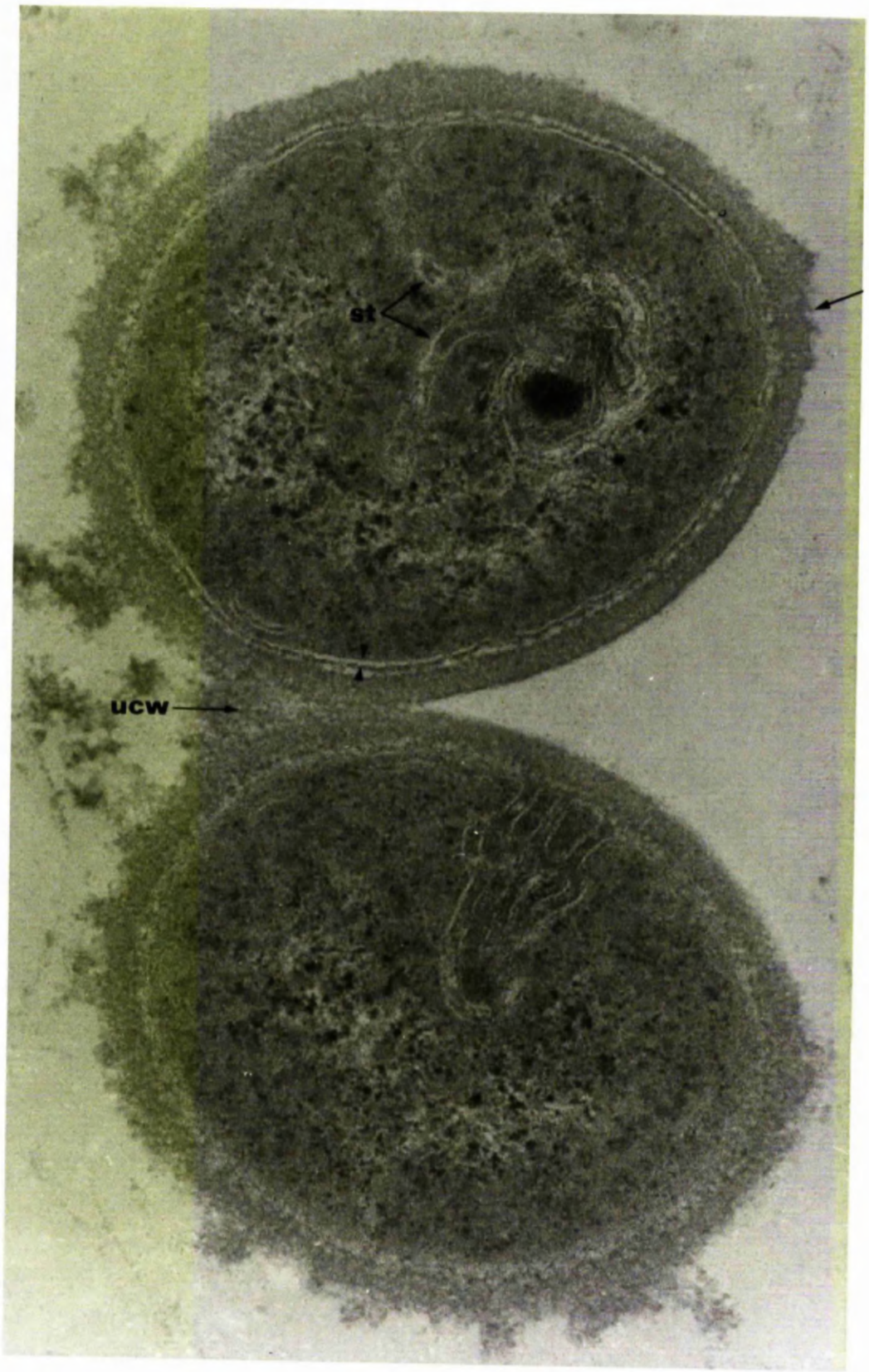
Magnification X 117 300



EXPLANATION OF PLATE 44

Thin section of two cells of M. lysodeikticus St.O at the final stages of cell septum cleavage. Cleavage along the electron-transparent zone has almost been completed leaving the cells attached by remaining unruptured cell wall (ucw). Note the smooth profile of cleaved cell wall and the ragged profile of cell wall ruptured at the onset of cell separation (arrow). The stalks (st) attached to the convoluted mesosome in the upper cell probably connect it to developing septa. Note the multiple connections between the internal membrane system of the lower cell and the cell periphery. Small arrows point to the inner and outer leaflet of the peripheral membrane.

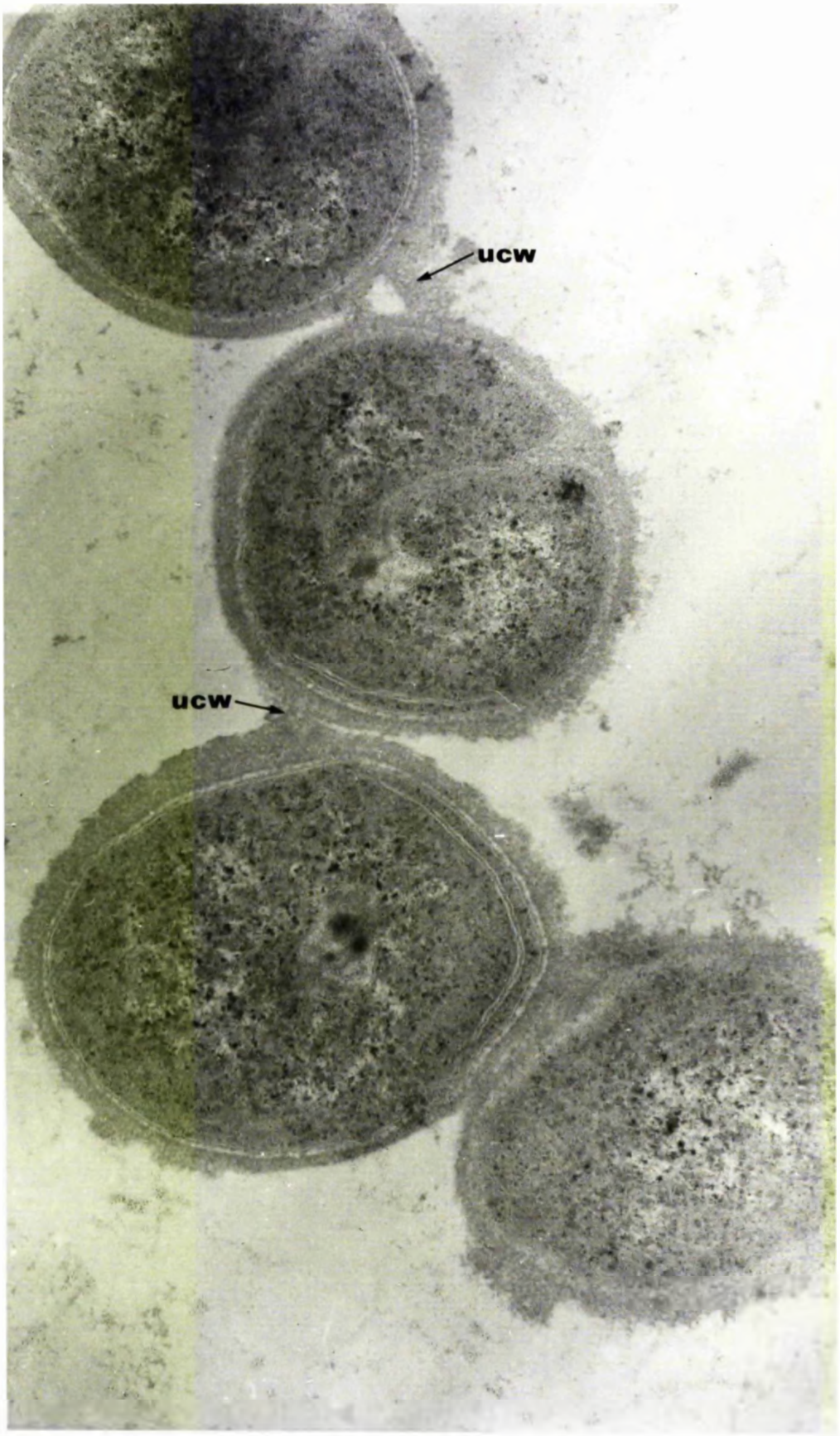
Magnification X 117 300


st
ucw

EXPLANATION OF PLATE 45

Thin section of a chain of cells of M. lysodeikticus St.O. Note that cells remain attached after division by an unruptured part of wall of the parent cell (ucw).

Magnification X 53 700


ucw
ucw

EXPLANATION OF PLATE 46

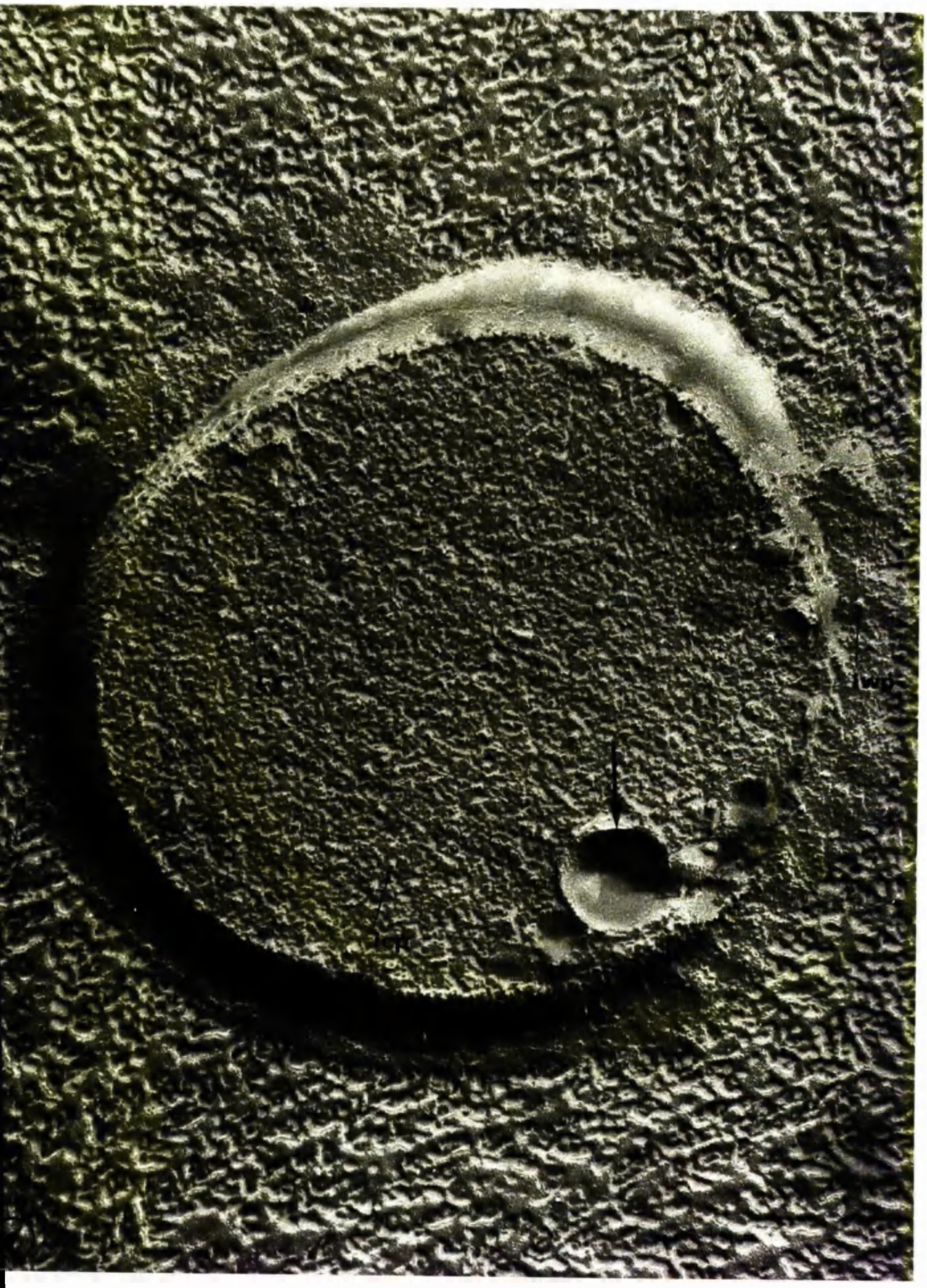
Replica of a cross-fractured unetched cell of M. lysodeikticus St.0 in 20% (v/v) glycerol. Note the presence of intrawall particles (iwp) and of similar structures (icp) in the cytoplasm (cy). Vesicle (arrow) shows a smooth-surfaced concave fracture. Wall (w).

Magnification X 121 200

EXPLANATION OF PLATE 47

Replica of a cross-fractured cell of M. lysodeikticus St.A infiltrated with 20% (v/v) glycerol. The wall (w) shows presence of intrawall particles (iwp), similar structures (icp) being observed in the cytoplasm (cy). Vesicle (arrow) shows a smooth concave fracture face.

Magnification X 151 000



EXPLANATION OF PLATE 48

Replica of a cross-fractured unetched cell of M. lysodekticus St.0 infiltrated with 20% v/v glycerol. A centrally located vesicle and several smaller peripheral ones are revealed (arrows). Both concave and convex fractures are smooth-surfaced.

Magnification X 90 900

EXPLANATION OF PLATE 49

Replica of a cross-fractured unetched cell of M. lysodeikticus St.A infiltrated with 20% (v/v) glycerol. Two round vesicles (arrows) revealing smooth convex fracture faces are revealed at opposite poles of the cell. Note the invagination revealed (large arrow) by the cross-fractured peripheral membrane (cpm), possibly indicating the site of new septum formation.

Magnification X 150 600

EXPLANATION OF PLATE 50

Replica of a fractured unetched dividing cell of M. lysodeikticus St.A impregnated with glycerol. Intrawall particles (iwp) can be observed in the cell wall (w) and in developed cell septum (s). Note the occurrence of peripheral vesicles (arrows).

Magnification X 199 500

W
FWP

EXPLANATION OF PLATE 51

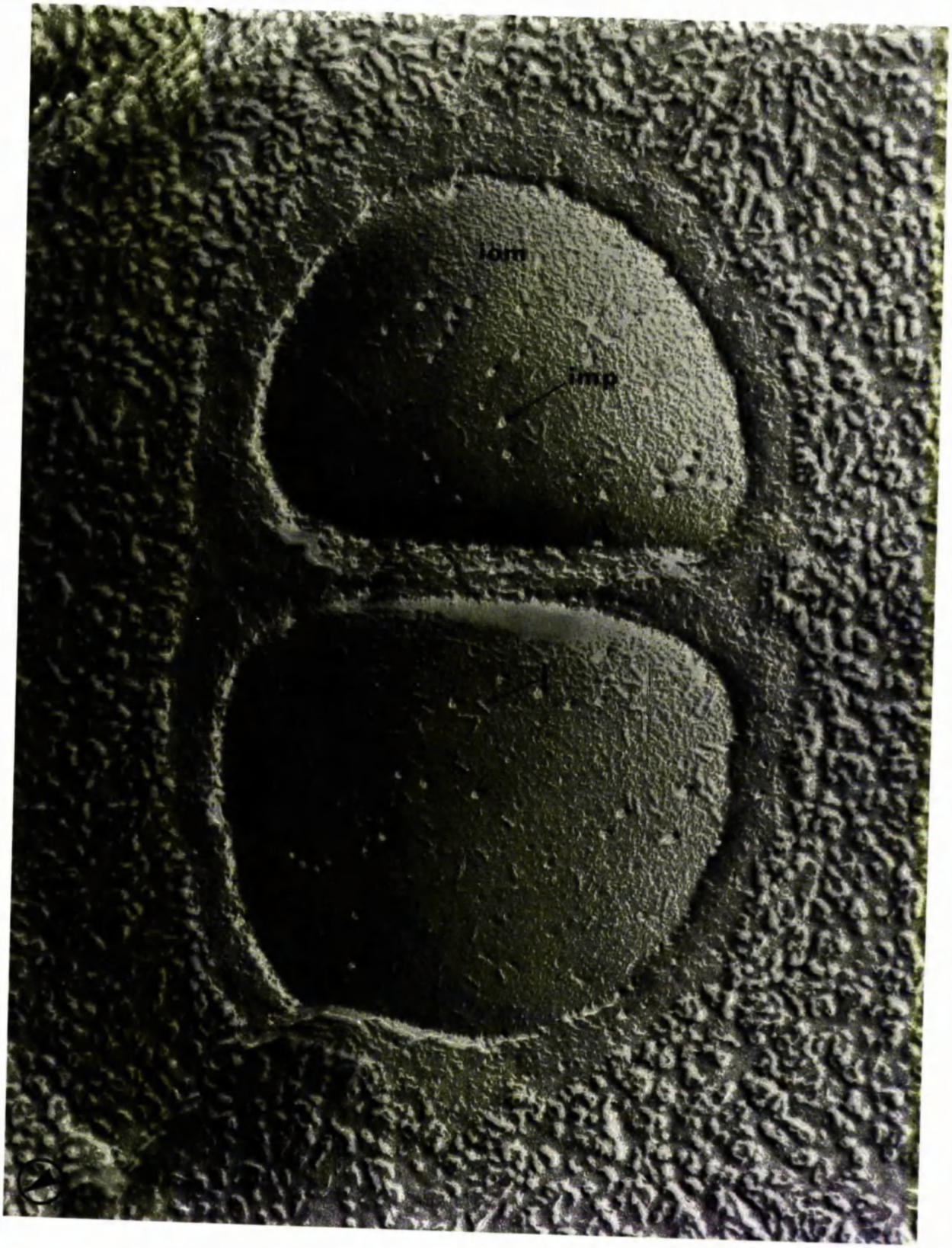
Replica of a cross-fractured unetched cell of M. lysodeikticus St.A impregnated with glycerol. Note the cross-fractured internal membrane system (arrow).

Magnification X 150 600

EXPLANATION OF PLATE 52

Replica of two freeze-fractured unetched cells of M. lysodeikticus St.A. Both cells reveal concave fracture faces of peripheral membrane (iom) and show few intramembrane particles (imp) and many linear depressions (l).

Magnification X 150 600


lom
imp

EXPLANATION OF PLATE 53

Replica of two freeze-fractured unetched cells of M. lysodeikticus St.0 impregnated with glycerol. The concave fracture face of the peripheral membrane (iom) of the upper cell reveals linear depression (l) and occasional intramembrane particles (imp). The convex fracture face of the peripheral membrane of the lower cell (oim) reveals many intramembrane particles and numerous rod-shaped structures (r) after connected to the cell wall (w) and the concealed septa (s).

Magnification X 119 700


imp
I
iom

EXPLANATION OF PLATE 54

Replica of a complex fracture involving two cells of M. lysodeikticus St.A impregnated with glycerol. The concave fracture face (iom) of the upper cell reveals linear depressions (l) and occasional intramembrane particles (imp). The convex fracture face (oim) of the lower cell shows many intramembrane particles. Rod-shaped structures (r) on its surface connect the membrane and cell wall and also connect the membrane and the hidden cell septum (s). Only very occasional rod-shaped structures appear to connect with the cytoplasm (cy).

Magnification X 181 800

EXPLANATION OF PLATE 55

Replica of two freeze-fractured unetched cells of M. lysodeikticus St.A impregnated with glycerol. Note the rod-shaped structures (r) apparently connecting the convex fracture face of the peripheral membrane (oim) of the lower cell with the developed cell septum (s).

Magnification X 150 600

EXPLANATION OF PLATE 56

Replica of freeze-fractured unetched cells of M. lysodeikticus St.0 impregnated with glycerol. Note the rod-shaped structures (r) apparently connecting the convex (oim) and concave (iom) membrane fracture face of the two cells A can be seen to be continuous with the developed cell septum (s).

Magnification X 117 300


A
olm

EXPLANATION OF PLATE 57

Replica of a freeze-fractured, unetched, dividing cell of M. lysodeikticus St.O impregnated with glycerol. Note the smooth-surfaced vesicle (arrow) partly revealed by fracture from the convex membrane fracture face (oim) into the cytoplasm (cy).

Magnification X 90 900

EXPLANATION OF PLATE 58

Replica of a freeze-fractured unetched cell of M. lysodeikticus St.A after impregnation with glycerol. A break in the fracture plane through the membrane has revealed a vesicle (arrow) in the cell interior.

Magnification X 90 900

EXPLANATION OF PLATE 59

Replica of a complex fracture in two freeze-fractured unetched cells of M. lysodeikticus St.O impregnated with glycerol. See symbols list for interpretation.

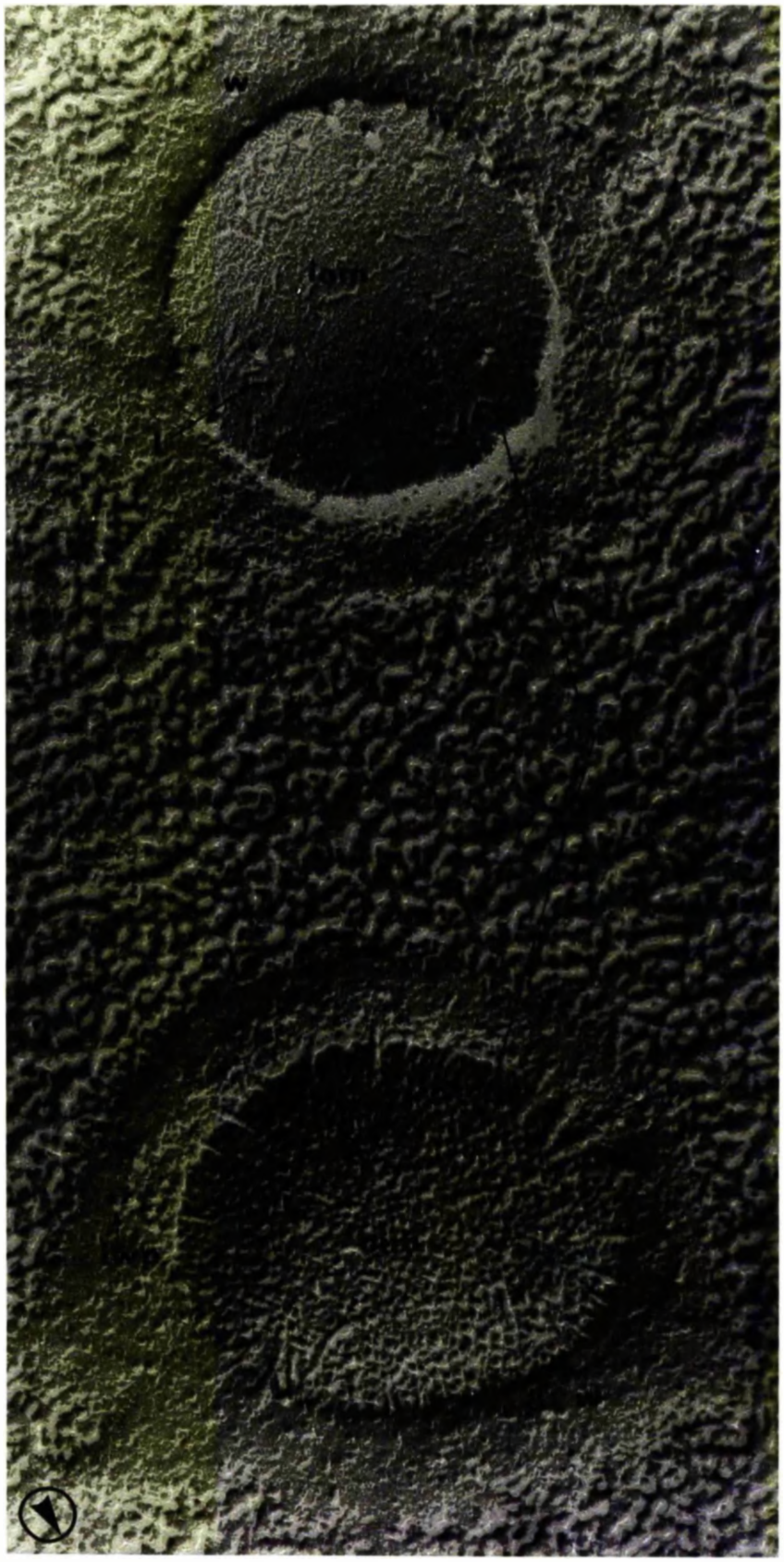
Magnification X 151 500


iom
imp
olm

EXPLANATION OF PLATE 60

Replica of two freeze-fractured unetched cells of *M. lysodeikticus* St.A impregnated with glycerol. See symbols list for interpretation.

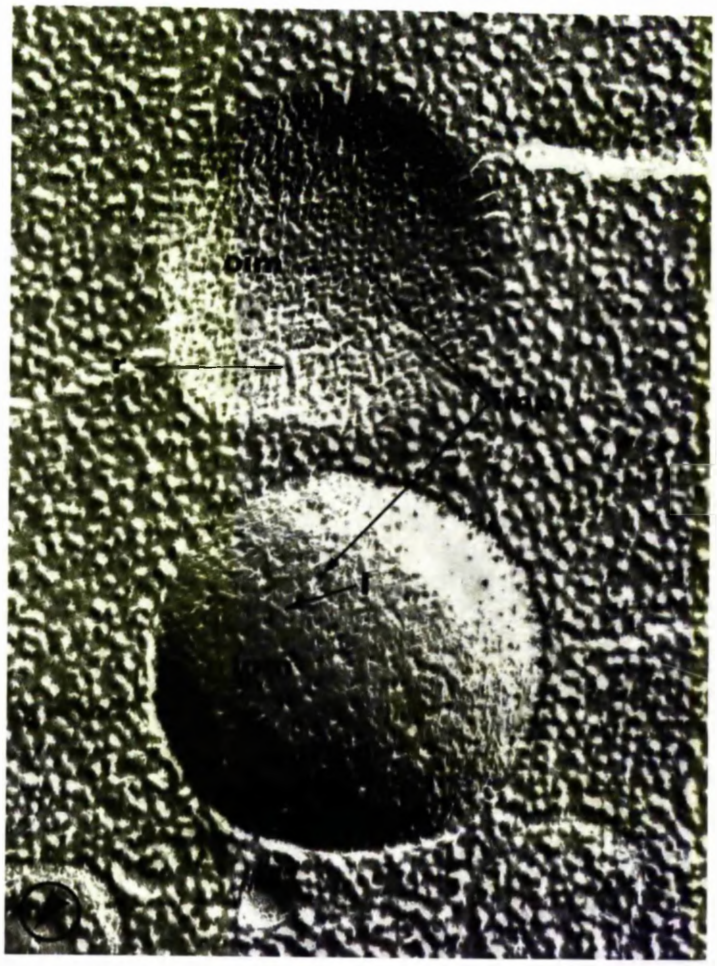
Magnification X 150 600



EXPLANATION OF PLATE 61

Replica of freeze-fractured unetched "standard" membranes of M. lysodeikticus St.0 suspended in glycerol. The convex fracture face (oim) reveals many intramembrane particles (imp) and rod-shaped structures (r) whereas the concave fracture face (iom) reveals fewer intramembrane particles and also linear depressions (l).

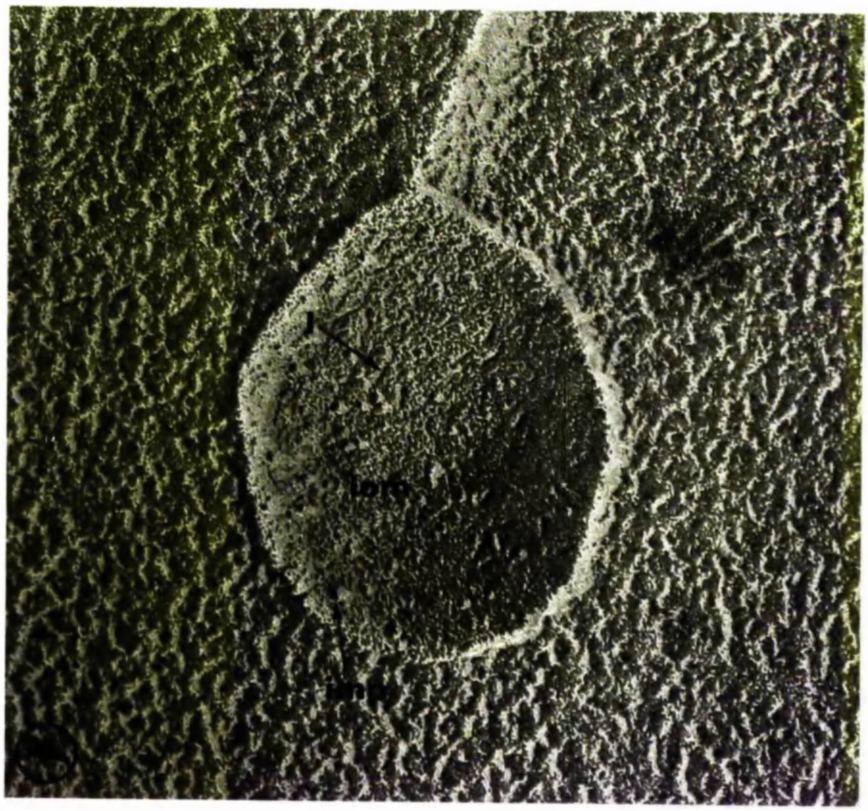
Magnification X 117 300


OIM
I

EXPLANATION OF PLATE 62

Replica of freeze-fractured*unetched* "standard" membrane isolated from *M. lysodeikticus* St.A and suspended in glycerol. This concave fracture (iom) reveals linear depression (l) and some intramembrane particles (imp).

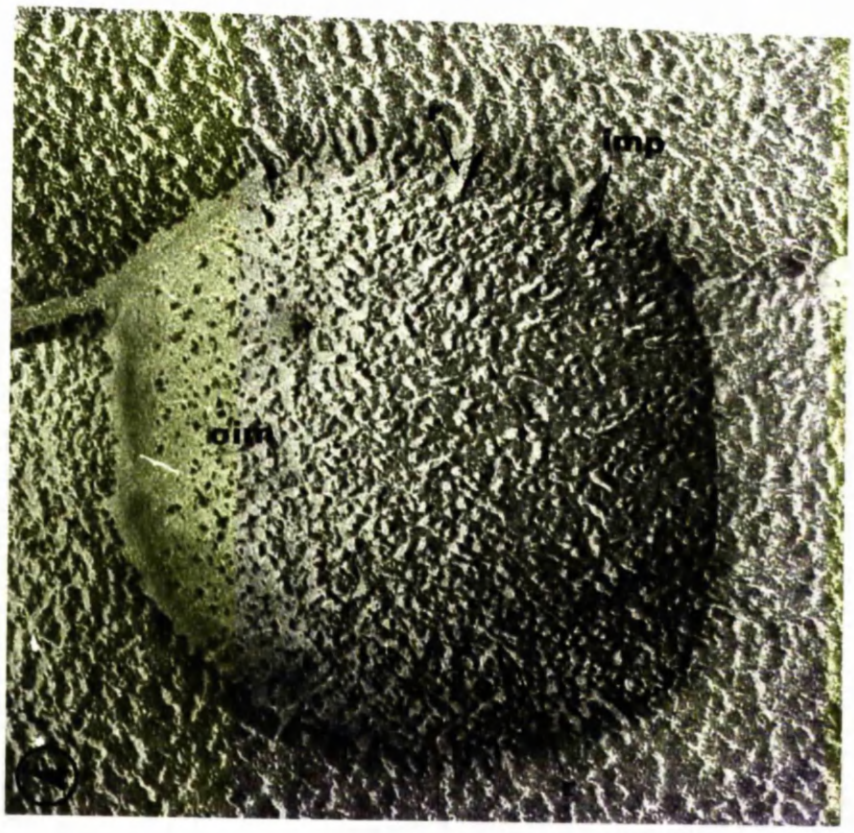
Magnification X 151 200



EXPLANATION OF PLATE 63

Replica of freeze-fractured unetched "standard" membrane from M. lysodeikticus St.A suspended in glycerol. This convex fracture (oim) reveals a similar distribution of intramembrane particles (imp) and rod-shaped structures (r) observed in convex fractions of peripheral membrane in situ.

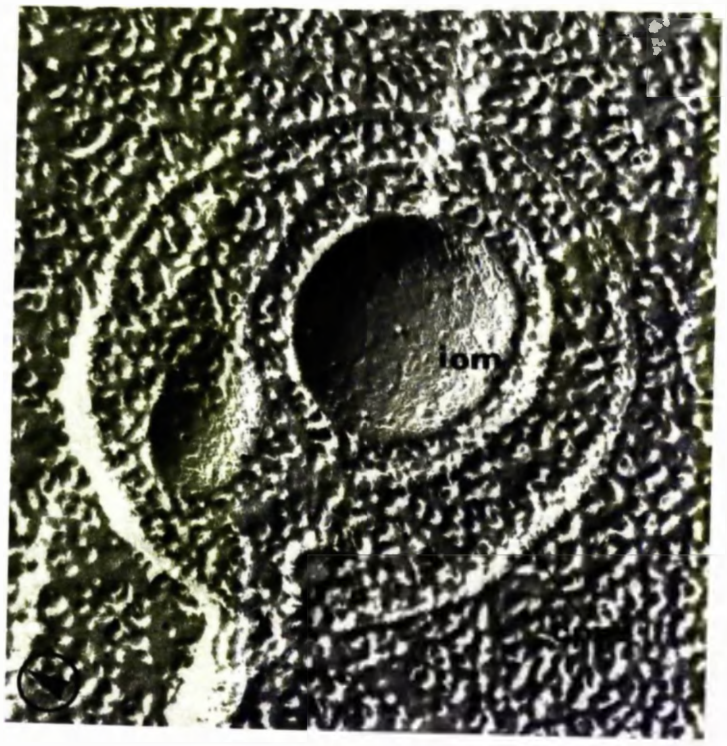
Magnification X 151 200


imp
oim

EXPLANATION OF PLATE 64

Replica of freeze-fractured unetched "standard" membranes from M. lysodeikticus St.O. The concentric vesicles show cross-fracturedmembrane (cpm) and also concave fractures (iom).

Magnification X 117 300


iom

EXPLANATION OF PLATE 65

Replica of freeze-fractured unetched "standard" membranes from M. lysodeikticus St.A. The outer two concentric vesicles show cross-fractured membrane (cpm). The membrane contained by these vesicles has fractured so as to reveal the outer surface of the inner half of the membrane (oim) in both convex and concave relief.

Magnification X 151 200


oim
oim
epm

EXPLANATION OF PLATE 66

Replica of freeze-fractured unetched "standard" membranes isolated from M. lysodeikticus St.A and suspended in glycerol. Both the inner surface of the outer half of the membrane (iom) and the outer surface of the inner half of the membrane (oim) are revealed as both convex and concave fractures.

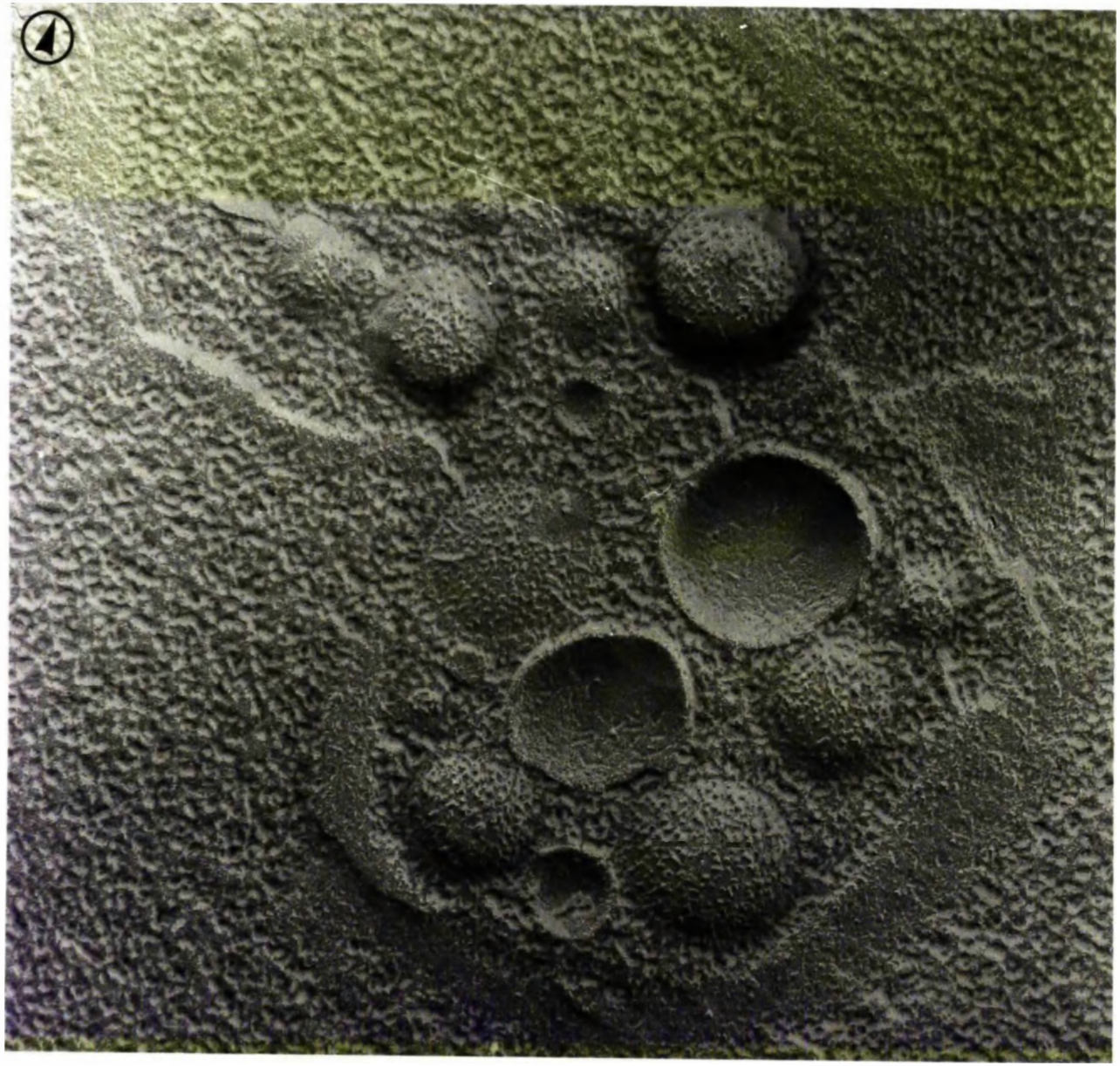
Magnification X 151 200

iom
iom
dim
om

EXPLANATION OF PLATE 67

Replica of a freeze-fractured unetched autolysing cell of M. lysodeikticus St.O. The membrane system, although extensively disrupted, reveals fracture faces similar to those observed in whole cells and isolated membranes.

Magnification X 117 300



EXPLANATION OF PLATE 68

Replica of freeze-fractured and etched fixed protoplasts of M. lysodeikticus St.O. Etching has revealed the relatively smooth outer surface of the outer half of the protoplast peripheral membrane (oom) and shows rod-shaped structures (r) connecting it to the outer surface of the inner half of the peripheral membrane (oim). This latter fracture face is densely covered with intramembrane particles (imp) similar to the corresponding fracture face in situ.

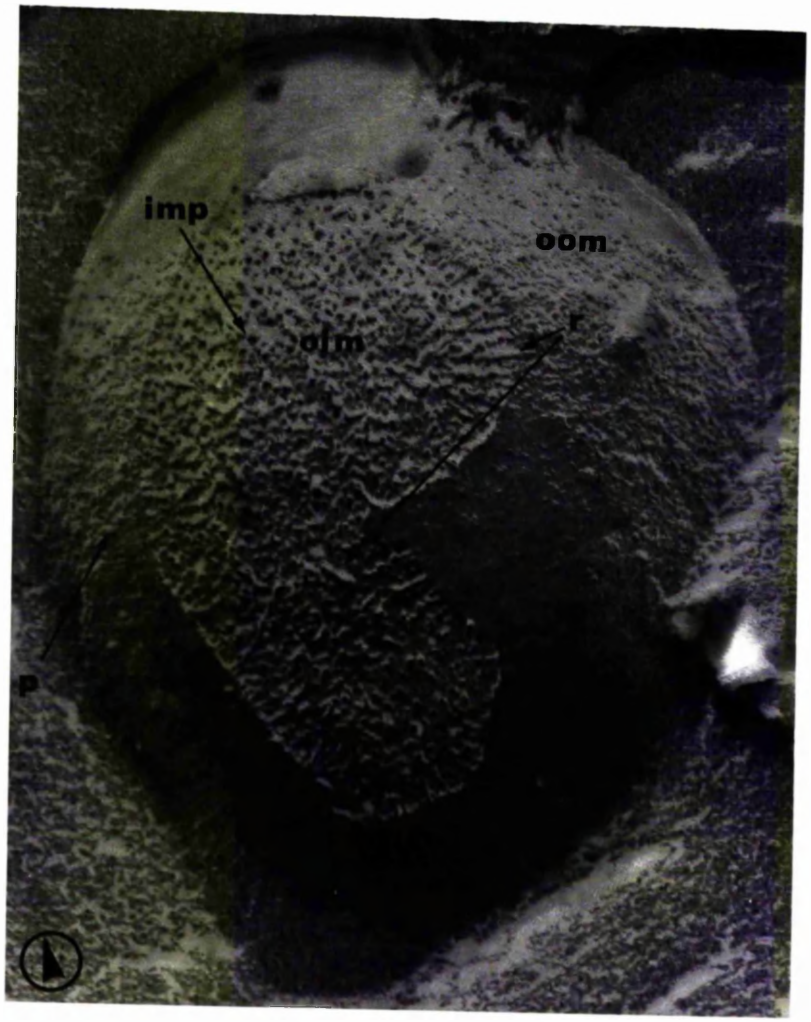
Magnification X 117 300


oim
oom

EXPLANATION OF PLATE 69

Replica of freeze-fractured and etched fixed protoplast of M. lysodeikticus St.O revealing the outer surface of both halves of the peripheral membrane (oim and oom), rod-shaped structures (r) and intramembrane particles (imp). At low shadowing angles the apparently smooth outer membrane surface (oom) reveals an undulating topography with numerous protuberances (p).

Magnification X 117 300


imp
oom
olm
r
P

EXPLANATION OF PLATE 70

Replica of a freeze-fractured and etched fixed protoplast of M. lysodeikticus St.O. Note the area (a) of the convex fracture face (oim) depleted in intramembrane particles (imp). No rod-shaped structures (r) are evident on this area or connecting it with the etched outer surface (oom). Note also the numerous craters (c) formed during the course of fracture below the membrane.

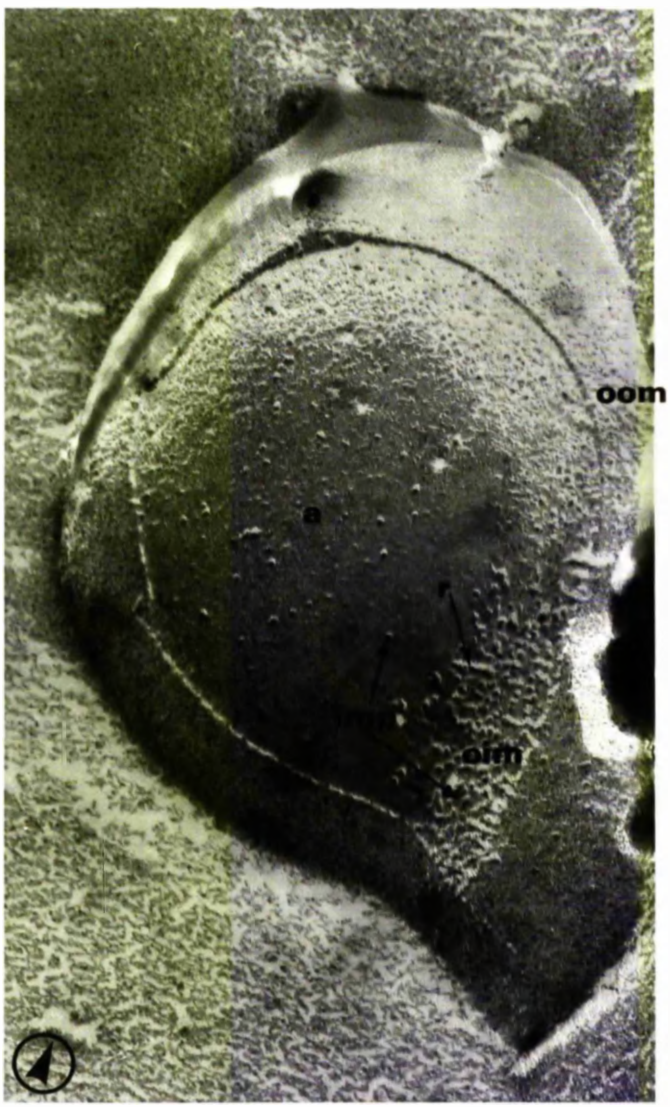
Magnification X 117 300


olm
c
c
imp

EXPLANATION OF PLATE 71

Replica of a freeze-fractured and etched fixed protoplast of M. lysodeikticus St.O. Note the extensive area (a) of the outer surface of the inner half of the peripheral membrane (oim) with relatively few intramembrane particles (imp). This area (a) is clearly continuous with a part of the fracture face carrying a normal distribution of intramembrane particles (imp) and rod-shaped structures (r). No rod-shaped structures are evident either on this area (a) or connecting it with the etched outer membrane surface (oom).

Magnification X 117 300


oom
e
r
oim

EXPLANATION OF PLATE 72

Replica of a freeze-fractured and etched fixed protoplast of M. lysodeikticus St.O. Note the line of small depressions (d) on the etched outer membrane surface (oom) which follow closely the edge of the etched surface (e).

Magnification X 117 300

EXPLANATION OF PLATE 73

Replica of a freeze-fractured and etched fixed protoplast of M. lysodeikticus St.O. Note the line of small depressions (d) of the outer membrane surface (oom) apparently extrapolating the edge of the etched surface (e). The relatively smooth nature of the outer surface of the outer half of the peripheral membrane (oom) is revealed, although at low shadowing angles numerous small protuberances (p) are observed.

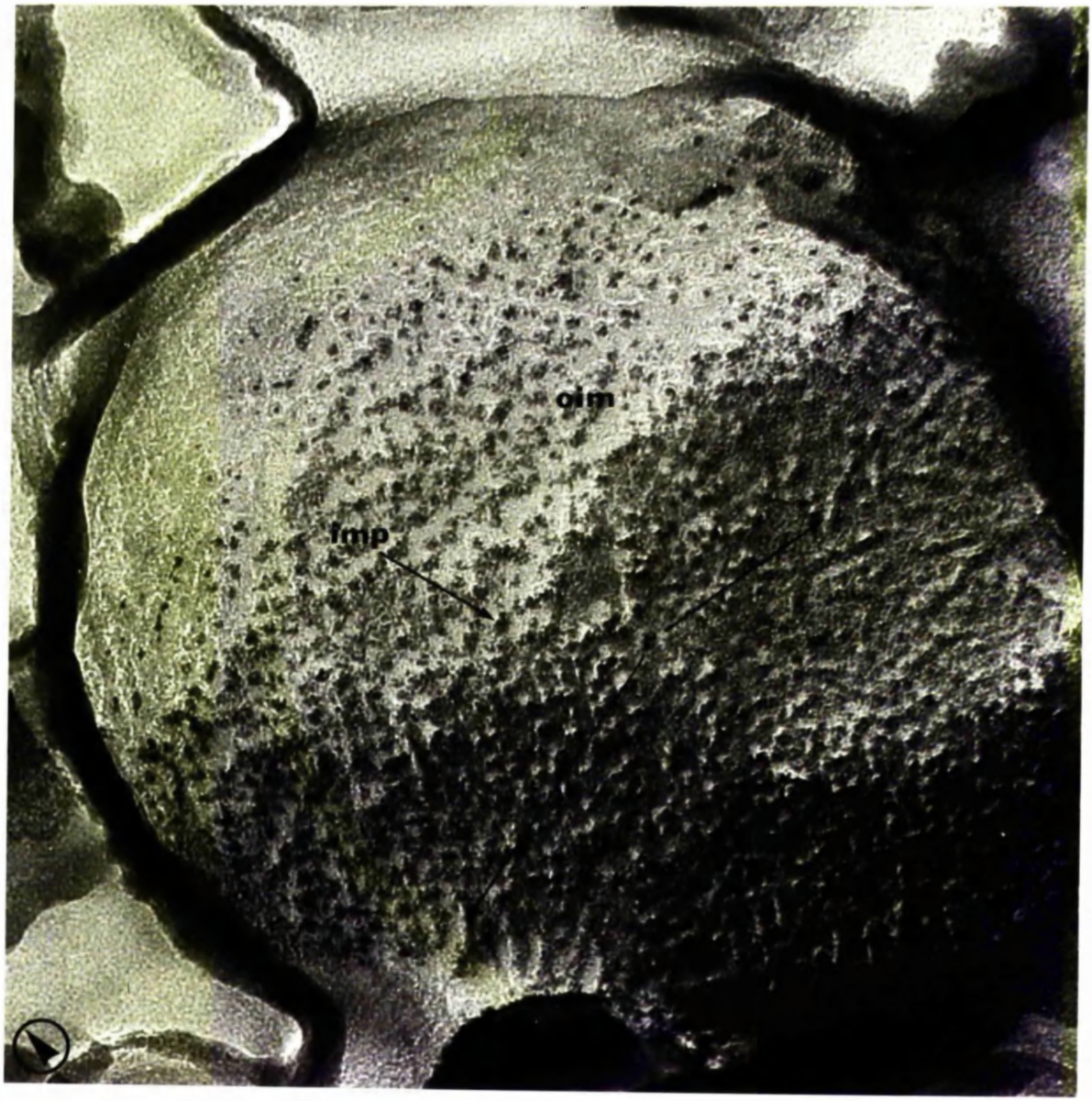
Magnification X 117 300


oom
d
p
e

EXPLANATION OF PLATE 74

Replica of freeze-fractured and etched "standard" membrane preparation from M. lysodeikticus St.O. The convex fracture reveals the outer surface of the inner half of the peripheral membrane and shows its dense covering with intramembrane particles (imp) and rod-shaped structures (r).

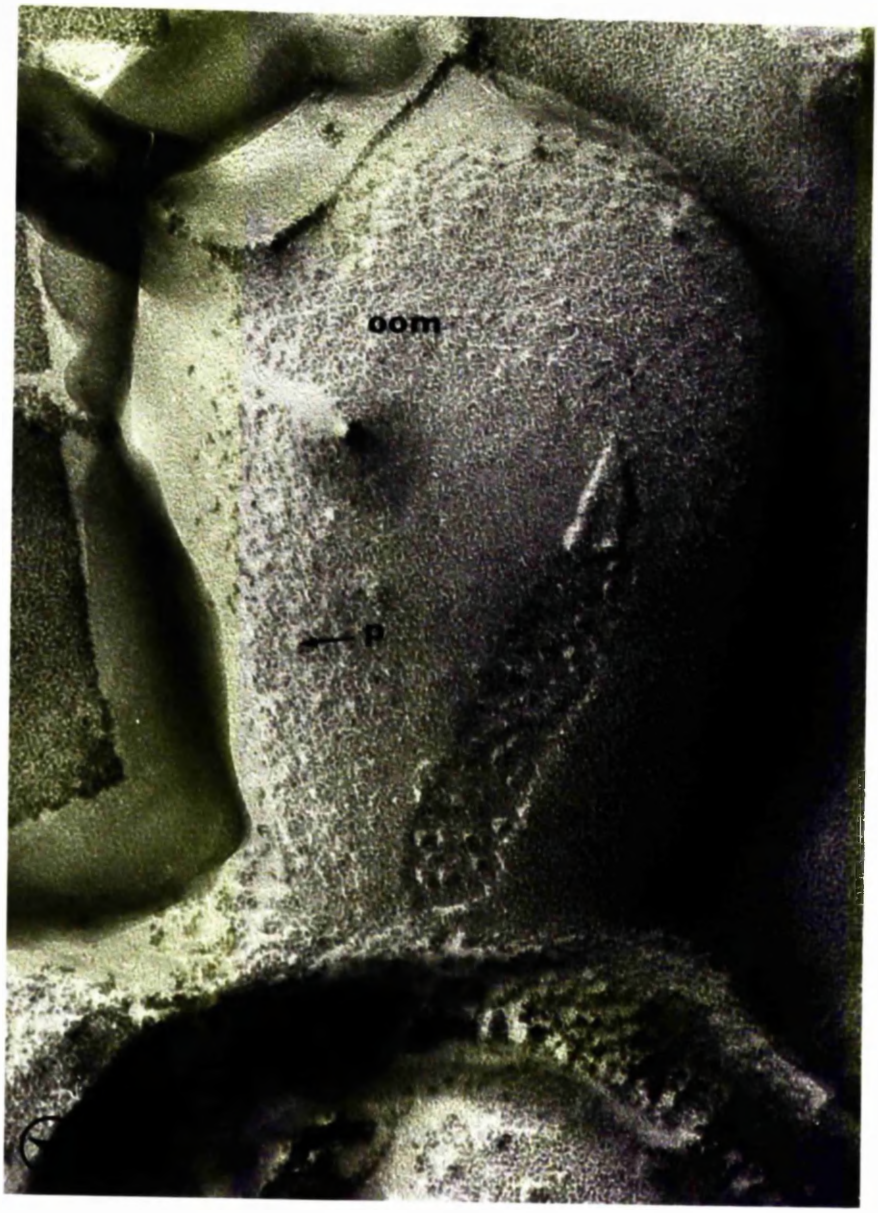
Magnification X 234 600


oim
fmp

EXPLANATION OF PLATE 75

Replica of freeze-fractured and etched "standard" membranes from M. lysodeikticus St.O. The etched surface (oom) shows small protuberances (p) at low shadowing angles.

Magnification X 234 600


oom
P

EXPLANATION OF PLATE 76

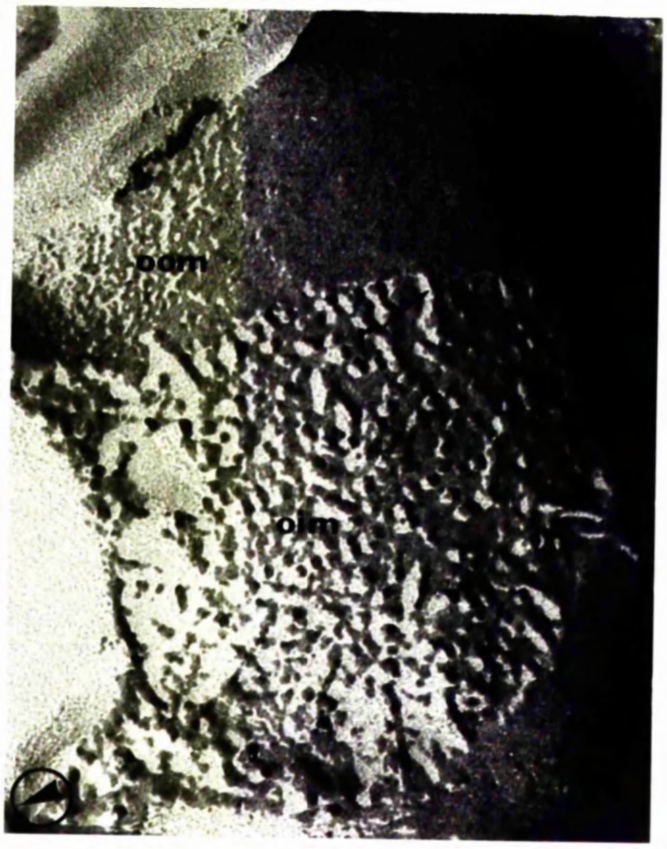
Replica of freeze-fractured and etched "standard" membranes from M. lysodeikticus St.A. This convex fracture reveals the outer surface of the inner half (oim) of the membrane with its numerous intramembrane particles. Note also the relatively smooth outer membrane (etched) surface (oom).

Magnification X 234 600

EXPLANATION OF PLATE 77

Replica of freeze-fractured and etched "standard" membranes from M. lysodeikticus St.A. Rod-shaped structures (r) connect to etched surface (oom) with the convex fracture face (oim).

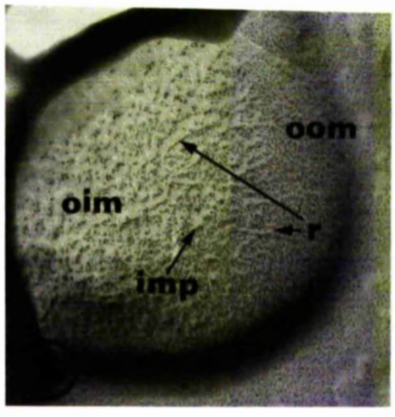
Magnification X 234 600


pom
oim

EXPLANATION OF PLATE 78

Replica of freeze-fractured and etched "standard" membranes isolated from M. lysodeikticus St.0 and subjected to ionic shock. Intramembrane particles (imp) and rod-shaped structures (r) on the convex fracture surface (oim) and connecting with the etched surface (oom) are visible.

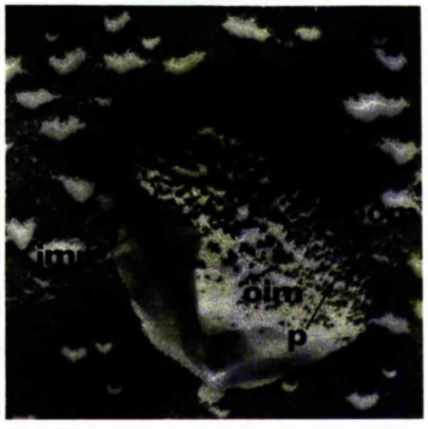
Magnification X 117 300


oom
oim
r
imp

EXPLANATIONS OF PLATE 79

Replica of freeze-fractured and etched "standard" membranes isolated from M. lysodeikticus St.A and subjected to ionic shock. Intramembrane particles (imp) are evident on the convex fracture face (oim) as are protuberances (p) on the etched surface (oom) at low shadowing angles.

Magnification X 117 300


im
olm
p

EXPLANATION OF PLATE 80

Replica of freeze-fractured and etched "standard" membranes isolated from M. lysodeikticus St.0 and treated with EDTA. The convex fracture face (oim) reveals a dense population of intramembrane particles (imp) and also the occurrence of rod-shaped structures (r).

Magnification X 151 200


imp
oim

EXPLANATION OF PLATE 81

Replica of freeze-fractured and etched "standard" membranes isolated from M. lysodeikticus St.O and treated with EDTA. The rod-shaped structures (r) on the convex fracture surface (oim) are often clearly continuous with the relatively smooth etched surface (oom).

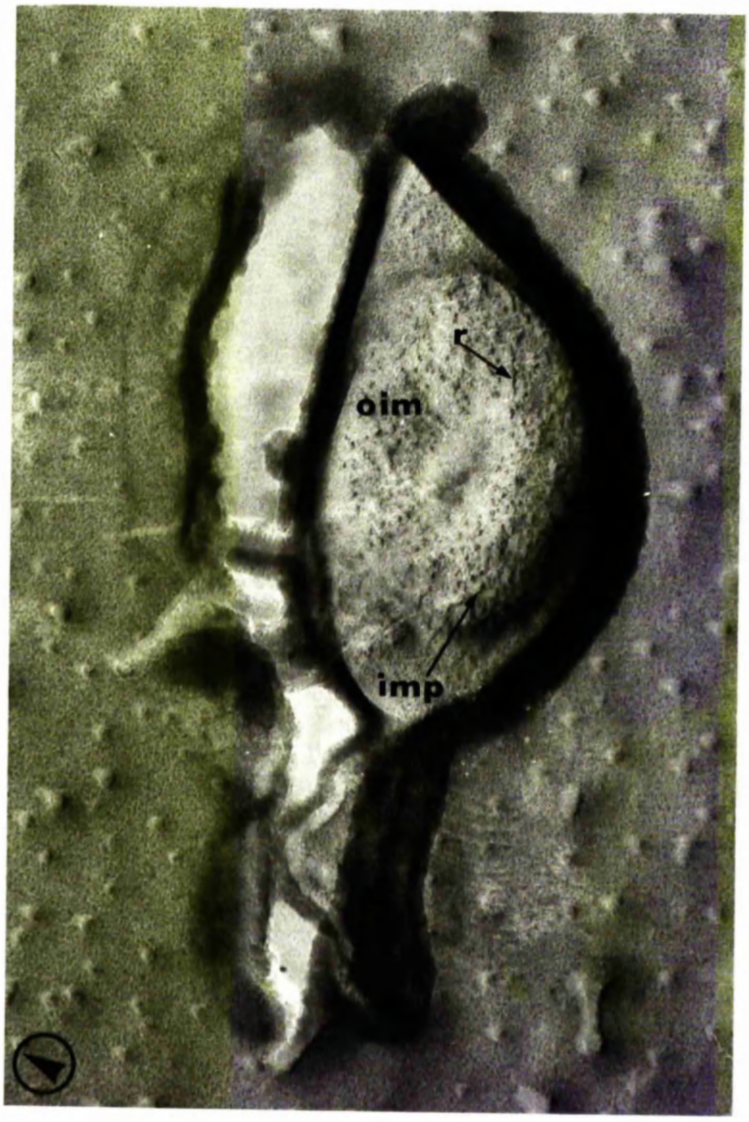
Magnification X 151 200


oom
olm

EXPLANATION OF PLATE 82

Replica of freeze-fractured and etched "standard" membranes isolated from M. lysodeikticus St.A and treated with EDTA. Intramembrane particles (imp) and rod-shaped structures (r) are evident on the convex fracture face (oim).

Magnification X 151 200


r
oim
imp

EXPLANATION OF PLATE 83

Replica of freeze-fractured and etched "standard" membranes isolated from M. lysodeikticus St.A and treated with EDTA. The relatively smooth nature of the etched outer membrane surface (oom) is revealed.

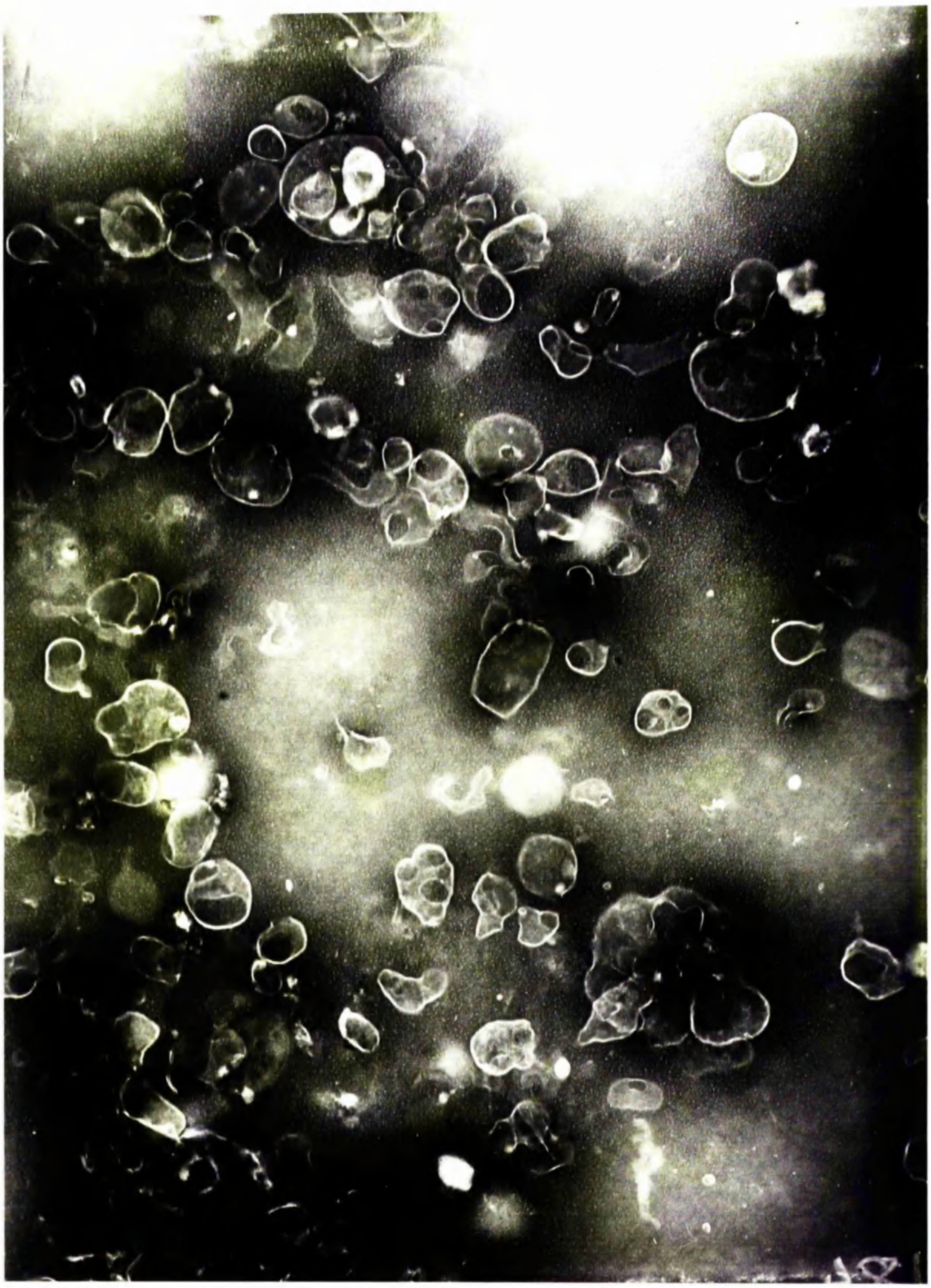
Magnification X 117 300


oom

EXPLANATION OF PLATE 84

Negatively stained preparation of membrane residue obtained following treatment of "standard" membranes from M. lysodeikticus St.0 with ionic shock. Note the absence of surface particles present in micrographs of "standard" membranes (cf. Plate 26).

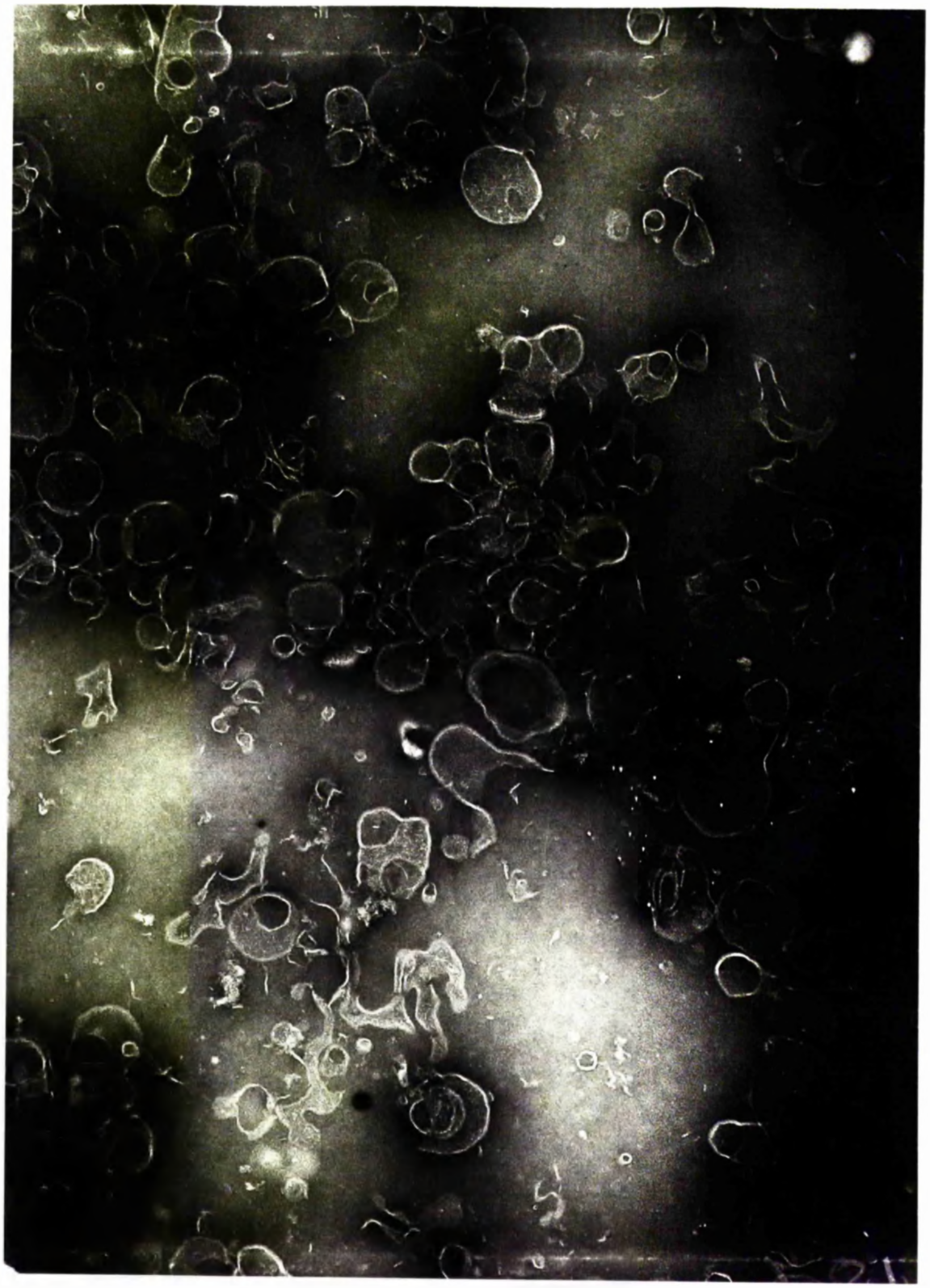
Magnification X 50 000



EXPLANATION OF PLATE 85

Negatively stained preparation of membrane residues obtained following treatment of "standard" membranes from M. lysodeikticus St.O with EDTA in dilute buffer (0.005M tris buffer). Note the absence of surface particles found on "standard" membrane preparations (cf. Plate 26).

Magnification X 50 000



EXPLANATION OF PLATE 86

Replica of freeze-fractured unetched mesosomal membrane isolated from M. lysodeikticus St.0 and suspended in glycerol. Mesosomal membranes appear as spherical vesicles and fractures reveal a dense covering of intramembrane particles (imp) on the outer surface of the inner half of the mesosomal membrane (oiv). The inner surface of the outer half of the mesosomal membrane (iov) reveals the occasional intramembrane particle.

Magnification X 145 200


oiv
iov
imp

EXPLANATION OF PLATE 87

Replica of freeze-fractured unetched mesosomal membrane isolated from M. lysodeikticus St.O and suspended in glycerol. Convex (oiv) and concave (iov) fracture faces of the mesosomal membrane reveal a distribution of intramembrane particles (imp) similar to that observed for isolated peripheral membrane. Note the absence of rod-shaped structures and linear depression on the convex and concave fracture faces respectively.

Magnification X 145 200

EXPLANATION OF PLATE 88

Replica of freeze-fractured unetched mesosomal membrane isolated from M. lysodeikticus St.O and suspended in glycerol. Note the vesicle A which reveals the presence of rod-shaped structures on its convex fracture surface (oim). Vesicle A was the only one of its kind observed in many hundreds of fields. Note also vesicle B which reveals a relatively smooth convex fracture face, devoid of intramembrane particles.

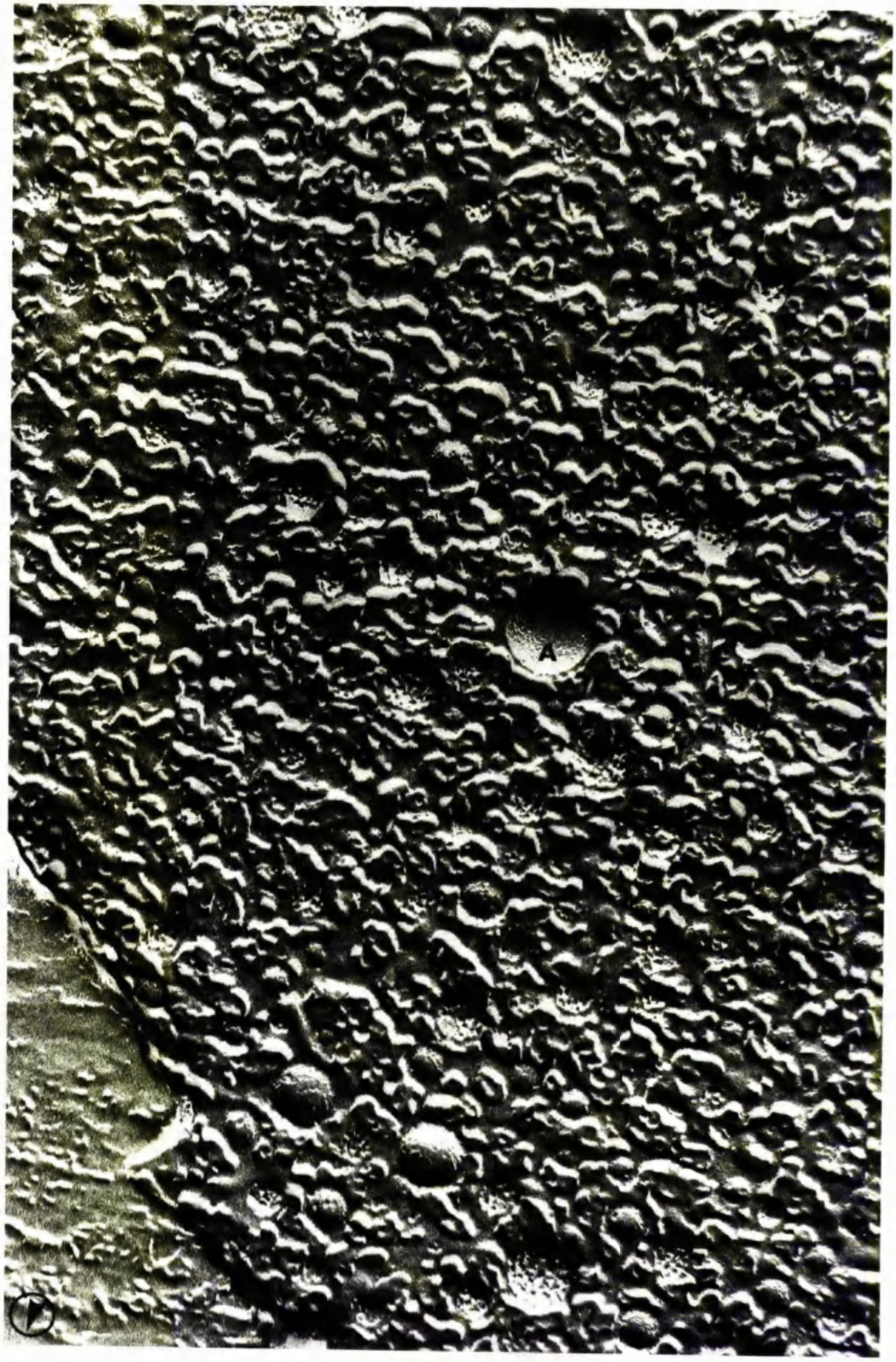
Magnification X 117 300


A
B

EXPLANATION OF PLATE 89

Replica of freeze-fractured unetched mesosomal membrane isolated from M. lysodeikticus St.O and suspended in glycerol. Note vesicle A which has a diameter almost twice that of other vesicles and reveals a smooth surfaced convex fracture face devoid of intramembrane particles.

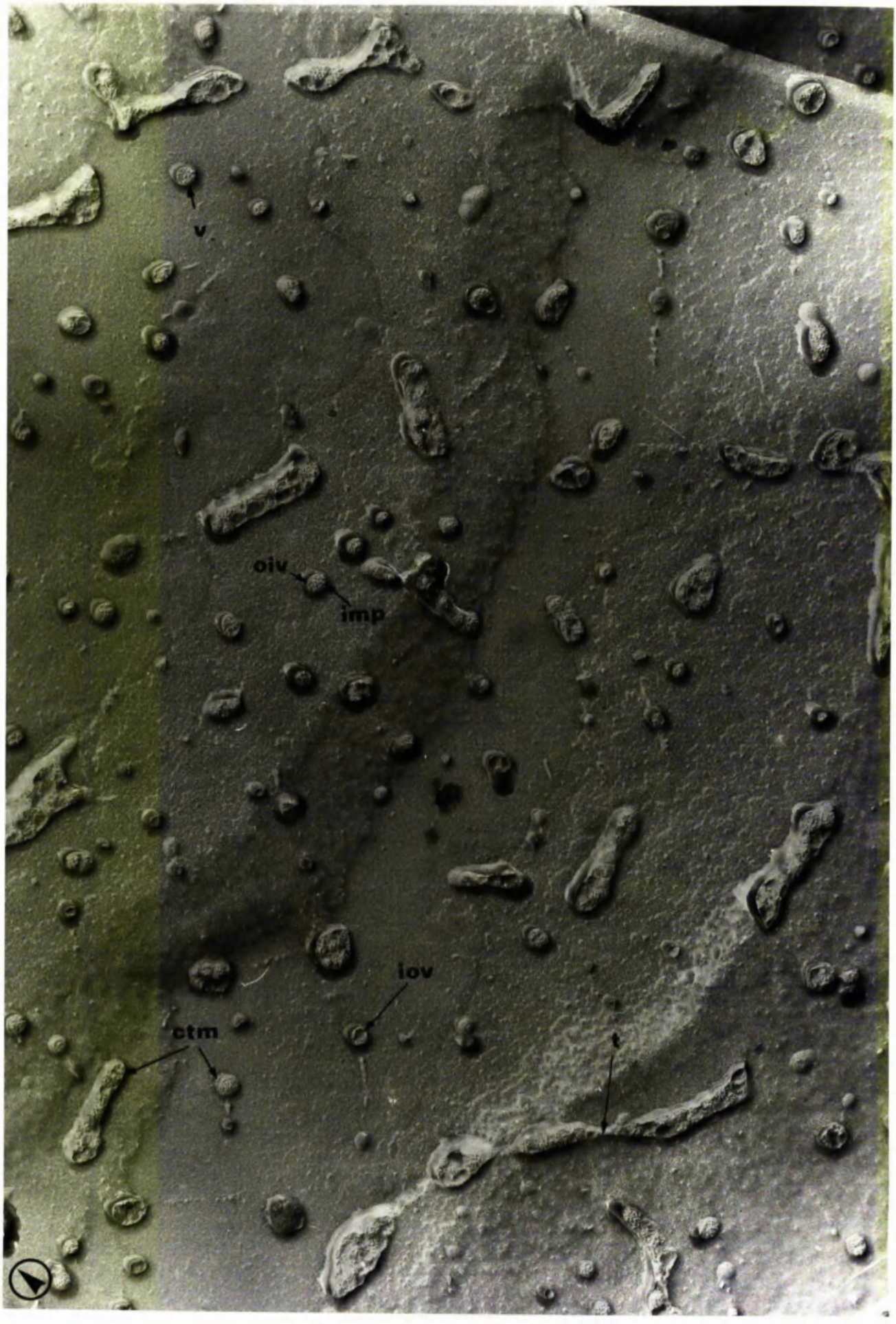
Magnification X 117 300


A

EXPLANATION OF PLATE 90

Replica of freeze-fractured and etched mesosomal membrane isolated from M. lysodeikticus St.O. Note the presence of spherical vesicles (v) and of tubular mesosomal membrane (t). Convex fractures of vesical membrane (oiv) reveal many intramembrane particles (imp). Concave fractures of vesical membrane (iov) reveal few intramembrane particles. Occasionally cross-fractured membrane (ctm) can be visualised around such vesicles. Note the complex nature of fractures across mesosomal tubules.

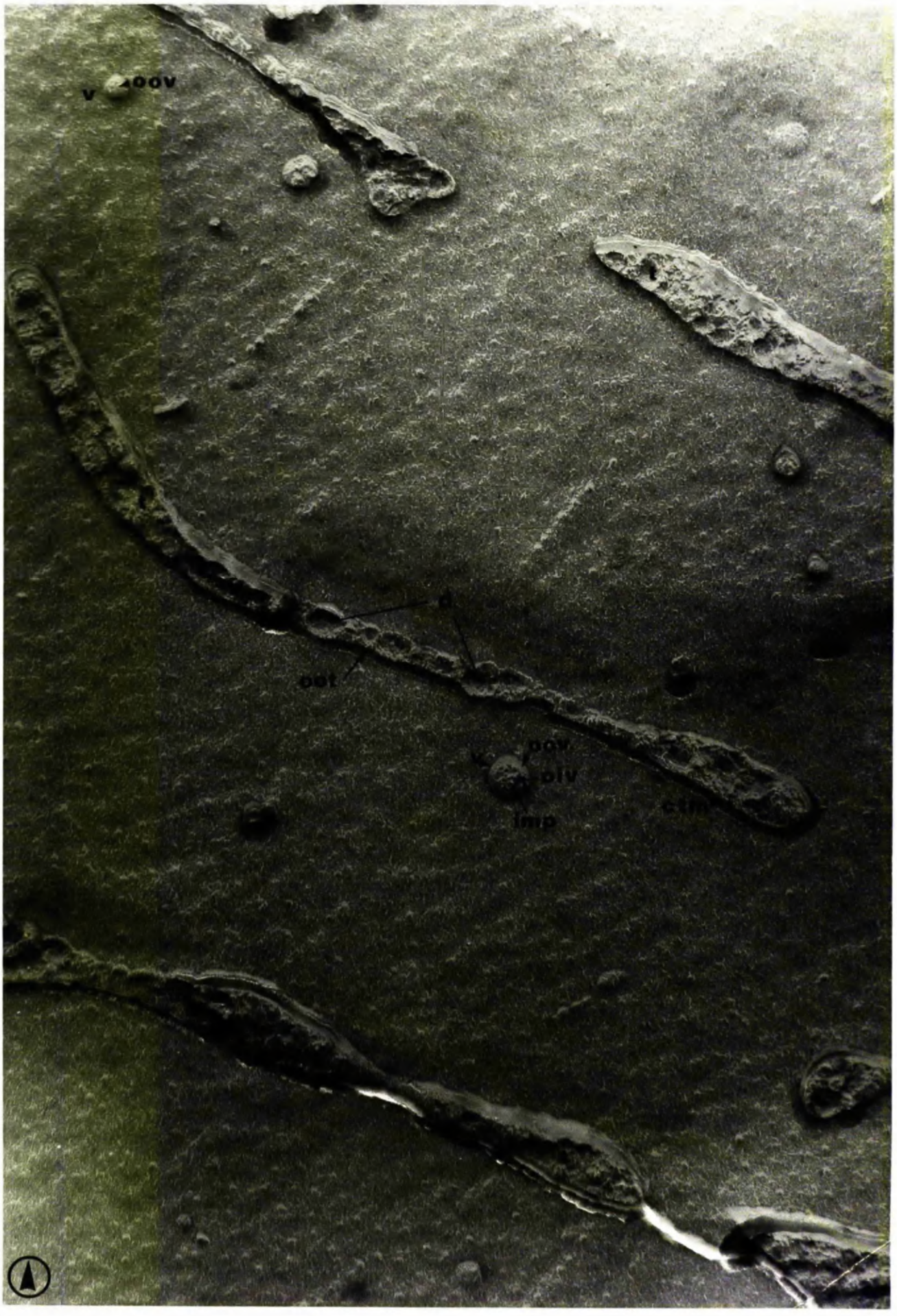
Magnification X 72 600


v
oiv
imp
iov
ctm

EXPLANATION OF PLATE 91

Replica of freeze-fractured and etched mesosomal membrane isolated from M. lysodeikticus St.O. Note the presence of long tubules (t) and also small vesicles (v). The convex etched surface of both tubules (oot) and of vesicles (oov) appears smooth, and the convex fracture through vesical membrane (oiv) reveals many intramembrane particles (imp). Freeze-fractured tubules reveal cross-fractured tubule membrane (ctm) and numerous smooth-surfaced depressions (d), which may represent the inner surface of the outer half of intratubular vesicles.

Magnification X 90 900


v
oov
oot
pov
olv
imp

EXPLANATION OF PLATE 92

Replica of freeze-fractured and etched mesosomal membrane isolated from M. lysodeikticus St.O. Vesicles (v) revealing a convex fracture face (oiv) show numerous intramembrane particles (imp). Larger tubules (t) show cross-fractured tubular membrane (ctm), numerous smooth depressions (d) and particle-studded convex areas (itv).

Magnification X 90 900


v
oiv
imp
itv
t

EXPLANATION OF PLATE 93

Replica of freeze-fractured and etched mesosomal membrane isolated from M. lysodeikticus St.O. The large branched mesosomal tubule reveals a smooth etched surface (oot) and a cross fractured tubular membrane (ctm). Within the tubule are numerous smooth-surfaced concave depressions (d) and also convex particle (imp) studded regions (itv).

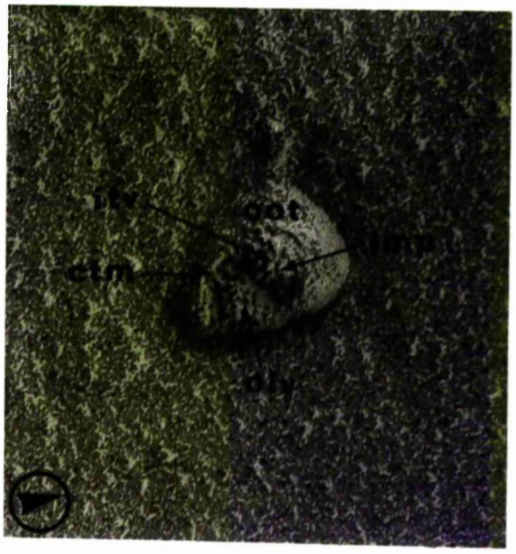
Magnification X 117 300


ltv
ptm
lmp

EXPLANATION OF PLATE 94

Replica of freeze-fractured and etched mesosomal membrane isolated from M. lysodeikticus St.O. This small tubule reveals a smooth etched surface (oot), an apparently cross-fractured outer membrane (ctm) and a particle (imp) studded convex fracture face (oiv) of intratubular vesicle membrane (itv).

Magnification X 117 300


ctn

EXPLANATION OF PLATE 95

Replica of freeze-fractured and etched mesosomal membrane isolated from M. lysodeikticus St.O. The tubule membrane is invariably cross fractured (ctm). The contents of the tubule itself are revealed as consisting of concave smooth-surfaced depressions (d) and also of intratubular vesicular structures (itv) carrying a dense population of intramembrane particles (imp) on their convex fracture face (oiv). These structures are interspersed with areas having a similar topography to that of the ice table (double headed arrow). Note also the intratubular vesicles displaying a smooth-surfaced convex fracture (small arrow).

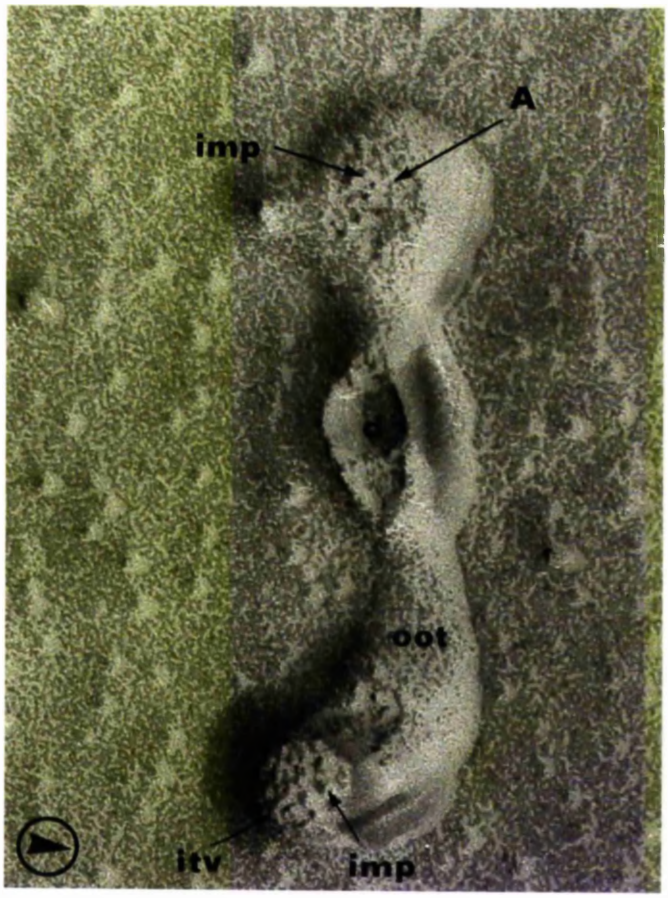
Magnification X 117 300


ctm
itv
imp
d
oiv

EXPLANATION OF PLATE 96

Replica of freeze-fractured and etched mesosomal membrane isolated from M. lysodeikticus St.O. The tubule appears to contain intratubular vesicular structures (itv) carrying a dense population of intramembrane particles (imp) on their convex fracture face. A smooth-surfaced depression (d) is also evident. Note particle-studded fracture face A, which may represent the outer surface of the inner half of the mesosomal tubule membrane.

Magnification X 181 800


A
imp
oot
itv
imp

EXPLANATION OF PLATE 97

Replica of freeze-fractured unetched membrane residue DO 1 suspended in glycerol.

Magnification X 117 300

Table 1. The occurrence of mesosomes in Gram-positive bacteria

Genus	Species	Reference
Actinomyces	A. naesulandii	
	A. israelii	Overman & Pine, 1963
	A. propionicus	
Arthrobacter	A. marinus	Cobet & Jones, 1971
	A. pascens	Stevenson, 1968
Bacillus	B. anthracis	Moberly, Shafa & Gerhardt, 1966
	B. cereus	Ellar & Lundgren, 1966
	B. coagulans	Ohye & Murrell, 1962
	B. fastidiosus	Leadbetter & Holt, 1968
	B. licheniformis	Rogers, 1970
	B. marcerans	Conti, Jacobs & Gray, 1968
	B. megaterium	Ellar, Lundgren & Slepecky, 1967
	B. mycoides	Robinow, 1962
	B. popillae	Mitruka, Costilow, Black & Pepper, 1967
	B. stearothermophilus	Abram, 1965
	B. subtilis	Grandboulan & Leduc, 1967
	B. thuringiensis	Aronson, Bowe & Swafford, 1967
Brevibacterium	B. halotolerans	Sasson & Delaporte, 1969
Clostridium	Cl. bifermentans	Samsonoff, Hashimoto & Conti, 1971
	Cl. botulinum	Takagi, Nakamura & Ueda, 1965
	Cl. pasteurianum	Mackey & Morris, 1970
	Cl. pectinovorum	Hoeniger & Headley, 1968
	Cl. perfringens	Hoeniger, Stuart & Holt, 1968
	Cl. tetani	Takagi, Nakamura & Ueda, 1965

Genus	Species	Reference
Corynibacterium	C.avis	Hard, 1965
	C.diphtheriae	Barksdale, 1970
	C.minutissimum	Montes & Black, 1967
	C.rubrum	Serrano, Tablante de San Blas & Imaeda, 1972
Dermatophilus	D.congolensis	Gordon & Edwards, 1963
Diplococcus	D.pneumoniae	Tomasz, Jamieson & Ottolenghi, 1964
Geodermatophilus	unnamed sp.	Ishiguro & Wolfe, 1970
Halobacterium	H.halobium	Stoeckenius & Rowen, 1967
Lactobacillus	L.acidophilus	Glauert, 1962
	L.bifidus	Kojima, Suda, Hotta, Hamada & Suganoma, 1970
	L.casei	Brown, Edwards & Van Demark, 1968
	L.corynoides	Schotz, Abo-Elnaga & Kandler, 1965
	L.plantarum	Kakefuda, Holden & Utech, 1967
Listeria	L.monocytogenes	Kawata, 1963
Micrococcus	M.albus	Van Iterson, 1962
	M.cryophilus	Mazanec, Kocur & Martinec, 1966
	M.lysodeikticus	Salton & Chapman, 1962
	M.roseus	Murray, 1960
Mycobacterium	unnamed spp.	Freer, Kim, Krauss, Beaman & Barksdale, 1969; Koike & Takeya, 1961
	M.leprae	Imaeda & Ogura, 1963
	M.lepraemurium	Imaeda & Ogura, 1963
	M.konsassii	Schaefer & Lewis, 1965
	M.phlei	Petitprez, Roos & Tacquet, 1967
Nocardia	N.asteroides	Farshtchi & McClung, 1967
Sarcina	S.maxima	Holt & Canale-Parola, 1967
	S.ventriculi	Holt & Canale-Parola, 1967

Genus	Species	Reference
Sporolactobacillus	S.inulinus	Fitz-James, 1968
Sporosarcina	S.ureae	Mazanec, Kocur & Martinec, 1965
Staphylococcus	S.aureus	Popkin, Theodore & Cole, 1971
	S.epidermidis	Conti et al., 1968
Streptococcus	unnamed sp.	Ranhard, Leonard & Cole, 1971
	S.faecalis	Higgins & Shockman, 1970
	S.lactis	Thomas, Lyttleton, Williamson & Batt, 1969
	Lactobacterium pentoaceticum	Kats & Kharat'yan, 1969
Streptomyces	S.cinnamonensis	Chen, 1964
	S.coelicolor	Glauert & Hopwood, 1960
	S.noursei	Stuart, 1959
	S.venezuelae	Bradley & Ritzi, 1968
	S.viridochromogens	Rancourt & Lechevalier, 1964

Table 2. The occurrence of mesosomes in Gram-negative bacteria

Genus	Species	Reference
Acetobacter	A. subozydans	Claus & Roth, 1964
Achromobacter	unnamed sp.	Wiebe & Chapman, 1968
Acinetobacter	unnamed sp.	Thornley & Glauert, 1968
Alcaligenes	A. faecalis	Beer, 1960
Asticcacaulis	A. excentricus	Poindexter & Cohen-Bazire, 1964
Azobacter	A. agilis	Pangborn, Marr & Robrish, 1962
	A. chroococcum	Tchan, Birch-Anderson & Jenson, 1962
	A. vinelandii	Oppenheim & Marcus, 1970
Bacteroides	unnamed sp.	Bladen & Waters, 1963
Bdellovibrio	B. bacteriovorus	Burnham, Hashimoto & Conti, 1968
Bordetella	B. pertussis	Richter & Kress, 1967
Borrelia	B. recurrentis	Ludyic, 1964
Brucella	B. abortus	de Petris, Karlsbad & Kessel, 1964
Caulobacter	C. bacteroides	Poindexter & Cohen-Bazire, 1964
	C. crescentus	
	C. fusiformis	
Erwinia	E. amylovara	Huang & Goodman, 1970
Escherichia	E. coli	Pontefract, Bergeron & Thatcher, 1969
Fusibacterium	F. polymorphum	Takagi, Ueyama & Ueda, 1963
Haemophilus	H. vaginalis	Criswell, Stenback, Black & Gardner, 1972
Leptospira	unnamed sp.	Nauman, Holt & Cox, 1969
	L. pomona	Ritchie & Ellinghausen, 1965

Genus	Species	Reference
Leptoctrichia	L. buccalis	Holstad & Selvig, 1969
Moraxella	unnamed sp.	Ryter & Piechaud, 1963
Neisseria	N. gonorrhoeae	Fitz-James, 1964
Proteus	P. vulgaris	Nermut & Ryc, 1964
Pseudomonas	unnamed sp.	Wiebe & Chapman, 1968
	Ps. aeruginosa	Carrick & Berk, 1971
Rhizobium	unnamed sp.	Dixon, 1964
	Rh. trifolii	Dart & Mercer, 1963
Spirillum	S. serpens	Vanderwinkel & Murray, 1962
Treponema	T. pallidum	Ryter & Pillot, 1963
	T. microdentium	Listgarten, Loesche & Socransky, 1963
Veillonella	unnamed sp.	Bladen & Mergenhagen, 1964
Vibrio	V. marinus	Felter, Kennedy, Colwell & Chapman, 1970
unnamed marine bacterium		D'aoust & Kushner, 1971

Table 3. The occurrence of mesosomes in gliding bacteria

Chondrococcus columnaris	Pate & Ordal, 1967
Chondromyces apiculatus	Abadie, 1968
Cytophaga marinoflava	Valentine & Chapman, 1966
Leucothrix mucor	Snellen & Raj, 1970
Myxobacter FP-1	Shilo, 1970
Myxococcus xanthus	Voelz, 1965
Stigmatella aurantiaca	Reichenbach, Voelz & Dwarkin, 1969

Table 4. Reports of the isolation of "mesosome fractions" from various species of bacteria

Species	Reference
Bacillus licheniformis NCTC 6346	Rogers, Reaveley & Bardett, 1967; Reaveley, 1968; Reaveley & Rogers, 1969; Burdett & Rogers, 1972.
Bacillus licheniformis 749/C	Sargent et al., 1969b; Sargent & Lampen, 1970.
Bacillus megaterium KM	Fitz-James, 1967, 1968; Ellar, 1969; Daniels, 1971; Ellar, Thomas & Postgate, 1971.
Bacillus subtilis NCTC 3610	Ferrandes, Chaix & Ryter, 1966.
Bacillus subtilis 168	Fitz-James, 1967.
Bacillus subtilis Marbarg SMY	Ferrandes et al., 1970.
Lactobacillus casei ATCC 7469	Thorne & Barker, 1969, 1971, 1972.
Lactobacillus plantarum ATCC 8014	Thorne & Barker, 1972.
Listeria monocytogenes strain 42	Ghosh & Murray, 1969.
Micrococcus lysodeikticus NCTC 2665	Ellar & Freer, 1969; Ellar, 1969; Ellar, Thomas & Postgate, 1971.
Staphylococcus aureus ATCC 6538P	Popkin et al., 1971; Theodore, Popkin & Cole, 1972.

Table 5. Wavelengths and coefficients of molar extinction used for calculating content of cytochromes in membrane preparations and residues from M.lysodeikticus

(after Simakova et al., 1969)

Cytochrome	Wavelength (nm)			Molar extinction E
	λ max	λ' min	λ'' min	($mM^{-1} \cdot cm^{-1}$)
a_{601}	600	580	620	16.0
b_{560} } b_{556} }	560	580	540	20.0
c_{550}	550	580	540	19.1

Table 6. Comparison of the reported chemical, biochemical and physical properties of isolated mesosomal and peripheral membrane fractions

It should be noted that data relating to radioactive labelling experiments have not been included in this survey. The author reference is A - Rogers et al., 1967; B - Reaveley, 1968; C - Reaveley & Rogers, 1969; D - Rogers, 1970; E - Sargent et al., 1969a; F - Daniels, 1971; G - Rogers, 1970; H - Ferrandes et al., 1966; I - Fitz-James, 1967; J - Ferrandes et al., 1970; K - Frehel et al., 1970a; L - Patch & Landman, 1970; M - Thorne & Barker, 1969, Barker & Thorne, 1970; N - Thorne & Barker, 1971, 1972; O - Thorne & Barker, 1972; P - Ghosh & Murray, 1969; Q - Popkin et al., 1971; R - Theodore et al., 1971; S - Ellar, 1969; T - Ellar & Freer, 1969; U - Ellar, Thomas & Postgate, 1971; V - Rogers, 1970.

Superscript legend

1. Over 95% of the total succinate dehydrogenase activity reported to be in the peripheral membrane fraction.
2. Two different peripheral membrane fractions analysed.
3. Mesosomal membrane reported to be deficient in succinate dehydrogenase.
4. Four different isoenzymes detected and estimated in polyacrylamide gels containing DOC. Actual values are 2.9, 2.4, 0.89 and 0.51.
5. Estimated as in 4.
6. No activity detected in either fraction.
7. Estimated both spectrophotometrically (0.039) and polarographically (0.034).

8. Mesosomal membrane reported to be deficient in ATP-ase activity.

9. Mesosomal membrane reported to have higher specific activity.

10. Ratio of specific activity of mesosomal membrane to that of whole protoplasts.

11. More lipid reported to be present in mesosomal membrane.

12. Approx. half the phospholipid content in mesosomal membrane.

13. Lipid estimated as phospholipid.

14. Mesosomal membrane claimed to have twice as many "wall precursors" as peripheral membrane.

15. Values expressed as % of total cellular lipid phosphorus.

16. Quantitative differences noted in membranous components of two fractions following elution of membrane bound to cadmium lauroylsarcosinate crystals.

17. Phospholipid ratios were similar in both membrane fractions viz. diphosphatidylglycerol 67%; phosphatidylglycerol 27%; phosphatidyl inositol 6%.

18. Fatty acid composition of both fractions similar, both containing mainly C_{15} branched compounds.

19. In presence of sodium dodecyl sulphate.

20. In presence of sodium deoxycholate.

21. No experimental results presented.

22. Values expressed as % dry wt. of protein as estimated by the method of Lowry et al., 1951.

23. Values probably an underestimate as level of mevalonic acid in growth medium was less than saturated.

24. Amino acid analysis of purified mesosomal vesicles also reported (Thorne & Barker, 1969).

25. Rhamnose only estimated and expressed as a % of protein content, the latter estimated by the method of Lowry et al., 1951.

26. Values computed from reported values of content of other membrane components.

27. P.M. and M.M. are abbreviations for peripheral membrane and mesosomal membrane respectively.

Specific Activity of mesosomal membrane/ specific activity of peripheral membrane

Organism	Author Reference Number	Succinate dehydrogenase	$NADH_2$ dehydrogenase
B. licheniformis NCTC 6346	A	$\leqslant 5\%$[1].	-
	B	-	-
	C	0.066[2].	-
	D	0.056	-
B. licheniformis 749/C	E	-	0.10
B. megaterium KM	F	-	-
	G	0.060	0.67
B. subtilis NCTC 3610	H	-	-
B. subtilis 168	I	-	-
B. subtilis Marburg SMY	J	0.027	-
	K	-	-
B. subtilis SB 108	L	0.47	2.9 - 0.51[4].
L. casei ATCC 7469	M	-	-
	N	-	-
L. plantarum ATCC 8014	O	-	-
L. monocytogenes St. 42	P	1.80	2.81
S. aureus 6538 P	Q	-	-
	R	-	-
	S	zero[21.3].	similar[21].
	T	-	-
M. lysodeikticus NCTC 2665	U	zero[21.3]	-
	V	0.031	0.48

Specific Activity of mesosomal membrane/specific activity of peripheral membrane

ıor rence ber	Malate dehydrogenase	Lactate dehydrogenase	Glutamate dehydrogenase	Glucose-G-dehydrogenase	$NADH_2$-cyto-chrome-c-reductose
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	0.58 0.34 2.
	-	-	-	-	0.30
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
	3.3 5.	-	0.36	-	-
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
	-	2.77	-	None 6.	-
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
	-	-	-	-	-
	0.44	-	-	-	-

Specific Activity of mesosomal membrane/specific activity of peripheral membrane

Author Reference Number	$NADH_2$ oxidase	Ferricyanide reductase	ATP-ase	Acid phosphatase	Alkaline phosphatase
A	-	-	-	-	-
B	-	-	-	-	-
	0.23[2.]	0.82		0.64[2.]	
C	0.27	1.29[2.]	-	0.60	-
D	0.28	-	-	-	-
E	-	-	-	-	-
F	-	-	-	-	-
G	-	-	-	-	-
H	-	-	-	-	-
I	-	-	-	-	-
	0.039[7.]				
J	0.034	-	-	-	-
K	-	-	-	-	-
L	-	-	1.54[5.]	-	-
M	-	-	-	-	-
N	-	-	0.037	-	-
O	-	-	0.043	-	-
P	-	-	-	-	-
Q	-	-	-	-	-
R	-	-	-	-	-
S	-	-	-	-	-
T	-	-	-	-	-
U	-	-	zero[21.8.]	>1[9.]	>1[9.]
V	-	-	-	-	-

Specific Activity of mesosomal membrane/specific activity of peripheral membrane		Chemical properties					
		Protein (% dry wt. membrane)		Lipid (% dry wt. membrane)		Protein Lipid ratio	
ice α-Glucosidase	Penicillinase	P.M.[27.]	M.M.[27.]	P.M.	M.M.	P.M.	M.M.
-	-	-	-	-	-	-	-
-	-	43-49	44	18-25	17	2.14	2.59
-	-	46,45[2.]	44	22,31[2.]	17	1.85 / 1.40[2.]	2.58
-	-	-	-	-	-	-	-
0.049[10.]	5.56	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	1.82[13.]	0.75[13.]
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	69	66	-	-	-	-
-	-	60[26.]	55[26.24.]	48[22.]	60[22.]	2.1	1.7
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	56	58	-	-	2.9[13.]	2.8[13.]
-	-	-	-	-	-	-	-
-	-	56	41	25	34	2.24	1.21
-	-	-	-	~40	~40	different[21.]	
-	-	-	-	< MM[21.11]	> PM[21.11.]	-	-
-	-	similar		PM[12.]	½ PM[12.]	different[21.]	
-	-	-	-	-	-	-	-

Chemical Properties

thor erence nber	RNA (% dry wt. membrane) P.M.	RNA (% dry wt. membrane) M.M.	DNA (% dry wt. membrane) P.M.	DNA (% dry wt. membrane) M.M.	Carbohydrates (% dry wt. membrane) P.M.	Carbohydrates (% dry wt. membrane) M.M.	Bactoprenol (% dry wt. total protein) P.M.	Bactoprenol (% dry wt. total protein) M.M.
A	-	-	-	-	-	-	-	-
3	13-15	2-10	-	-	-	-	-	-
]	11,12[2].	6.7	-	-	-	-	-	-
)	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-
F	-	-	-	-	-	-	-	-
;	-	-	-	-	-	-	-	-
[	-	-	-	-	-	-	-	-
:	-	-	-	-	-	-	-	-
.	-	-	-	-	-	-	-	-
:	-	-	-	-	-	-	-	-
,	-	-	-	-	-	-	-	-
:	27.9[22].	3.8[22].	3.9[22].	0[22].	1.1[25].	0[25].	0.05[23].	0.1[23].
	-	-	-	-	-	-	0.26	0.25
	-	-	-	-	-	-	0.11	0.08
	1.6	2.1	0.86	1.7	-	-	-	-
	-	-	-	-	-	-	-	-
	14	8	-	-	4	4	-	-
	-	-	-	-	-	-	[14].	[14].
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-

Chemical Properties

Total phosphorus (% dry wt. membrane)		Lipid soluble phosphorus (% dry wt. membrane)		Lipid Carbohydrate (% dry wt. membrane		Acid soluble phosphorus (% total membrane P)	
P.M.	M.M.	P.M.	M.M.	P.M.	M.M.	P.M.	M.M.
-	-	95.7[15.]	6.3[15]	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
1.2	1.3	0.55	0.77	-	-	-	-
5.7[22.]	0.8[22.]	0.35[22.]	0.02[22.]	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	6.9	17.6
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	[21.] different [21.]		-	-
-	-	-	-	-	-	-	-

Chemical Properties

Author reference number	Chloroform soluble phosphorus (% membrane P)		Methanol soluble phosphorus (% membrane P)		Nucleic acid phosphorus (% membrane P)		Protein bound phosphorus (% membrane P)	
	P.M.	M.M.	P.M.	M.M.	P.M.	M.M.	P.M.	M.M.
A	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-
D	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-
F	-	-	-	-	-	-	-	-
G	-	-	-	-	-	-	-	-
H	-	-	-	-	-	-	-	-
I	-	-	-	-	-	-	-	-
J	-	-	-	-	-	-	-	-
K	-	-	-	-	-	-	-	-
L	-	-	-	-	-	-	-	-
M	-	-	-	-	-	-	-	-
N	-	-	-	-	-	-	-	-
O	-	-	-	-	-	-	-	-
P	25.6	17.1	2.5	1.9	11.6	26.0	30-41	25-35
Q	-	-	-	-	-	-	-	-
R	-	-	-	-	-	-	-	-
S	-	-	-	-	-	-	-	-
T	-	-	-	-	-	-	-	-
U	-	-	-	-	-	-	-	-
V	-	-	-	-	-	-	-	-

Chemical Properties

chor erence iber	Yield (% total membrane)		Carotenoid content		Menaquinone content		Individual phospholipid ratios	
	P.M.	M.M.	P.M.	M.M.	P.M.	M.M.	P.M.	M.M.
A	-	-	-	-	-	-	-	-
B	-	10.8	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-
D	-	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-
F	16.	16.	-	-	-	-	-	-
G	-	-	-	-	-	-	-	-
H	-	-	-	-	-	-	-	-
I	-	-	-	-	-	-	-	-
J	-	-	-	-	-	-	-	-
K	-	-	-	-	-	-	-	-
L	82	14	-	-	-	-	-	-
M	-	-	-	-	-	-	-	-
N	-	-	-	-	-	-	-	-
O	-	-	-	-	-	-	-	-
P	-	-	-	-	-	-	-	-
Q	-	-	-	-	-	-	-	-
R	-	-	-	-	-	-	-	-
S	-	-	-	-	-	-	-	-
T	-	-	-	-	-	-	-	-
U	-	-	21. different	21.	21. different	21.	21.17. similar	21.17.
V	-	-	-	-	-	-	-	-

thor erence nber	Chemical Properties: Fatty acid composition P.M.	Chemical Properties: Fatty acid composition M.M.	Physical Properties: Density (g/cc^3) P.M.	Physical Properties: Density (g/cc^3) M.M.
A	-	-	-	-
B	21. identical	21.	-	-
C	-	-	1.21 2. 1.25	1.25
D	-	-	-	-
E	-	-	-	-
F	-	-	-	-
G	-	-	-	-
H	-	-	-	-
I	-	-	-	-
J	-	-	1.15	1.10
K	-	-	-	-
L	-	-	1.17	1.18
M	-	-	-	-
N	-	-	-	-
O	-	-	-	-
P	-	-	-	-
Q	-	-	-	-
R	-	-	-	-
S	-	-	> 1.127	1.127
T	-	-	-	-
U	21.18. similar	21.18.	-	-
V	-	-	-	-

Physical Properties

Author Reference Number	Disc gel electrophoresis pattern
A	-
B	Quantitative increase in intensity of 3 bands[19.] in mesosome preparation
C	-
D	-
E	-
F	-
G	- -
H	-
I	-
J	-
K	-
L	Quantitative increase in some bands in mesosome[20.] preparation stated to be not significant
M	-
N	-
O	-
P	-
Q	-
R	-
S	-
T	Differences noted[21.19.]
U	-
V	-

Author Reference Number	Cytochrome characteristics
A	-
B	-
C	Mesosome preparation had additional peak at 500 nm. *a* type cytochrome to higher λ . But basically similar.
D	-
E	-
F	-
G	-
H	Cytochromes ($a + a_3$), b, c, y, z all in mesosome preparation, none in peripheral membrane.
I	-
J	Peripheral membrane richer in cyt. ($a + a_3$)[21].
K	Mesosomes reported to have unique cytochrome[21].
L.	Mesosome fraction had no λ_{max} at 598 nm. Also peripheral membrane richer in *c* : Basically similar.
M	-
N	-
O	-
P	-
Q	-
R	-
S	-
T	Mesosomes deficient in *a*, *b*, and *c* type cytochromes[21].
U	-
V	-

Table 7. Some properties of total membrane fractions from M. lysodeikticus St.A

"Standard" membranes were prepared by the method of Owen & Freer (1970b), dialysed against distilled water and lyophilized. Values for protein, total hexose, glucose and total phosphorus are expressed as % of dry wt. of membrane. Protein was determined by both the method of a) Gornall et al., (1949) and of b) Lowry et al., (1951).

Membrane preparation	Age of culture (h)	Yield of membrane (% dry wt. cell)	Protein		a/b Ratio	Total hexose	Glucose	Total Phosphorus
			a	b				
9	10	22.1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
10	12	22.6	56.4	20.3	2.78	5.9	0.29	0.71
11	12	20.9	55.5	20.4	2.72	8.2	N.D.	0.80
12	17	22.4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
13	17	23.8	47.6	18.8	2.53	9.3	N.D.	0.74
14	18	22.4	N.D.	N.D.	N.D.	N.D.	0.22	N.D.
15	18	N.D.	52.3	24.4	2.14	7.1	N.D.	0.96
16	18	19.4	57.1	21.0	2.72	6.9	0.30	0.77
17	36	29.7	53.7	23.8	2.26	6.8	N.D.	0.74

Table 8. Some properties of total membrane fractions from *M. lysodeikticus* St.0.

"Standard" membranes were prepared by the method of Owen & Freer (1970b), dilaysed against distilled water and then lyophilized. Values for protein, total hexose, glucose and total phosphorus are expressed as % of dry wt. of membrane. Protein was determined by both the method of a) Gornall *et al.*, (1949) and b) Lowry *et al.*, (1951).

Membrane preparation	Age of culture (h)	Yield of membrane (% dry wt. cell)	Protein a	Protein b	a/b Ratio	Total Hexose	Glucose	Total Phosphorus
1	12	N.D.	49.8	37.9	1.31	3.7	N.D.	1.32
2	18	N.D.	43.9	33.1	1.33	6.9	N.D.	1.28
3	18	14.1	47.3	35.1	1.35	5.9	0.09	1.28
4	18	10.1	50.1	39.9	1.26	4.1	N.D.	1.42
5	21	13.5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
6	21	16.6	50.4	39.2	1.29	6.7	N.D.	1.10
7	21	9.6	49.1	37.5	1.31	5.0	N.D.	1.26
8	24	16.5	46.6	35.4	1.32	4.3	N.D.	1.23

Table 9. Reported chemical composition of membrane (total) fractions isolated from M. lysodeikticus

Values, except where indicated, are expressed as a percentage of dry wt. of the membrane. Protein was determined either by method of a) Gornall et al., 1949 and b) Lowry et al., 1959.

Superscript legend

1. Protein estimation based on amino acid analysis.

2. Method of determination not documented.

3. Expressed as the percentage of the total cellular protein as estimated by the method of Lowry et al., 1951.

4. Value includes DNA content.

References	Age of culture used (h)	Membrane Yield (% dry wt. of cell)	Protein a	Protein b	Total Lipid	Total carbohydrate	Hexosamines	RNA	Vitamin K_2 (μmoles/g dry wt. membrane)	Carotenoid	(μmoles/g dry wt. membrane) cytochrome a_{601}	cytochrome b_{560}	cytochrome c_{550}	Total Phosphorus	Total Nitrogen
Gilby et al., 1958	20	8.6	50[1]		28.0	18.9	2.7	-	-	0.25	-	-	-	1.16	8.36
Gilby & Few, 1958	-	-	-	-	-	-	-	-	-	0.14	-	-	-	-	-
Gel'man et al., 1960	-	10	64[2]		-	12.6	-	-	-	-	-	-	-	-	10.25
MacFarlane, 1961a	-	-	-	-	41.1	-	-	-	-	-	-	-	-	-	-
Bishop & King, 1962	48	9.8	-	-	-	-	-	-	13.0	-	-	-	-	-	-
Cho et al., 1964	24	-	-	-	25.4	-	-	-	-	-	-	-	-	-	-
MacFarlane, 1964	-	-	-	-	25.5	-	-	-	-	-	-	-	-	-	-
Salton & Freer, 1965	24	25.2	63	-	26.1	-	-	-	-	-	-	-	-	0.8	-
Salton & Freer, 1965	48	23.1	63	-	25.5	-	-	-	-	-	-	-	-	0.7	-
Butler et al., 1967	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-
Montague & Moulds, 1967	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5-0.6	-
Salton, 1967d; Nachbar & Salton, 1970b	-	-	64.5	49	23.8	4.2	-	1-2	-	-	-	-	-	-	-
Salton & Schmitt, 1967a	24	-	-	-	-	-	-	-	12.1-7.8	~0.1	-	-	-	-	-
Lukoyanova & Taptykova, 1968	24	-	-	-	-	-	-	-	-	-	0.18	0.56	0.60	-	-
Ellar, Munoz & Salton, 1971	-	8.8[3]	-	-	-	-	-	2.5[4]	-	-	-	-	-	-	-

Table 10. Lipid and carbohydrate content of isolated total membrane fractions from M. lysodeikticus

Values, except where noted, refer to percentage dry wt. of membrane. Values in parenthesis refer to percentage of the total lipid content of the membrane (% dry wt.). Values which are singly underlined are expressed as a percentage of the total phospholipid phosphorus content of the membrane, and those doubly underlined, as a percentage of the total membrane phosphorus.

Superscript legend

1. Trace amounts only detected

2. Value is the sum of phosphatidyl glycerol and glycolipid.

3. Detected as a glyco_lipo_protein complex bound to butanol insoluble membrane residues.

T10b

Reference	Sugars detected	Amino sugars detected	Phosphorus (% dry wt. lipid)	Total phospholipid	Phospholipids: Phosphatidic acid	Diphosphatidyl Glycerol	Phosphatidyl Glycerol	Phosphatidyl Inositol	Other Lipids	Diglycerides	Glycolipids	Mannosyl diglyceride	Di Mannosyl diglyceride	Mannan	Lipo amino acids
Gilby et al., 1958	Mannose Galactose[1.]	-	3.0	22.4 (80)	+	+	+	-	5.6 (20)	+	-	-	-	suggested	-
MacFarlane, 1961a	Mannose	-	-	27.7 (47)		68	24[a.]	5	8.9 (22)	+	24[2.]	+	-	-	4.6 (11)
MacFarlane, 1961b	-	-	-	-	-	4.0	72.0	12.9	+	-	+	+	-	-	-
MacFarlane, 1964	Mannose	-	-	24	-	16 (63)		2 (8)	7.5 (29)	-	6 (24)	+	-	(9)	-
Lennarz, 1964; Lennarz & Talamo, 1964, 1965	Mannose	-	-	-	-	-	-	-	+	-	+	-	+	-	-
Salton & Freer, 1965	Mannose, Glucose Galactose, Ribose	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Butler et al., 1967	Mannose	Glucosamine Muramic acid[1.]	-	-	-	(~80)	+	-	-	-	-	-	-	-	-
Nachbar & Salton, 1970a	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-
Ellar, Thomas & Postgate, 1971	-	-	-	-	-	(67)	(27)	(6)	-	-	-	-	-	-	-
de Siervo & Salton, 1971	-	-	-	(82)	-	(36)	(34)	(12)	(18)	(12)	(6)	-	-	-	-
Pollock et al., 1971	-	-	-	-	-	60	30	8	-	-	-	-	-	-	-
Whiteside et al., 1971	-	-	-	(82)	-	56	33	9	+	+	+	-	-	-	-
Estrugo et al., 1972	-	-	-	-	-	-	-	-	-	-	+[3.]	-	-	-	+[3.]

Table 11. Some properties of detergent insoluble residues of membranes from M. lysodeikticus St.A.

Results relating to the sodium deoxycholate (DOC) insoluble fraction refer to residue DA 1 (see Methods). All results are expressed as % of dry wt. of the residue. Protein was determined by both the method of a) Gornall et al., (1949) and that of b) Lowry et al., (1951).

			Detergent Insoluble Residue						
Membrane preparation	Detergent used	Yield of residue	Protein a	Protein b	a/b ratio	Total Hexose	Glucose	Total Phosphorus	Residual SDS
10	SDS	52.1	N.D.	N.D.	N.D.	N.D.	N.D.	0.08	N.D.
11	SDS	51.7	50.8	11.4	4.46	9.6	0.26	0.13	4.8
16	SDS	39.6	52.6	11.6	4.53	8.7	N.D.	0.10	N.D.
17	SDS	50.5	50.3	12.1	4.16	10.4	0.46	0.15	3.6
18	SDS	31.8	47.1	11.6	4.06	7.8	N.D.	0.18	5.9
19	SDS	47.2	50.0	10.7	4.70	8.0	0.36	0.14	2.8
20	SDS	-	47.7	12.6	3.79	7.9	N.D.	0.15	6.2
21	SDS	-	45.5	N.D.	N.D.	9.4	N.D.	N.D.	N.D.
9	DOC	49.0	N.D.	N.D.	N.D.	N.D.	N.D.	0.18	-
13	DOC	62.5	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	-
16	DOC	42.4	51.3	12.7	4.04	8.9	N.D.	0.23	-
Mean value	SDS	45.5	49.1	11.7	4.28	8.8	0.36	0.13	4.7

Table 12. Assignments of absorption peaks in infra-red spectra of membrane fractions, membrane residues and extracts of membranes from M. lysodeikticus

Wavenumber (cm^{-1})	Assignment
~ 3300	O-H stretch, N-H stretching
3070	C-H stretching
2955	CH_3-asymmetric stretching
2930	CH_2-asymmetric stretching
2870	CH_3-symmetric stretching
2850	CH_2-symmetric stretching
1735	ester C = O stretching
~ 1655	Amide I
~ 1540	Amide II
1465 and 1430	CH_2 and CH_3 deformations
1380	CH_3-symmetrical deformation
1365	CO_2^- symmetrical stretching
~ 1230	P = O stretching
1195	C - O - C asymmetrical stretching
1170	C - O - C symmetrical stretching
1060	P - O - C stretching
720	CH_2 rocking

Table 13. Amino acid analysis of membranes and membrane residues from M. lysodeikticus strains O and A

"Standard" membranes were prepared (Owen & Freer, 1970b) from cells after 18 h growth and deoxycholate-insoluble (DA 1) and SDS-insoluble residues obtained as described in Methods. The amino acid composition of membrane samples and membrane residues were determined on acid hydrolysates of lyophilized preparations in a Locarte Model 4 automatic amino acid analyser. Figures in brackets represent the molar ratio of individual amino acids relative to lysine.

Legend

a) Quantitative determination of methionine not possible in this system due to coelution with glucosamine.

b) Methionine not detected by paper chromatography.

c) Presence of methionine detected by paper chromatography.

µmoles/mg dry wt. membrane or membrane residue

	Standard Membranes St. 0	Standard Membranes St. A	Membrane residues from St. A: DOC-insoluble residue DA1		Membrane residues from St. A: SDS-insoluble residue	
Asx	0.307	0.118	0.033	(0.04)	0.004	(0.01)
Thr	0.220	0.088	0.025	(0.03)	0.002	(0.00)
Ser	0.189	0.077	0.104	(0.11)	0.056	(0.07)
Glx	0.328	0.573	0.967	(1.05)	0.827	(1.08)
Pro	0.191	0.072	0.000	(0.00)	0.000	(0.00)
Gly	0.375	0.584	0.976	(1.06)	0.802	(1.05)
Ala	0.481	1.000	1.847	(2.01)	1.545	(2.02)
Cys_1	0.004	0.000	0.000	(0.00)	0.000	(0.00)
Val	0.305	0.121	0.016	(0.02)	0.000	(0.00)
Met	0.112	a, c	a, b	-	a, b	-
Ile	0.145	0.060	0.010	(0.01)	0.001	(0.00)
Leu	0.342	0.145	0.025	(0.03)	0.002	(0.00)
Tyr	0.071	0.026	0.007	(0.01)	0.000	(0.00)
Phe	0.135	0.054	0.008	(0.01)	0.000	(0.00)
His	0.074	0.028	0.004	(0.00)	0.000	(0.00)
Lys	0.121	0.428	0.920	(1.00)	0.766	(1.00)
Arg	0.265	0.098	0.007	(0.01)	0.000	(0.00)
Muramic acid	0.000	0.067	0.108	(0.12)	0.094	(0.12)
Glucosamine	0.000	0.159	0.316	(0.34)	0.247	(0.32)
Recovery (%)	46.0	60.3	64.5	-	52.6	-

Table 14. Comparison of amino acid molar ratios in membrane preparations from M.lysodeikticus

"Standard" membranes were prepared from cells after 18 h growth (Owen & Freer, 1970b) and amino acid analysis performed as indicated in the legend of Table 13. Molar ratios (normalised with respect to lysine) are compared with amino acid molar ratios reported by Grula et al., (1967) and by Gilby et al., (1958).

Superscript legend

1. Estimated by paper chromatography.
2. Value is the sum of serine plus glycine.
3. Value is the sum of valine plus methionine.
4. Value is the sum of leucine plus isoleucine plus phenylalanine.
5. Results from an automatic amino acid analysis of reaggregated SDS-dispersed membranes.
6. Quantitative determination of methionine not possible in this system due to its co-elution with glucosamine.
7. Small amounts of methionine detected by paper chromatography. This value is therefore an overestimate.

	Standard membranes St.0	Grula et al., 1967[5].	Gilby et al., 1958[1].	Standard membranes St.A.	Molar ratio St.A / Molar ratio St.0
Asx	2.54	1.04	0.65	0.28	0.11
Thr	1.82	0.98	0.46	0.21	0.12
Ser	1.56	0.86	1.86[2].	0.18	0.12
Glx	2.71	1.02	1.49	1.34	0.50
Pro	1.58	0.52	0.43	0.17	0.11
Gly	3.10	1.63	1.86[2].	1.36	0.44
Ala	3.96	1.96	2.70	0.70	0.18
Cys_1	0.03	0.00	0.00	0.00	0.00
Val	2.53	1.11	0.81[3].	0.28	0.11
Met	0.93	0.20	0.81[3].	-[6].	-[6].
Ile	1.20	0.64	1.62[4].	0.14	0.12
Leu	2.83	1.47	1.62[4].	0.34	0.12
Tyr	0.59	0.20	0.14	0.06	0.10
Phe	1.12	0.58	1.62[4].	0.13	0.12
His	0.61	0.32	-	0.07	0.11
Lys	1.00	1.00	1.00	1.00	1.00
Arg	2.19	0.90	0.56	0.23	0.11
Muramic acid	0.00	trace	-	0.16	∞
Glucos-amine	0.00	-	-	0.37[7].	∞

Table 15. Reported chemical composition of isolated cell walls from M. lysodeikticus

Values are expressed as a percentage of the dry wt. of the wall except where indicated.

Superscript legend

1. Figures referring to amino acid analysis derived from results of paper chromatography not automatic amino acid analysis.

2. Figures are the average of two sets of results.

Author Reference	Hexose sugar	Glucose	Phosphorus	Nitrogen	Amino sugar	Protein	Molar ratio					
							Glucosamine	Muramic Acid	Alanine	Glutamate	Glycine	Lysine
Brumfitt, 1959[1.]	-	3.2	0.17	-	20.9	63	0.48	0.50	2.9	1.2	2.6	1.0
Perkins & Rogers, 1959[1.2.]	-	4.1	-	-	34	55	0.72	0.93	2.3	1.0	1.4	1.0
Ghuysen & Salton, 1960[1.]	-	-	-	-	38	48	0.75	0.89	2.5	0.83	0.76	1.0
Czerkawski, Perkins & Rogers, 1963	-	8.1	-	-	35.7	54.2	0.95	0.75	1.85	1.0	1.07	1.0
Salton, 1964b[1.]	7.5	3.5 - 5.8	0.09	8.7	16-22	-	-	-	2.6	1.0	1.0	1.0
Prasad & Litwack, 1965	-	-	0.16	-	20.4	44	0.8	0.9	2.7	1.1	1.6	1.0
Sharon et al., 1966	-	-	-	-	-	36	-	-	2.69	1.00	1.26	1.00
Katz & Strominger, 1967	-	-	-	-	-	23	1.4	1.1	2.20	1.18	1.29	1.00
Montague & Moulds, 1967	-	-	0.11	-	-	-	-	-	-	-	-	-
Schleifer & Kandler, 1967	-	-	-	-	34.8	36	1.23	1.04	2.00	1.08	1.13	1.00
Campbell et al., 1969	-	4.7	0.08	-	24.6	-	-	-	1.91	0.93	0.98	1.00

Table 16. Some properties of total membrane fractions from M. lysodeikticus St.A prepared under different conditions

Cells were harvested after 12 h and after 36 h growth, washed and resuspended to $\frac{1}{2}$ or 1/20 the original culture volume in tris buffer. Lysozyme was added to final concn. of 100 µg/ml and the suspensions incubated for either 45 min or 135 min at 30°C, Total membranes from these suspensions were isolated and washed as described by Owen & Freer (1970) and the preparation of SDS-insoluble membrane residues performed as in Methods. Determination of protein, total hexose, glucose, total phosphorus were made on lyophilised membrane preparations and expressed as % dry wt. membrane. Protein was determined by both the method of a) Gornall et al., (1949) and b) Lowry et al., (1951). The gross chemical analysis of SDS-insoluble membrane residues were also determined and the results found to compare favourably with those shown in Table 11. Note the increase in i) Lowry protein ii) total phosphorus and the decrease in i) membrane yield and yield of SDS-residue ii) a/b ratio iii) total hexose on both prolonged incubation with lysozyme and incubation at a relatively dilute cell suspension.

Age of culture (h)	Concn. factor	Cell concn. mg/ml	Digestion time (min)	Membrane yield (% dry wt. cell)	Yield SDS residue (% dry wt. cell)	Total membrane				
						Protein a	Protein b	a/b Ratio	Total hexose	Total phosphorus
12	20	51.9	45	20.9	10.8	55.5	20.4	2.72	8.2	0.80
12	20	51.9	135	15.7	5.0	45.7	20.0	2.29	6.3	1.16
12	2	5.19	45	10.8	0.0	45.1	30.2	1.49	6.2	1.55
12	2	5.19	135	7.9	0.0	44.5	37.8	1.18	4.9	1.60
36	20	51.4	45	29.7	15.0	53.7	23.8	2.26	6.8	0.74
36	20	51.4	135	26.7	12.6	48.7	23.9	2.04	7.6	0.80
36	2	5.14	45	15.6	4.0	51.2	26.4	1.94	6.2	0.95
36	2	5.14	135	11.6	1.7	58.0	41.2	1.41	4.1	1.40

Table 17. Effect of Mg^{2+} concentration on protoplast stability

Protoplasts of M. lysodeikticus were prepared in 0.8 M sucrose/tris buffer containing various Mg^{2+} concentrations. Suspensions were swirled for 2 h at 85 rev./min at 30°C and then sedimented. The E_{260} values of the protoplast supernatant fractions were subsequently determined. Note the increase in % E_{260} released from protoplasts prepared at Mg^{2+} concentrations above 2 mM.

Concn. of $MgCl_2$ (mM)	0	1	2	5	10	20	40
Leakage (% E_{260} released)	19.0	6.1	5.6	5.9	5.8	5.7	5.7

Table 18. Effect of plasmolysis on stability and membrane release from protoplasts

Protoplast suspensions were prepared from cells plasmolysed in 2.0 M sucrose/tris buffer and from control cells held in 0.8 M sucrose/tris buffer. Suspensions were held at 30°C for 1 h after cell wall digestion. E_{260} and E_{446} values were then determined on the protoplast supernatant fractions. A similar experiment was performed omitting cell wall digestion. Mg^{2+} concentration throughout the experiments was 10 mM. Note that plasmolysis of cells prior to protoplast formation effects a doubling of the membrane released.

	Protoplasts		Whole Cells	
	Plasmolysed	No Plasmolysis	Plasmolysed	No Plasmolysis
Membrane release (% E_{446} released)	8.0	4.1	0.0	0.0
Leakage (% E_{260} released)	4.2	2.7	1.2	1.5

Table 19. The effect of temperature and ionic shock on membrane release and protoplast integrity

Cells were plasmolysed in tris buffered 2.0 M sucrose containing 40 mM Mg^{2+} and protoplasts were prepared at the temperatures stated. In experiments involving no ionic shock (Expt. a), protoplasts were diluted with 1.5 vol. of tris buffer containing 40 mM Mg^{2+} at the desired temperature. Ionic shock (Expt. b) was achieved by dilution with 1.5 vol. of tris buffer at the appropriate temperature containing Mg^{2+} at a concentration of 6.67 mM (final Mg^{2+} concentration of 20 mM). After dilution, cell protoplast suspensions were held for 2 h at their respective temperatures. E_{260} and E_{446} values were determined for each protoplast supernatant fraction.

	Expt. type	Temp.(°C).....	25	30	35	40
Membrane release	a		5.2	5.5	5.2	4.7
(% E_{446} released)	b		5.5	6.3	6.6	6.4
Leakage	a		3.1	3.5	4.1	4.4
(% E_{260} released)	b		3.2	4.1	4.6	5.1

Table 20. Effect of swirling and ionic shock of protoplast suspensions on mesosomal membrane release.

Cells were plasmolysed in tris buffered 2.0 M sucrose containing 40 mM Mg^{2+}, and protoplasts prepared by incubation with lysozyme at 37°C. In experiments involving no ionic shock (Expt. a), protoplasts were diluted with 1.5 vol. of tris buffer containing 40 mM Mg^{2+}. In experiments involving ionic shock (Expt. b), suspensions were diluted with 1.5 vol. of tris buffer, 6.67 mM with respect to Mg^{2+}. Suspensions were held at 37°C for 2 h or swirled as described in "Methods". E_{260} and E_{446} values were determined for each protoplast supernatant fraction.

	Expt. Type	Protoplast swirled 2 h	Protoplasts held for 2 h
Membrane release	a	6.2	6.4
(% E_{446} released)	b	7.6	7.4
Leakage	a	6.4	6.2
(% E_{260} released)	b	7.1	7.4

Table 21. The effect of ionic shock at various Mg^{2+} concentrations on the release of membrane from protoplast suspensions

Cells were plasmolysed in tris buffered sucrose containing Mg^{2+} at concentrations of 40, 20, 10 and 5 mM and protoplasts prepared by incubation with lysozyme at 37°C. In experiments involving no ionic shock (Expt. a), suspensions were diluted with 1.5 vol. of tris buffer containing Mg^{2+} at the concentration present in the original protoplast suspension. In suspensions subjected to ionic shock (Expt. b), protoplast suspensions were diluted with 1.5 vol. of tris buffer containing sufficient Mg^{2+} to give a final concentration of half the original value. Protoplast suspensions were held for 2 h at 37°C before E_{260} and E_{446} values were determined for supernatant fractions.

	Expt. Type	Initial Mg^{2+} concn. (mM)	40	20	10	5
Membrane release	a		4.6	6.3	8.9	12.3
(% E_{446} release)	b		6.3	9.1	13.0	
Leakage	a		4.9	5.7	6.7	9.0
(% E_{260} release)	b		6.7	7.9	10.2	

Table 22. Comparison of the properties of mesosomal and peripheral membranes prepared at different Mg^{2+} concentrations

Cells of M. lysodeikticus St.0 were plasmolysed in buffered sucrose containing Mg^{2+} at various concentrations. Following incubation with lysozyme, protoplast supernatant fractions were prepared as described in Methods, maintaining the $[Mg^{2+}]$ constant throughout the procedure. Washed mesosomal and peripheral membranes were then prepared as described in Methods. Protein was determined on membrane fractions by both the method of a) Gornall et al., (1949) and b) Lowry et al., (1951).

* Succinate dehydrogenase was assayed at 30°C. Figures in brackets denote the ratio of specific activities of mesosomal to corresponding peripheral membrane preparations.

Membrane type	Mg^{2+} concn. (mM)	Yield of Membrane (% dry wt.cell)	Properties of Membrane: Protein a (wt. %)	Protein b (wt. %)	a/b ratio	Extractable lipid (wt. %)	Total Phosphorus (wt. %)	Total hexose (wt. %)	Total pentose (wt. %)	Carotenoid (wt. %)	Nucleic acid (wt. %)	Succinate dehydrogenase* (E_{600}/min/mg membrane)
Peripheral	10	11.5	47.0	43.4	1.08	25.8	1.63	4.7	0.2	0.153	3.9	1.34
Peripheral	20	11.8	46.2	40.6	1.14	24.5	1.60	4.8	0.3	0.153	4.3	1.27
Peripheral	40	13.4	48.2	41.0	1.18	25.7	1.57	4.6	0.7	0.141	6.5	0.79
Mesosomal	10	1.76	38.5	29.3	1.31	N.D.	1.38	19.3	1.7	0.137	6.9	0.077 (0.057
Mesosomal	20	1.34	40.9	28.4	1.44	N.D.	1.20	20.5	0.6	0.132	3.7	0.067 (0.053
Mesosomal	40	0.91	40.1	27.8	1.44	N.D.	1.14	19.3	0.7	0.125	4.3	0.059 (0.075

T22

Table 23. Comparison of the autolytic activities of mesosomal and peripheral membranes prepared at different Mg^{2+} concentrations

Cells of M. lysodeikticus St.0 were plasmolysed in buffered sucrose containing Mg^{2+} at various concentrations. Following incubation with lysozyme, protoplast supernatant fractions were prepared as described in Methods, maintaining the Mg^{2+} concentration constant throughout the procedure. Washed mesosomal and peripheral membranes were then prepared as described in Methods and the presence of autolytic activity determined by assay (see Methods) on between 500 µg - 1500 µg dry wt. of fresh membrane. Lysozyme at a final concentration of 1.0 µg/ml was assayed for comparison and displayed an activity of 96,500 units/mg. Note that the ratio of specific activity for mesosomal membrane to that for the corresponding peripheral membrane preparation is constant and thus independent of Mg^{2+} concentration.

Membrane type	Mg^{2+} concn. during preparation (mM)	Autolytic activity (units/mg dry wt. membrane)		Ratio: Autolytic activity of preparation / Autolytic activity of corresponding peripheral membrane preparation
		Expt. 1	Expt. 2	Expt. 1
Peripheral	40	2.6	N.D.	1.0
Peripheral	20	2.9	N.D.	1.0
Peripheral	10	7.2	N.D.	1.0
Mesosomal	40	43	37	16.5
Mesosomal	20	42	47	14.5
Mesosomal	10	110	72	15.3

Table 24. Comparison of the chemical properties of mesosomal and peripheral membrane prepared at 10 mM Mg^{2+}

Protoplast suspensions of M. lysodeikticus St.0 were obtained from plasmolysed cells, the Mg^{2+} concentration being held at 10 mM throughout the procedure. Peripheral membrane and mesosomal membrane preparations were obtained from sedimented protoplasts and protoplast supernatant fractions respectively as described in Methods.

Protein was determined on membrane fractions by both the method of a) Gornall et al., (1949) and b) Lowry et al., (1951).

* The yield of peripheral membrane is expressed as % dry wt. of the cell and that of mesosomal membrane as the % dry wt. of the total isolated membrane.

Membrane preparation	Membrane type	Yield*	(wt. %) Protein a	Protein b	a/b ratio	Extract-able lipid (wt. %)	Total Phosphorus (wt. %)	(wt. %) Total Hexose	Total Pentose (wt. %)	Glucose (wt. %)	Nucleic acid (wt. %)	Carotenoid (wt. %)
24	Peripheral	13.1	50.0	42.3	1.18	N.D.	1.58	4.8	N.D.	0.04	6.2	0.140
25	Peripheral	13.6	50.8	39.9	1.27	26.8	1.60	3.6	N.D.	0.07	6.6	0.149
26	Peripheral	N.D.	52.5	45.7	1.15	26.6	1.67	5.8	0.6	N.D.	3.6	0.137
27	Peripheral	11.5	47.0	43.4	1.08	25.8	1.63	4.7	0.2	N.D.	3.9	0.153
24	Mesosomal	13.1	36.2	26.5	1.37	N.D.	N.D.	N.D.	N.D.	N.D.	3.5	0.124
25	Mesosomal	7.7	38.7	27.5	1.41	24.2	1.14	16.8	N.D.	N.D.	4.1	0.115
26	Mesosomal	6.6	38.7	28.6	1.35	28.0	1.10	21.9	1.0	N.D.	6.0	0.110
27	Mesosomal	13.2	38.5	29.2	1.31	N.D.	1.38	19.3	1.7	N.D.	6.9	0.137

Table 25. Comparison of enzymic activities of isolated mesosomal and peripheral membrane

Protoplast suspensions of M. lysodeikticus St.O were obtained from plasmolysed cells, the Mg^{2+} concentration being held at 10 mM throughout the procedure. Peripheral membrane and mesosomal membrane preparations were obtained from sedimented protoplasts and protoplast supernatant fractions respectively, as described in Methods. Dehydrogenase activity was assayed on fresh membrane suspensions at 25°C.

*Succinate dehydrogenase in membrane preparations 23, 24 and 27 were assayed at 15°C, 25°C and 30°C respectively. It was necessary to ensure that membrane preparations only attained assay temperature immediately prior to reaction, in order to prevent thermal inactivation of the enzyme (Owen & Freer, 1970b).

Membrane Preparation	Membrane type	Dehydrogenase activity E^{1cm}_{600}/min/mg membrane		
		succinate*	Malate	$NADH_2$
23	Peripheral	0.428	0.958	N.D.
24	Peripheral	0.602	0.739	6.440
27	Peripheral	1.348	N.D.	N.D.
23	Mesosomal	0.080	0.048	N.D.
24	Mesosomal	0.082	0.029	0.376
27	Mesosomal	0.077	N.D.	N.D.

Table 26. Extractable and "Bound" lipid in membranes from M. lysodeikticus St. 0.

Extractable lipid and bound lipid were determined gravimetrically on lyophilized membrane preparations as described in Methods.

Membrane preparation	Membrane type	Membrane	Extractable lipid			Bound Lipid					
						Ether soluble			Acetone/methanol soluble		
		Total P content (wt. %)	Dry wt. (% membrane)	Total P content (wt. %)	% of membrane P	Dry wt. (% of membrane)	Total P content (wt. %)	% of membrane P	Dry wt. (% of membrane)	Total P content (wt. %)	% of membrane P
26	Peripheral	1.67	22.9	2.64	36.2	8.4	N.D.	N.D.	6.8	N.D.	N.D.
27 (Expt. a)	Peripheral	1.53	24.4	2.44	38.8	10.2	0.08	0.5	9.6	6.57	41.2
27 (Expt. b)	Peripheral	1.58	25.8	2.59	43.7	11.3	N.D.	N.D.	6.5	N.D.	N.D.
26	Mesosomal	1.10	28.0	2.07	52.7	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Table 27. Cytochrome content of mesosomal and peripheral membranes isolated from M. lysodeikticus St.0

Protoplast suspensions of M. lysodeikticus St.0 were obtained from plasmolysed cells, the Mg^{2+} concentration being held at 10 mM throughout the procedure. Peripheral and mesosomal membrane preparations were obtained from sedimented protoplasts and protoplast supernatant fractions respectively as described in Methods.

The cytochrome content was estimated on membrane suspensions clarified by ultrasound or by the addition of Triton X-100 and reduced with sodium dithionite. The values reported are the mean of the number of estimations indicated in parenthesis.

Legend

a) A cytochrome-a_{601} content of 0.03 μmoles/g dry wt. membrane indicated. See however Results.

Membrane preparation	Membrane type	Conc. Triton-X-100 (%,v/v)	Cytochrome content (μmoles/g dry wt. membrane)			
			a_{601}	$(b_{560} + b_{556})$	b_{556}	c_{550}
24	Peripheral	0.0	0.19(1)	0.61(1)	-	0.54(1)
25	Peripheral	0.0	N.D.	N.D.	-	N.D.
24	Peripheral	1.0	0.18(2)	0.67(2)	-	0.55(2)
25	Peripheral	1.0	0.18(3)	0.64(3)	-	0.57(3)
24	Mesosomal	0.0	(a)	-	0.53(4)	-
25	Mesosomal	0.0	(a)	-	0.47(1)	-
24	Mesosomal	1.0	(a)	-	N.D.	-
25	Mesosomal	1.0	(a)	-	0.50(1)	-

Table 28. Differential centrifugation of deoxycholate extracted membrane

"Standard" membranes (14.7 mg dry wt. membrane/ml) from M. lysodeikticus St.0 were extracted with 1% sodium deoxycholate and residues DO 1, DO 2 and DO 3 prepared as described in Methods.

Membrane Preparation	Deoxycholate insoluble residue (% dry wt. of membrane)			
	DO 1	DO 2	DO 3	Total
3	33.6		N.D.	(33.6)
4 (Expt. 1)	17.3	18.4	3.4	39.1
4 (Expt. 2)	19.6	15.7	3.5	38.8

Table 29. Some features of freeze-fractured/freeze-etched vegetative bacterial cells

Author reference

1. Cagle, Vela & Plister, 1972
2. Meyer & Richter, 1971
3. Holt & Leadbetter, 1969
4. Remsen, 1966
5. Ghosh et al., 1969
6. Sleytr, 1970b
7. Sleytr, 1970a
8. Nanninga, 1968
9. Remsen, 1968
10. Nanninga, 1969
11. Nanninga, 1971a
12. Nanninga, 1971b
13. Abram & Davis, 1970
14. Remsen, Watson & Trupper, 1970
15. Sleytr & Krebs, 1971
16. Hoeniger, Stuart & Holt, 1968
17. Fiil & Branton, 1970
18. Bayer & Remsen, 1970
19. Nanninga, 1970
20. Van Gool & Nanninga, 1971
21. Remsen & Lundgren, 1966
22. Veltri & McAlear, 1971
23. Veltri & McAlear, 1972
24. Sleytr & Kocur, 1971
25. Berstein, 1969
26. Tillack, Branton & Razin, 1970
27. Tourlellote, Clark & Razin, 1970
28. Remsen, Valois & Watson, 1967
29. Watson & Remsen, 1969
30. Watson & Remsen, 1970
31. Van Gool, Lambert & Laudelout, 1969
32. Beaman & Shankel, 1969
33. De Voe, Costerton & MacLeod, 1971
34. Young, Chao, Turnbill & Philpott, 1972
35. Buckmire & Murray, 1970
36. Pooley, Shockman, Higgins & Porris-Juan, 1972
37. Hurst & Stubbs, 1969
38. Wildermuth, 1971
39. Wildermuth, Wehrli & Horne, 1971
40. Jackson & Black, 1970

Table 29. Some features of freeze-fractured/freeze-etched vegetative bacterial cells

		Membrane-wall connections	"Smooth" areas on peripheral membrane	Morphology of mesosomal membrane fracture faces		
				Particle Studded	Cross fractions Evident	Smooth Surfaced
	AZOBACTER					
1	A. vinelandii	+				
	Aquatic bacterium					
2	unnamed sp.		+		-	+
	BACILLUS					
3	B. anthracis	+				
4	B. cereus	+			-	+
3	B. cereus	+	+	+	±	+
3	B. fastidiosus	+				
5	B. licheniformis	+			6.	+
3	B. megaterium					
3	B. marcoides	+				
3	B. polymyxa	+				1.
3	B. psychrophilus	+				
6	B. sphaericus	+				
7	B. stearothemophilus	+	+	+	-	
8	B. subtilis	+			-	+
9	B. subtilis			+	±	+
10	B. subtilis				-	+2.
11	B. subtilis				+	+
12	B. subtilis					

		Membrane-wall connections	"Smooth" areas on peripheral membrane	Morphology of mesosomal membrane fracture faces		
				Particle Studded	Cross fractions Evident	Smooth Surfaced
	BDELLOVIBRIO					
13	B. bacteriovorus	+				
	CHROMATIUM					
14	C. buderi	+				
	CLOSTRIDIUM					
15	Cl. nigrificans		+5.	+	-	
16	Cl. perfringens	+				
	ESCHERICHIA					
17	E. coli	+	+	+	-	
18	E. coli	+	+			
19	E. coli					
20	E. coli	+	+5.			
	FERROBACILLUS					
21	F. ferroxidans	+				
	HYDROGENOMONAS					
22	H. eutropha					
23	H. eutropha	+	+5.		-	+
	MICROCOCCUS					
24	M. cryophilus			+	-	
	MYCOPLASMA					
25	M. gallisepticum					
26	M. laidlawii					
27	M. laidlawii		+			
	NITROCYSTIS					
28	N. oceanus					
29	N. oceanus					
30	N. oceanus					

		Membrane-wall connections	"Smooth" areas on peripheral membrane	Morphology of mesosomal membrane fracture faces		
				Particle Studded	Cross fractions Evident	Smooth Surfaced
	NITROBACTER					
31	N. winogradskii	+				
	NOCARDIA					
32	Nocardia sp.					
	PSEUDOMONAS					
33	Pseudomonas sp.	+				
34	Ps. saccharophila					
	SPIRILLUM					
35	S. serpens	+				
	SPOROSARCINA					
3	S. ureae	+				
	STREPTOCOCCUS					
	S. faecalis	3.				
36						
37	S. lactis	4.				
	STREPTOMYCES					
38	S. coelicolor					
39	S. coelicolor					
	TREPONEMA					
40	T. pallidum					

Legend

1. Showing "cratered" morphology.
2. Mesosomal "stalk" also visualized.
3. Rod-shaped structures observed connecting cells of a mutant deficient in autolytic enzyme activity.
4. Large conical protrusions observed on membrane fracture faces.
5. "Network" arrangement of intramembrane particles observed.
6. Vesicles evidently periplasmic.