INTERNATIONAL

Review of Cytology

EDITED BY

G. H. BOURNE J. F. DANIELLI

ASSISTANT EDITOR
K. W. JEON

VOLUME 69

INTERNATIONAL

Review of Cytology

EDITED BY

G. H. BOURNE
St. George's University School of Medicine
St. George's, Grenada
West Indies

J. F. DANIELLI
Worcester Polytechnic Institute
Worcester, Massachusetts

ASSISTANT EDITOR
K. W. JEON
Department of Zoology
University of Tennessee
Knoxville, Tennessee

VOLUME 69

1981

ACADEMIC PRESS *A Subsidiary of Harcourt Brace Jovanovich, Publishers*
New York London .Toronto Sydney San Francisco

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 52-5203

ISBN 0-12-364469-0

PRINTED IN THE UNITED STATES OF AMERICA

81 82 83 84 9 8 7 6 5 4 3 2 1

Contents

List of Contributors v

The Structure and Functions of the Mycoplasma Membrane

D. B. Archer

I. Introduction 1
II. Membrane Isolation 2
III. Membrane Structure 3
IV. Membrane Functions 20
V. Immunogenicity 33
VI. Conclusions and Prospects 34
References 35

Metabolic Cooperation between Cells

M. L. Hooper and J. H. Subak-Sharpe

I. Introduction 46
II. Detection of Metabolic Cooperation Using Variants Incapable of Incorporating Nucleotide Precursors into Their Nucleic Acid 47
III. Extension and Generality of the Phenomenon 55
IV. Quantification of Metabolic Cooperation 67
V. Genetics of Metabolic Cooperation 72
VI. Properties of Permeable Junctions 78
VII. Incidence and Specificity of Permeable Junction Formation 85
VIII. Kinetics of Permeable Junction Formation and Breakdown 87
IX. Possible Functions of Metabolic Cooperation 88
X. Conclusions 95
References 98
Note Added in Proof 104

The Kinetoplast as a Cell Organelle

V. D. KALLINIKOVA

I. Introduction 105
II. The Mitochondrial Nature of the Kinetoplast 107
III. The Phenomenon of Dyskinetoplasty 132
IV. The Kinetoplast and General Problems of Cytology 143
V. Conclusion 149
References 150

Chloroplast DNA Replication in *Chlamydomonas reinhardtii*

STEPHEN JAY KELLER AND CHING HO

I. Introduction 157
II. Structure of the Chloroplast Chromosome 158
III. Replication of the Chloroplast DNA *in Vivo* 162
IV. Replication of Chloroplast DNA in Isolated Chloroplasts 166
V. Chloroplast DNA Polymerase 176
VI. Summary and Conclusions 185
References 188

Nucleus-Associated Organelles in Fungi

I. BRENT HEATH

I. Introduction 191
II. Terminology 192
III. Morphology 193
IV. Composition 207
V. Functions 208
VI. Phylogenetic Aspects 217
VII. Conclusions 217
References 218

Regulation of the Cell Cycle in Eukaryotic Cells

ROSALIND M. YANISHEVSKY AND GRETCHEN H. STEIN

I. Introduction 223
II. Cycling Cells 224
III. Quiescent Cells 238
References 254

The Relationship of *in Vitro* Studies to *in Vivo* Human Aging

EDWARD L. SCHNEIDER AND JAMES R. SMITH

I. Introduction 261
II. Selection of Donors of Skin Fibroblast Cultures 262
III. Establishment of Skin Fibroblast Cell Cultures 263
IV. Cell Population Studies 263
V. Studies on Cloned Cells 267
VI. Discussion and Conclusions 267
References 269

Cell Replacement in Epidermis (Keratopoiesis) via Discrete Units of Proliferation

C. S. POTTEN

I. Introduction 272
II. General Structure of Mouse Epidermis 272
III. Epidermal Cell Kinetics 284
IV. Stem Cell Concepts 292
V. Clonal Regeneration Studies 295
VI. Implications and Complications of the Stem Cell Concept in the EPU 303
VII. Summary and Conclusions 312
References 315

INDEX 319
CONTENTS OF PREVIOUS VOLUMES 323

List of Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

D. B. ARCHER (1), *John Innes Institute, Norwich NR4 7UH, England*

I. BRENT HEATH (191), *Biology Department, York University, Toronto, Ontario M3J 1P3, Canada*

CHING HO[1] (157), *Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio 45221*

M. L. HOOPER[2] (45), *Institutes of Genetics and Virology, University of Glasgow, Glasgow G11 5JS, Scotland*

V. D. KALLINIKOVA (105), *Biological Department, Moscow University, Moscow 117234, USSR*

STEPHEN JAY KELLER (157), *Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio 45221*

C. S. POTTEN (271), *Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, England*

EDWARD L. SCHNEIDER (261), *National Institute on Aging, National Institutes of Health, Bethesda, Maryland 20205*

JAMES R. SMITH (261), *W. Alton Jones Cell Science Center, Old Bard Road, Lake Placid, New York 12946*

GRETCHEN H. STEIN[3] (223), *Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309*

J. H. SUBAK-SHARPE (45), *Institutes of Genetics and Virology, University of Glasgow, Glasgow G11 5JS, Scotland*

ROSALIND M. YANISHEVSKY (223), *Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309*

[1]Present address: College of Medicine, University of Cincinnati, Cincinnati, Ohio 45267.

[2]Present address: Department of Pathology, University of Edinburgh Medical School, Edinburgh EH8 9AG, Scotland.

[3]Present address: Cancer Biology Program, NCI/Frederick Cancer Research Center, P.O. Box B, Frederick, Maryland 21701.

INTERNATIONAL REVIEW OF CYTOLOGY, VOL. 69

The Structure and Functions of the Mycoplasma Membrane

D. B. ARCHER

John Innes Institute, Norwich, England

I. Introduction 1
II. Membrane Isolation 2
III. Membrane Structure 3
A. Lipid 3
B. Protein 14
IV. Membrane Functions 20
A. Enzymic Activities 20
B. Transport 25
C. Motility 26
D. Surface Adherence and Pathogenicity 29
E. Mycoplasma Membranes and Viruses 30
V. Immunogenicity 33
VI. Conclusions and Prospects 34
References 35

I. Introduction

Mycoplasma is the trivial name used in this article to include all members of the class Mollicutes. They are small prokaryotic organisms that are distinguished from bacteria by the absence of peptidoglycan and that are bounded by the plasma membrane, which is their only membrane; although, some species possess capsular material exterior to the membrane. Many mycoplasmas are pathogenic, disease being caused in a number of animal, plant, and insect hosts. Mycoplasma membranes are of particular interest not only because of their involvement in disease due to adhesion to host cells, but also because they provide a system that is most suited to many investigations into the structure and function of biomembranes. Two principal reasons explain their suitability in biomembrane research. First, mycoplasmas grow in synthetic culture media, and controlled alterations in membrane composition can be introduced by manipulation of the medium content. Second, mycoplasma membranes are easily prepared free from other cellular material. Mycoplasma membranes have been the subject of a number of recent reviews (Tourtellotte, 1972; Razin, 1975, 1978a, 1979; Razin and Rottem, 1976; Rottem, 1979), and in this article, much of the earlier work on mycoplasma membranes will be noted by reference to these reviews.

ISBN 0-12-364469-0

II. Membrane Isolation

The composition of a membrane can be altered by its method of isolation. Peripheral membrane proteins may be lost from the membrane by severe procedures, or extramembranous material such as cytoplasmic proteins may be incompletely removed. Peripheral membrane proteins are those proteins bound to membrane surfaces and removable from the membrane by alteration of the ionic strength of the suspending medium or by chelating agents (Singer and Nicolson, 1972). Integral membrane proteins are intimately associated with the lipid bilayer and normally require detergent action for their removal.

Many mycoplasmas are sensitive to osmotic shock, and this provides a simple and effective means of preparing membranes (Razin, 1963). Razin and Rottem (1974) have described a procedure for the isolation of *Acholeplasma laidlawii* membranes by osmotic shock, differential centrifugation, and a suitable washing procedure. Not all mycoplasmas are as sensitive to osmotic lysis as *A. laidlawii* and even osmotically sensitive organisms can become resistant upon aging (Razin, 1963). Divalent cations protect mycoplasmas against osmotic lysis (Razin, 1964) and should be absent from the lytic step. The osmotic sensitivity of mycoplasmas is increased by glycerol loading prior to osmotic shock (Rottem *et al.*, 1968), and a number of osmotically insensitive mycoplasmas are rendered sensitive by this method.

Mycoplasma membranes contain sterol (distinguishing these organisms from other prokaryotes), although acholeplasmas need not contain sterol. Digitonin complexes with cholesterol (Moore and Baumann, 1952) and causes lysis of mycoplasmas (Smith and Rothblatt, 1960). Digitonin-induced lysis has been used to prepare membranes of osmotically insensitive mycoplasmas (Rottem and Razin, 1972; Archer, 1975a; Alexander and Kenny, 1977; Masover *et al.*, 1977) and has the advantage that divalent cations do not interfere but the disadvantage that the digitonin is retained in the membrane, resulting in an increased hexose content of the membrane (Archer, 1975a). As with osmotic lysis, digitonin produces membranes that resemble fractured mycoplasma ghosts and are easily sedimented (Rottem and Razin, 1972). Other methods of producing mycoplasma membranes, including alternate freezing and thawing, ultrasound, high pH, and gas cavitation, have been reviewed elsewhere (Razin, 1975). Freezing and thawing often results in incomplete lysis, high pH may be damaging to the membranes, and ultrasound, although a most effective means of cell lysis, produces small membrane fragments that can be difficult to sediment. Mechanical methods (such as the Hughes press), other membrane active compounds (like saponins) other than digitonin (Chu and Horne, 1967), polyene antibiotics (Rottem, 1972; Archer, 1976), and tetanolysin (Rottem *et al.*, 1976) lyse mycoplasmas but do not provide convenient methods for membrane isolation.

Purity of a mycoplasma membrane preparation is normally judged by electron

microscopic examination. Chemical, enzymic, and membrane density characterization may be used where reference data are available. These criteria have been fully discussed by Razin (1975) and Razin and Rottem (1976). *A. laidlawii* membranes are about 10 nm thick, have a typical trilaminar appearance when examined by electron microscopy, and have a density between 1.140 gm cm^{-3} and 1.181 gm cm^{-3} according to growth conditions (Kahane and Razin, 1969a; Razin, 1975).

III. Membrane Structure

Mycoplasma membranes contain all the cell lipid and one-quarter to one-half of the cell protein (Razin, 1975, 1978a). The gross chemical composition of the membranes varies with species but generally falls within the range of 50 to 60% protein, 30 to 40% lipid, and 1 to 3% carbohydrate (Smith, 1971; Razin, 1975). The membranes also contain some inorganic ions (Kahane *et al.*, 1973), and traces of nucleic acids are often detected in membrane preparations but can be removed by addition of nucleases when endogenous nucleases have not already removed the material (Razin, 1975). However, ribosomes (Razin, 1969; Maniloff and Morowitz, 1972) and deoxyribonucleic acid (Smith and Hanawalt, 1969; Quinlan and Maniloff, 1972) have some association with mycoplasma membranes and could be regarded as legitimate minor membrane components. Capsular material of some mycoplasmas clearly has a membrane association, but this article will discuss only the lipid and protein components, and nature of individual compounds, their disposition and roles within the membrane, and their relationships with each other.

A. Lipid

1. *Lipid Composition*

The lipid composition of mycoplasma membranes is dependent upon a number of factors, principally medium content and age of culture. *Mycoplasma* species are incapable of *de novo* fatty acid or sterol biosynthesis, whereas *Acholeplasma* species can synthesize straight-chain saturated fatty acids from acetate (Smith, 1971; Romijn *et al.*, 1972) and can elongate medium-chain-length unsaturated fatty acids (Smith, 1971; Rottem and Barile, 1976; Saito *et al.*, 1978). *Acholeplasma* species are also incapable of *de novo* sterol biosynthesis but, unlike *Mycoplasma*, do not require sterols for growth, although sterols are incorporated into acholeplasma membranes if present in the growth medium (Smith, 1971).

As with other biomembranes, mycoplasma membranes contain a complex mixture of lipids and these have been tabulated by Smith (1979). The phos-

pholipids are acidic, being mainly phosphatidyl glycerol and diphosphatidyl glycerol (cardiolipin) and occasionally acylphosphatidyl glycerol (Plackett *et al.*, 1969, 1970; Smith, 1971). Lysophosphatidic acid and phosphatidic acid are also encountered but probably are degradative products or biosynthetic precursors (Razin, 1975). Where other phospholipids have been detected (such as sphingomyelin, phosphatidylethanolamine, or phosphatidylcholine), they have in most cases been absorbed into the membrane from the growth medium (Plackett, 1967; Plackett *et al.*, 1969). Phosphatidylcholine appears not to be incorporated into the *A. laidlawii* membrane but probably is into the *Mycoplasma hominis* membrane (Kahane and Razin, 1977). Recently it was shown that the disaturated phosphatidylcholine of *Mycoplasma gallisepticum* is synthesized by insertion of a saturated fatty acid into medium-derived lysophosphatidylcholine (Rottem and Markowitz, 1979). In *Ureaplasma*, 5% of the phospholipid was identified as phosphatidylethanolamine, and this was probably synthesized by the organism (Romano *et al.*, 1972). Other phospholipids, unusual in mycoplasmas, are a sphingolipid of *Acholeplasma axanthum* (Plackett *et al.*, 1970; Mayberry *et al.*, 1973) and the plasmalogens of *Anaeroplasma* (Langworthy *et al.*, 1975).

Glycolipids in mycoplasmas are mainly monoglucosyl- and diglucosyldiglycerides (Smith, 1971). Phosphoglucolipids have also been described in mycoplasmas (Smith, 1972; Schiefer *et al.*,.1977a; Wieslander and Rilfors, 1977). The mono- and diglucosyldiglycerides and *A. laidlawii* have some physical properties similar to phosphatidylethanolamine (Wieslander *et al.*, 1978). This finding may be of importance since phosphatidylethanolamine is a common bacterial lipid and yet is absent from mycoplasmas. Longer polysaccharide chains are found in the lipopolysaccharide of *Thermoplasma acidophilum* (Mayberry-Carson *et al.*, 1974, 1975), *Acholeplasma*, and *Anaeroplasma* (Smith *et al.*, 1976). Mycoplasma lipopolysaccharide is chemically unrelated to bacterial lipopolysaccharide and has been detected in only one (*Mycoplasma neurolyticum*) of six *Mycoplasma* species examined (Smith *et al.*, 1976). External sugar residues in *T. acidophilum* have been visualized cytochemically after binding concanavalin A. This binding was interpreted as recognizing mannose in the lipopolysaccharide (Mayberry-Carson *et al.*, 1978). But mannose has been shown recently to be the most abundant sugar in a membrane glycoprotein isolated from the organism (Yang and Haug, 1979), so the concanavalin A-horseradish peroxidase-diaminobenzidine assay presumably visualizes both the lipopolysaccharide and the glycoprotein.

Terpenes are synthesized by *Acholeplasma* but not *Mycoplasma* (Smith, 1971). The terpenes found in *Acholeplasma* membranes are carotenoid derivatives and recently the presence of carotenoids was confirmed in *A. axanthum* (Smith and Langworthy, 1979), which is an organism previously supposed to lack them (Tully and Razin, 1969, 1970). In contrast to other acholeplasmas, the

Acholeplasma oculi lipopolysaccharide contains only neutral sugars and no amino sugars (Al-Shammari and Smith, 1979).

Thermoplasma acidophilum is the name given to a wallless prokaryote that grows optimally at 59°C and pH 2 and is presently included within the class Mollicutes (Darland *et al.*, 1970). [However, for many reasons its classification as a mycoplasma is unsatisfactory and its true taxonomic position is still a matter for discussion (Freundt and Edward, 1979).] This organism has many differences from other mycoplasmas, and two membrane-related differences are the nature of its lipids and the presence in *T. acidophilum* of an active flagellum anchored in the membrane. Apart from the lipopolysaccharides of *T. acidophilum* mentioned previously, other lipids present include neutral lipids, glycolipids, and phospholipids. However, unlike mycoplasmas, the fatty acyl ester-linked glycerol derivatives are largely replaced by ether-linked alkyl glycerol moieties (Langworthy *et al.*, 1972), although fatty acyl esters are present, particularly in the neutral lipid fraction (Ruwart and Haug, 1975). The glycerol ethers appear to be formed by the ether linkage of two glycerol molecules by long-chain isoprenoid alkyls of 40 carbons, which are theoretically capable of spanning the membrane (Langworthy, 1977), so that the *T. acidophilum* membrane lipid would contain a monolayer of these molecules rather than a bilayer. Such a membrane would still give a trilaminar appearance in the elctron microscope after negative staining but would not be capable of freeze-fracture along the hydrophobic core of the membrane. This is the case (Ververgaert, unpublished; cited by Razin, 1978a) and may explain the high rigidity of the membrane as judged by electron paramagnetic resonance spectra of introduced spin labels (Smith *et al.*, 1974). Ether-linked polyisoprenoids are a characteristic of the Archaebacteria, and it is thought that *Thermoplasma* might better be considered as belonging to the Archaebacteria rather than the Mollicutes (Woese and Fox, 1977; Balch *et al.*, 1979). The high protein content of the membranes (Ruwart and Haug, 1975) may also contribute to rigidity, but more information on the nature of membrane proteins from *T. acidophilum* is lacking.

T. acidophilum is actively motile and has a functional flagellum (Black *et al.*, 1979), as shown in Fig. 1. This contrasts with protoplasts of flagellated bacteria, which are nonmotile. More detailed structural analysis of the *T. acidophilum* flagellum is required before more comment on its mode of operation can be made, but some conflict with current theories of bacterial flagellar movement (e.g., Berg, 1974) must be expected. If more detailed structural analysis of the *T. acidophilum* flagellum reveals close similarity with bacterial flagella, it may be that the relatively rigid membrane of the organism, which probably has more fluid regions (Smith *et al.*, 1974), combines the dual roles played by the fluid membrane and rigid wall as required by current theories of bacterial flagella movement.

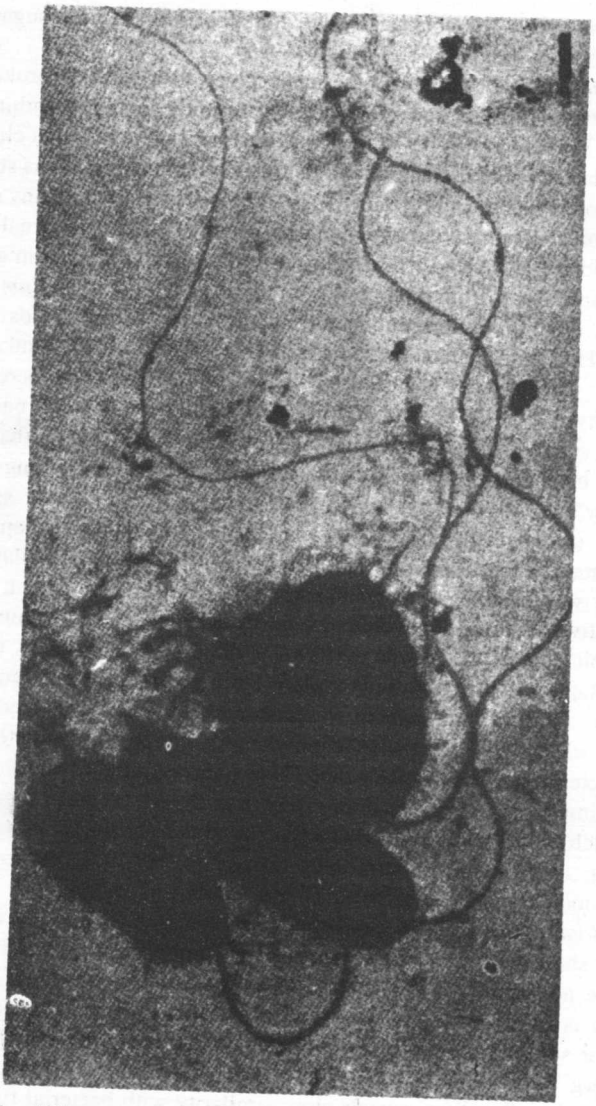

FIG. 1. Cells of *Thermoplasma acidophilum*, negatively stained, showing the presence of one flagellum per cell. Bar represents 250 nm. (Courtesy of Black *et al.*, 1979.)

2. *Lipid Fluidity*

Both cholesterol and fatty acid residues affect membrane fluidity in artificial lipid membranes (Ladbrooke *et al.*, 1968; de Gier *et al.*, 1969; Rothman and Engelman, 1972), and this is also true in mycoplasma membranes (Tourtellotte, 1972; Razin, 1975). The motion of the hydrocarbon chains within a membrane confers an overall fluidity on the membrane lipids and the fluidity increases with a rise in temperature. On cooling, the fluid liquid-crystalline phase changes reversibly to the rigid gel state and the transition occurs at a particular transition temperature that is dependent upon the nature of the hydrocarbon chains in the membrane. Sterol rigidifies fluid membranes but prevents crystallization of the hydrocarbon chains upon cooling and so, at high concentrations in the membrane (found in *Mycoplasma* spp. but not *Acholeplasma* spp.), the phase transition is abolished. Acholeplasmas are capable of some fatty acid biosynthesis, but in other mycoplasmas, the sterols and fatty acids in their membranes are derived from the growth medium where they must be supplied in an assimilable form. Detailed evaluation of the ability of individual sterols or fatty acids to satisfy growth requirements under a variety of conditions is best done by growth of the organisms in the defined medium, a condition that has been satisfied for *Mycoplasma mycoides* (Rodwell, 1969a,b) and *A. laidlawii* (Tourtellotte *et al.*, 1964). Lipid-poor media, with serum replaced by serum protein fractions and exogenously added lipid, have also been of value in membrane lipid studies (Razin, 1963; McElhaney and Tourtellotte, 1970).

Serum acts as a source of lipid in complex media, low density lipoproteins being the main cholesterol donor (Slutzky *et al.*, 1976, 1977). Fatty acids and sterols are normally carried by serum proteins, but sterols may also be made assimilable by yeast mannan (Archer, 1975a; Rodwell, 1977) or dispersion in lecithin vesicles (Kahane and Razin, 1977). Fatty acids may be supplied by Tween (Razin and Rottem, 1963; Razin and Tully, 1970) or as soluble fatty acid esters (Rodwell, 1969b, 1977). The sterol- and fatty acid-carrying functions of added serum protein fractions may be distinguished but are probably not totally independent of each other (Rodwell and Abbot, 1961; Rodwell, 1967, 1969a,b, 1971). For example, fatty acid-poor bovine serum albumin has been used as a fatty acid carrier, whereas a Pronase digest of the albumin carried sterol to facilitate the growth of *M. mycoides* (Rodwell, 1967).

The uptake of cholesterol into mycoplasma membranes is a nonenzymic, nonenergy-requiring process (Smith and Rothblat, 1960; Gershfeld *et al.*, 1974), although in *Mycoplasma capricolum*, the uptake of cholesterol is affected by cell growth (Clejan *et al.*, 1978). Rodwell (1963) found no indication of esterification of cholesterol nor of transformation into other sterols in *M. mycoides*, and this has been found to be so in all other *Mycoplasma* species examined (Smith, 1971; Razin, 1978a). Sterol esters, when found in mycoplasma membranes (Razin, 1967), are derived entirely from the medium (Argaman and Razin, 1965;

Rottem and Razin, 1973), although sterol esterase may afford entry of sterols into the mycoplasma membrane from sterol esters in the medium (Smith, 1971). Many sterols support the growth of mycoplasmas, but they are normally sterols with a relatively planar nucleus and a 3-β-hydroxyl group, which are the requirements for adequate sterol–lipid interaction within artificial membranes (de Kruyff *et al.*, 1973a; Brockerhoff, 1974). 3-*O*-Methyl ethers do support poor growth of *M. capricolum*, however (Odriozola *et al.*, 1978), and this work questions the absolute requirement for a 3-β-hydroxyl in sterol–lipid interactions in natural membranes (Lala *et al.*, 1979). Sterols with a 3-α-hydroxyl have been reported in acholeplasmas (de Kruyff *et al.*, 1972, 1973a) but, then, unlike *Mycoplasma* species, acholeplasmas do not have a sterol growth requirement. Similarly, coprostanol, which has a 3-β-hydroxyl but a relatively aplanar nucleus, does not support the growth of *Mycoplasma arthritidis* (Smith and Rothblat, 1960) or *Ureaplasma* (Rottem *et al.*, 1971) but is incorporated into the *A. laidlawii* membrane (de Kruyff *et al.*, 1973a). However, the effects of aplanar sterols and sterols with a 3-α-hydroxyl upon enzymic activity and erythritol flux in the *A. laidlawii* membrane are unlike the effects produced by cholesterol (de Kruyff *et al.*, 1973a,b). Both cholesterol and epicholesterol are incorporated into the *A. laidlawii* membrane to the same extent, but unlike cholesterol, epicholesterol has very little effect on permeability (de Kruyff *et al.*, 1972).

a. *Control of Lipid Fluidity in Mycoplasma.* *Mycoplasma* species depend on the growth medium for fatty acids and sterols, and they control membrane fluidity by selective incorporation of lipids into the membrane, being selective by lipid species and amount. Membrane lipid content is dependent upon medium composition, growth temperature, and age of culture. As cells age, the total lipid content of *Mycoplasma* decreases relative to protein (Razin, 1974a; Rottem and Greenberg, 1975). The fatty acid composition of *Mycoplasma* membranes can control the cholesterol content (Razin, 1974a), and in other instances, a change in cholesterol content has caused an alteration in fatty acid composition (Rottem *et al.*, 1973a; Archer, 1975a). It appears, then, that a change in any one parameter will be countered by a moderating effect so that membrane fluidity is maintained at a level suitable for cell growth, but it is not known whether changes in fatty acid incorporation from the growth medium are sufficient to maintain identical fluidity when the sterol content is altered. Cholesterol content of *Mycoplasma* membranes has been severely reduced but never eliminated (Rodwell *et al.*, 1972; Rottem *et al.*, 1973a). Rottem *et al.* (1973a) reduced the membrane cholesterol content of *M. mycoides* subsp. *capri* from 22 to 3% of membrane lipid and reported a subsequent increase in the ratio of saturated to unsaturated fatty acids of polar lipids from 1.15 to 1.56. Similar results were obtained by Archer (1975a,b) using cholesterol, ergosterol, and dehydroergosterol. Reduction in sterol content of the membrane lipid is paralleled by a reduction in total phospholipid and increases in the amounts of glycolipids and glycerides (Rottem

et al., 1973a; Archer, 1975a). Culture aging increased the ratio of saturated to unsaturated fatty acids and decreased the cholesterol content in *M. mycoides* subsp. *capri*, but these two factors were uncoupled by growth at lowered temperature when the cholesterol content was reduced without altering the fatty acids (Rottem *et al.*, 1973a). The control of membrane fluidity in *Mycoplasma* is limited by their inability to modify fatty acids and incorporation of sterol into the membrane from the growth medium seems to be the major factor in determining fluidity at a given temperature. Different *Mycoplasma* species incorporate different amounts of cholesterol into their membranes when grown under identical conditions (Rottem and Razin, 1973; Slutzky *et al.*, 1977; Razin, 1978a), and although isolated membranes show little control over the uptake of cholesterol, growing cells do seem to have a mechanism for controlling cholesterol uptake that is dependent on the supply of cholesterol in the medium, lipid arrangement within the membrane (Razin *et al.*, 1974), and lipid fluidity (Razin, 1978b). The change in fatty acid composition of membrane lipids in response to an alteration in the sterol content of the membrane is of particular interest because it implies that *Mycoplasma* species have a mechanism for selective incorporation of fatty acids into complex lipids, which is also a characteristic of bacteria (Cronan and Gelman, 1975) and *Acholeplasma* (Melchior and Steim, 1977). Certainly most enzymes involved in complex lipid biosynthesis are membrane bound (Smith, 1971) and would be expected to be influenced by fluidity changes caused by sterol, but whether other constraints such as temperature changes also affect fatty acid incorporation into complex lipids is not clear. Sterol content is the dominant factor in *Mycoplasma* membrane fluidity considerations and Rottem (1979) has suggested that the high quantities of sterol in *Mycoplasma* membranes may be necessary to prevent lipid crystallization at the growth temperature.

b. *Control of Lipid Fluidity in Acholeplasma.* As described previously, *Acholeplasma* are not obligate sterol requirers and are capable of biosynthesis of straight-chain saturated fatty acids and of elongating unsaturated fatty acids. They therefore have a potentially complicated and versatile means of controlling membrane fluidity. Quantitative changes in amounts of different lipids present in the membrane under altered growth conditions are complex (Christiansson and Wieslander, 1978). Shaw *et al.* (1968) reported that the total glycolipid content of *A. laidlawii* was unaffected by cholesterol, but Christiansson and Wieslander (1978) found that the molar ratio between the two most abundant glycolipids is largely determined by membrane fluidity, which in turn is affected by cholesterol. At high cholesterol contents in artificial lipid membranes, phase transitions are abolished (Ladbrooke *et al.*, 1968; Hinz and Sturtevant, 1972; Engelman and Rothman, 1972). Phase transitions can be detected by calorimetry [differential scanning calorimetry (DSC) and differential thermal analysis (DTA)], X-ray analysis, fluorescence, and electron paramagnetic resonance spectroscopy of introduced spin radicals. DSC was used to demonstrate that *M. mycoides* subsp.

capri grown with a high cholesterol content does not have a phase transition, whereas when the amount of cholesterol is reduced, a phase transition occurs (Rottem *et al.*, 1973b). In *A. laidlawii*, the cholesterol content does not reach a sufficiently high level to abolish the reversible phase transition, but the energy change of the transition is lowered by cholesterol and not by epicholesterol (de Kruyff *et al.*, 1972). Fluid membranes are more permeable to glycerol and erythritol than rigid ones and cholesterol, but not epicholesterol, reduces the permeability of *A. laidlawii* to both of these compounds (de Kruyff *et al.*, 1972, 1973a; McElhaney *et al.*, 1970, 1973).

The effects of cholesterol in *Acholeplasma* membranes are not entirely analogous to those in *Mycoplasma*. Cholesterol had only a small effect on the fatty acid composition of *A. laidlawii* (de Kruyff *et al.*, 1972) and did not alter the osmotic fragility of the organism (Razin *et al.*, 1966). As described previously, *Acholeplasma* contains terpenes and growth is inhibited by inhibition of polyterpene biosynthesis (Smith and Henrickson, 1966); this inhibition was reversed by cholesterol, and Smith (1964) has proposed that carotenoids and sterols have similar roles in the *Acholeplasma* membrane. As with cholesterol, carotenoid content did not affect the osmotic sensitivity of *A. laidlawii* (Razin *et al.*, 1966). However, *A. laidlawii* can grow in the absence of cholesterol without apparent carotenoid biosynthesis (Razin and Rottem, 1967), although Smith (1971) has suggested that under these conditions the colored polyterpene may have been substituted by an undetected fully saturated compound. Increasing carotenoid content in *A. laidlawii* rigidified the membrane (as judged by electron paramagnetic resonance studies) and caused only a slight change in fatty acid composition (Huang and Haug, 1974; results similar to those found with increasing cholesterol content), but Silvius *et al.* (1980) found only a slight effect of carotenoids on the phase transition of the lipid bulk. Permeability to glycerol was not affected as much by carotenoid content (Huang and Haug, 1974) as by cholesterol content (de Kruyff *et al.*, 1972). In summary, carotenoids and sterols do have some similarities of role in the *Acholeplasma* membrane despite structural dissimilarity, although a very close functional analogy probably does not exist.

Acholeplasmas use their capacity for biosynthesis of saturated fatty acids and elongation of unsaturated ones as part of the mechanism of regulating membrane fluidity (Saito *et al.*, 1977a, 1978; Silvius and McElhaney, 1978a,b; Silvius *et al.*, 1977). The regulation of membrane fluidity can involve a balance between the amount of bilayer in the rigid gel state and that in the more fluid liquid-crystalline state. The degree of fluidity in liquid-crystalline lipids may also vary, and it is supposed that an optimal fluidity exists for normal cell growth and function. At the growth temperature of *A. laidlawii*, the membrane contains both lipid states (Oldfield *et al.*, 1972; Romijn *et al.*, 1972; Stockton *et al.*, 1975), and up to one-half of the lipid may be in the gel phase without adversely affecting