Basic Biology Course

2 Electron Microscopy and Cell Structure

BASIC BIOLOGY COURSE

UNIT 1

MICROSCOPY AND ITS APPLICATION TO BIOLOGY

BOOK 2

Electron Microscopy and Cell Structure

MICHAEL A. TRIBE, MICHAEL R. ERAUT &
ROGER K. SNOOK
University of Sussex

CAMBRIDGE UNIVERSITY PRESS

CAMBRIDGE
LONDON · NEW YORK · MELBOURNE

Published by the Syndics of the Cambridge University Press
The Pitt Building, Trumpington Street, Cambridge CB2 1RP
Bentley House, 200 Euston Road, London NW1 2DB
32 East 57th Street, New York, NY 10022, USA
296 Beaconsfield Parade, Middle Park, Melbourne 3206, Australia

ISBNs:
0 521 20657 X hard covers
0 521 20557 3 limp covers

First published 1975

Printed in Great Britain
at the University Printing House, Cambridge
(Euan Phillips, University Printer)

Foreword

This book is part of a Basic Biology Course for undergraduates written by the Inter University Biology Teaching Project team at Sussex.

The main aim of this book has been to get you to look critically at the fine structure of plant and animal cells, as seen by the electron microscope. You will quickly realize the advantages conferred by the greater resolving and magnifying power of the electron microscope, but you should also appreciate its limitations and technical difficulties when interpreting electron micrographs. Most of the pictures that you will be asked to examine are taken from very thin sections and are essentially two-dimensional views of objects; so that from different two-dimensional aspects of the same object, you will be asked to deduce the apparent three-dimensional plan of that object.

As you work through the book, you will doubtless be struck by the amazing complexity of cells, which in turn should prompt you to ask the question 'Why?'. Although we have provided a brief synopsis of the functions of the various cell structures, we have not attempted to present evidence for their functions, nor have we attempted to answer the question 'Why such complexity?'. This has been intentional, since trials of these materials have shown that it is better to deal with only one or two concepts at a time. Consequently, evidence for function of cell organelles and answers to the question 'Why such complexity?' are dealt with in subsequent books in the series.

Finally, we should add that the book tries to explain in outline how the electron microscope works and how electron micrographs are produced. These explanations are given by means of an audio tape and slide sequence. Here our intention has not been to produce expert electron microscopists, but rather to avoid treating the electron microscope as if it were some magic black box.

Sussex. 1974

Michael A. Tribe
Michael R. Erault
Roger K. Snook

Acknowledgements

This book was developed under the auspices of the Inter University Biology Teaching Project and is the responsibility of the Sussex University project team. However, it owes a great deal to the students who studied and criticized our earlier versions and to many colleagues both at Sussex and elsewhere who made constructive suggestions for its improvement.

In particular we would like to thank the following:

the Nuffield Foundation for financially supporting the project from 1969-72;

Cambridge University Press for the continued interest and support in publishing the materials;

Mrs P. Smith, the project secretary;

Mr Colin Atherton, for photographic assistance;

Dr Doreen Ashhurst (Department of Anatomy, University of Birmingham), Dr A.M. Glauert (Strangeways Laboratory, Cambridge), Dr B.E.Juniper (University of Oxford) and Dr D.C. Spanner (Bedford College, University of London), for advice and constructive criticisms.

We are extremely grateful to the following for allowing us to use their electron micrographs:

H.J. Arnott, Dept. of Botany, University of Texas (fig. 27)

A. Bajer, Dept. of Biology, University of Oregon (fig. 13)

J.G. Bluemink & S. De Haat, Hubrecht Laboratory, Utrecht (fig. 6*c*)

H.T. Bonnett, Dept. of Botany, University of Oregon & E.H. Newcombe, Dept. of Botany, University of Wisconsin (fig. 24)

A. Boyd, Dept. of Anatomy, University College, London (figs. 66, 67)

D. Branton, Dept. of Botany, University of California, Berkeley (figs. 58, 59, 62, 63)

S. Bullivant, Dept. of Cell Biology, University of Auckland (fig. 61)

W.P. Cunningham, Dept. of Genetics and Cell Biology, University of Minnesota (fig. 26*b*)

C. de Duve, Dept. of Physiological Chemistry, University of Louvain, (5, page 72)

P. Echlin, Dept. of Botany, University of Cambridge (fig. 64)

D.W. Fawcett, Dept. of Anatomy, Harvard Medical School (figs. 19, 43)

N.T. Florendo, Dept. of Biology, University of Tennessee (fig. 25)

J. Freeman, Dept. of Pathology, Louisiana State University School of Medicine (fig. 39)

D.S. Friend, Dept. of Pathology, University of California, San Francisco Medical Center (figs. 40, 41)

M.S. Fuller, Dept. of Botany, University of California, Berkeley (fig. 12)

J.G. Gall, Dept. of Biology, University of Yale (fig. 14)

A.M. Glauert, Strangeways Research Laboratory, Cambridge (2, page 73)

A.D. Greenwood, Dept. of Botany, Imperial College, London (figs. 30, 3, page 72, 50)

C. Hackenbrock, Johns Hopkins Medical School (4, page 73, fig. 44)

J. Hall, School of Biological Sciences, University of Sussex (fig. 16, page 110)

P. Heighton, Dept. of Microbiology, University of Edinburgh (fig. 4)

H.E. Huxley, MRC Laboratory of Molecular Biology, Cambridge (6, page 73)

W.A. Jensen, Dept. of Botany, University of California, Berkeley (figs. 9, 10, 18, 54)

ACKNOWLEDGEMENTS

J.H. Judah & W.H. Butler, MRC Toxicology Laboratory, Carshalton (fig. 45)
B.E. Juniper, Dept. of Botany, University of Oxford (figs. 6*d*, 31, 32, 33, 34, 36, 46, page 107)
M.C. Ledbetter, Brookhaven National Laboratory, New York (figs. 35, 38)
R.G. Milne, Clinical Research Centre, Harrow (fig. 53)
H. Moor, Institut für Allgemeine Botanik, Zurich (fig. 60)
E. Munn, ARC Institute of Animal Physiology, Babraham (fig. 47)
D.H. Northcote, Dept. of Biochemistry, University of Cambridge (fig. 57)
R.B. Park, Dept. of Botany, University of California, Berkeley (figs. 9, 10, 18, 51)
J.C. Penland & H.C. Aldrich, Dept. of Botany, University of Florida (page 109)
K.R. Porter, Dept. of Biology, University of Colorado (figs. 20, 23)
A. Rich, Dept. of Biophysics, Massachusetts Institute of Technology (fig. 52)
J.D. Robertson, Harvard Medical School (fig. 6*a*, *b*)
A. Ryter, Institut Pasteur, Paris (figs. 2, 3, 5)
C.E. Schwerdt & R.C. Williams, Virus Laboratory, University of California, Berkeley (fig. 8)
L.K. Shumway, Program in Genetics and Dept. of Botany, Washington State University (fig. 29)
K. Taylor & S. Howells, School of Biological Sciences, University of Sussex (fig. 65)
Y. Uehara, Dept. of Zoology, University of Melbourne (fig. 7).
T.E. Weier & W.W. Thompson, Dept. of Botany, University of California, Davis (fig. 28)
W.G. Whaley & J.E. Kephart, Cell Research Institute, University of Texas (fig. 42)
J. Wiener, Dept. of Pathology, New York Medical College (fig. 15)

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2.0. Introduction

2.0.1. History

The microscope

Since 1660 when Anton von Leeuwenhoek started to observe small living objects by means of high-powered hand lenses, great advances have been made in light microscopy. The present-day microscope referred to in Book 1 bears little or no resemblance to the ones used by the early microscopists. Under the light microscope there is a limit at which small things can be seen clearly and further magnification only succeeds in blurring them. It was important therefore to find a microscope with much greater resolving power at a higher magnification. The knowledge that electrons have much shorter wavelengths than light waves prompted experimental work in the 1920s into the use of electromagnets to focus an electron beam. The development of suitable electromagnetic lenses enabled Knoll & Ruska during the years 1930-3 to produce the prototype electron microscope. Although the microscope was difficult to operate it was found to have much better resolving power and magnification than the light microscope. Their work resulted in the first commercial production of an electron microscope by the Siemens Co. (Germany) in 1939. From that time to the present, great technical improvements on the electron microscope have been made; these improvements have increased its performance and ease of operation.

During the last twenty years, problems associated with the electron microscope – i.e. (i) of cutting very thin sections, (ii) of finding suitable fixatives to faithfully preserve biological specimens, (iii) of finding suitable materials in which to embed the specimens, and (iv) of finding suitable stains – have been solved.

Mastery of these techniques has enabled present-day biologists to observe the fine detail of cells and large molecules.

The cell concept

It is not surprising to find that the emergence of the cell concept, which in turn has led to our present-day knowledge of cell ultrastructure and function, has followed the advances in microscopy. The cell concept states that the cell is the basic unit of life, because virtually all living organisms are composed of cells, and cells are the metabolic reaction sites in all organisms. Characteristically, yet uniquely, all living cells contain hereditary material, through which specific characters are passed on from one generation to the next, so that cells can only arise from pre-existing cells.

The present-day concept, which is also referred to as the cell theory, began with the work of the early seventeenth century microscopists. Notable amongst them was the Englishman, Robert Hooke, who in his *Micrographia* published in 1665, introduced the term cell' to describe the box-like structures which he observed in thin sections of cork under the microscope. It is perhaps paradoxical that the term 'cell' was first used in describing the structure of cork, because these cells are essentially dead; all that remains is the thickened cellulose wall which characterizes plant cells. Although many observations and descriptions of cells were undoubtedly made during the eighteenth century, it was not until the nineteenth century when the generalization that all living organisms were cellular in nature was proposed. Between 1838 and 1839 two German biologists, Mattias Schleiden (a

botanist) and Theodor Schwann (a zoologist), after extensive studies of a variety of tissues and organisms in their respective fields of study, came to the conclusion that the cell was the basic structural unit of all living things. Some twenty years later another German, Rudolf Virchow (1858), extended the cell theory to accommodate the metabolic principle and emphasized the continuity of living cells with the statement: '*omnis cellula e cellula*' – namely, all cells arise from (pre-existing) cells.

This book is essentially about the fine structure of cells as seen by the electron microscope.

2.0.2. Preknowledge requirements

An elementary description is assumed of the following terms:
cell, cell wall, limiting cell membrane, nucleus, chromosome, DNA and *RNA, chloroplast* and *chlorophyll, magnification* and *resolution.*

At the back of this book (section 2, page 113) there is a glossary of some important terms used in the text. Particularly important is a list of the major cell structures and organelles with a brief description of their functions. This can be found printed on the masking card. ***Please try to use both glossaries*** *as you work through the book.*

The main aim here is to identify organelles and examine their fine structure; evidence for their function has been presented in detail elsewhere, in Books 5 to 9 inclusive (Unit 3. 'Regulation within cells').

2.0.3. Objectives

At the end of this book you should be able to:

(*a*) List the essential structural features of the electron microscope and explain their function.
(*b*) Describe in outline the main methods of specimen preparation.
(*c*) Identify the preparatory method used on examining an electron micrograph, and be aware of likely side-effects of the preparation.
(*d*) Recognize the main cell organelles.
(*e*) Interpret the structure of cell organelles as revealed by electron micrographs.
(*f*) Estimate the relative sizes of cells and cell organelles.
(*g*) Describe two methods for isolating cell organelles.

Much of the book requires the use of a masking card and some parts will require a tape recorder and a slide viewer or projector. You are given instructions about working through the programmed section below.

2.0 4. Instructions on working through the programmed sections

In the programmed sections, questions and answers are arranged sequentially down the page. You are provided with a masking card and a student response booklet. When you come to a programmed section you will be given warning of it. Cover each page in turn, and move the masking card down to reveal two thin lines:

This marks the end of the first question on that page. Record your answer to the question under the appropriate section heading in the response booklet provided. Then *check* whether your answer is in agreement with the answer given. Obviously, you cannot be expected to reproduce precisely the same wording as the one given, especially as several frames give additional information or explanation in the answer. However, if your answer is essentially correct, move the masking card down the page to the next set of double lines and so on. If any of your answers are clearly incorrect retrace your steps and try to find out why you answered incorrectly. If you are still unable to understand the point of a given question, make a note of it and consult your tutor.

The single thick line

---

is a demarcation between one frame and the next.

2.1. Light versus electron microscopy

2.1.1. Resolution and magnification [Masking card needed]

1 Bacterial cells, which in general have dimensions lying between 1 μm and 4 μm, are resolved only with difficulty under the light microscope. At magnifications of 400 times natural size, large bacteria stained with methyl violet can just be seen as small, dark specks. Providing the resolution of the light microscope is good, higher magnifications (e.g. X 1000 or X 1500) do reveal a little more of the internal structure of bacterial cells.
The light micrograph (fig. 1) shows a single type of bacterial cell treated with Geimsa stain. Measure the length of cell X, and, assuming the actual length to be 3 μm calculate the magnification.

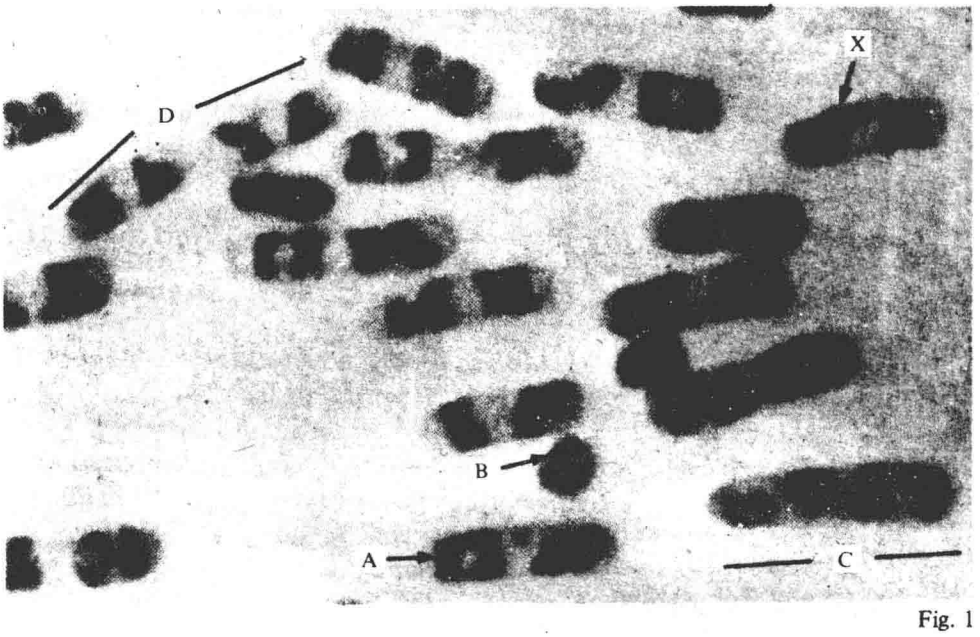

Fig. 1

Measured length is 2 cm. Magnification is 6600 times.

2 How has a magnification of 6600 times been achieved if the light microscope itself only magnifies 1500 times?

By photographically enlarging the picture of the bacteria obtained from the light microscope.
(*Note.* This technique is used frequently to obtain higher magnification of both light micrographs and electron micrographs.)

3 With reference to (fig. 1), what internal organization can be distinguished in cell X?

There are two densely stained bodies within the cell.

4 What inclusions of comparable size in relation to the rest of the cell have you seen in other cells?

Nuclei. The densely staining areas in bacterial cells are the equivalent of the nucleus in a plant or animal cell. These densely staining areas are called *chromatin bodies* or *nucleoids*. Under standard conditions of culture *Escherichia coli* (the bacterial species considered here) usually contains two or three nucleoids or chromatin bodies.

5 Apart from these chromatin bodies, can you resolve any other form of structural organization?

No; the regular shape of the cell might suggest that there is a limiting membrane, but it cannot be seen.

6 In fig. 1 some cells appear longer or shorter than cell X. Account for the differing lengths of cells A, B, C and D by matching each cell with the possible explanation selected from the list below.

1. Damage or distortion in preparation
2. Natural variation in length
3. A cell in the process of division
4. Two contiguous cells

A2, B1, C4, D3

7 Fig. 2 is an electron micrograph of the same type of bacterium as shown in fig. 1 (i.e. *Escherichia coli*). The picture has been obtained by cutting a very thin section of the bacterial cell along its longitudinal axis. (Details about how this is done will be given later.)
Measure the total length of the cell, and assuming the actual length to be 2.1 μm, calculate the magnification.

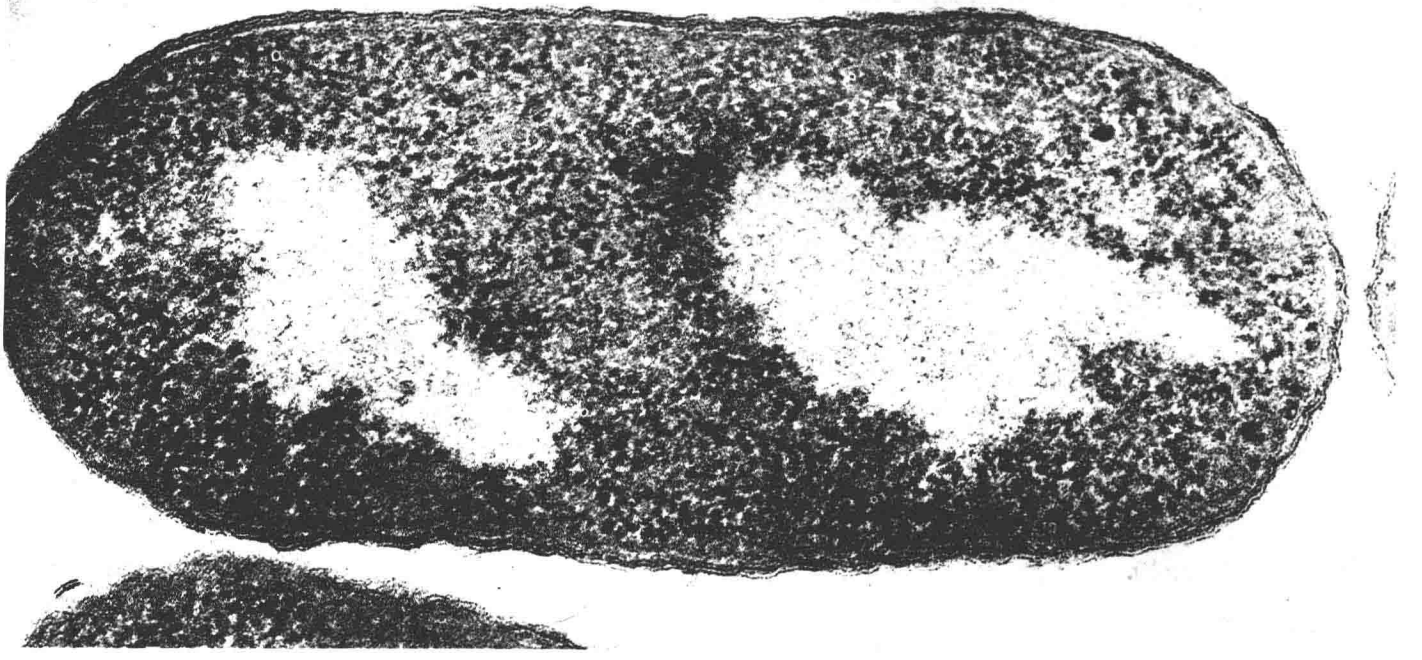

Fig. 2

Measured length 18 cm. Magnification 85 000.

8 Again with reference to fig. 2 what are the major differences between the chromatin bodies shown in fig. 1 and those shown here?

The chromatin bodies are better resolved, but in fig. 2 they are 'light' in appearance. This is because different staining has been employed and because an electron beam rather than a light source has been used (cf. the darkly staining, poorly resolved, chromatin bodies in the light micrograph, fig. 1).

9 The next electron micrograph (fig. 3) resolves another difficulty which we encountered in figs. 1 and 2. What is it?

Fig. 3

The cells at the centre of the picture are in the process of division. It therefore resolves the problem of whether the cells are contiguous (overlapping), or dividing, or the result of variation in length (the total length of the two cells here is 5 μm).

10 How do you account for some apparently very much smaller cells around the two dividing bacterial cells?

Variation in length is one possibility, but the more likely explanation here is that various bacterial cells have been cut in different planes of section (i.e. some have been cut longitudinally, some transversely and some obliquely).

11 With reference to figs. 2 and 3 how many chromatin bodies are apparently present, bearing in mind the answer to the previous frame? (Think carefully about this question before answering it.)

In most cases there appear to be two.
However, there could be alternative interpretations:
(*a*) there may only be one chromatin body present under certain circumstances;
or
(*b*) there may in fact be two chromatin bodies, but only one has been cut by the plane of section.
Alternatively, what appears to be two chromatin bodies (as seen by one plane of section) is actually one chromatin body if viewed in another plane of section, as shown in the micrograph of another strain of *E. coli* below (fig. 4).

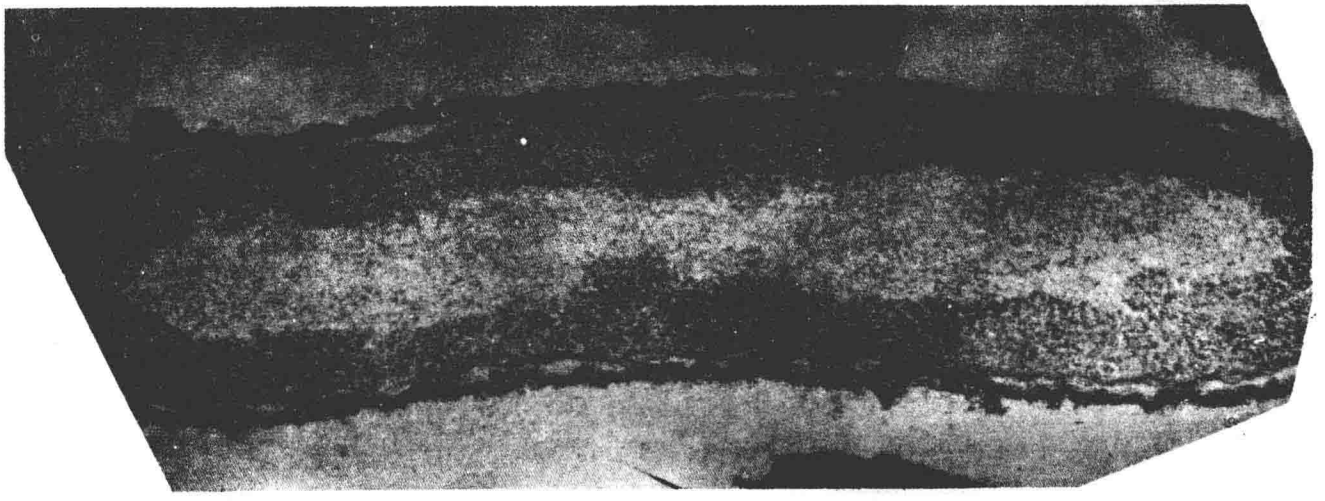

Fig. 4

Note. The explanation of whether one, two or more chromatin bodies are present is complex and depends upon the strain of *E. coli* used and the growth rate in relation to culture conditions and temperature. Under standard conditions there are usually two chromatin bodies.

However, our main aim here has been to get you thinking about the problems of resolution and interpretation of cellular structures.

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12 Again with reference to figs. 2, 3 and 4, what other structural features can be resolved?

(*a*) A limiting boundary is seen as two thin dark lines with an equally thin light band just separating them.
(*b*) A clear region is present just inside the limiting boundary (most marked in fig. 3).
(*c*) Surrounding the chromatin body/bodies, and inside the clear region is a darker area containing abundant minute, yet apparently randomly distributed, particles.
(*d*) Surrounding the darker area is a poorly defined, limiting membrane, just visible as two thin lines separated by an equally thin light band.

These features are indicated in fig. 5 below:

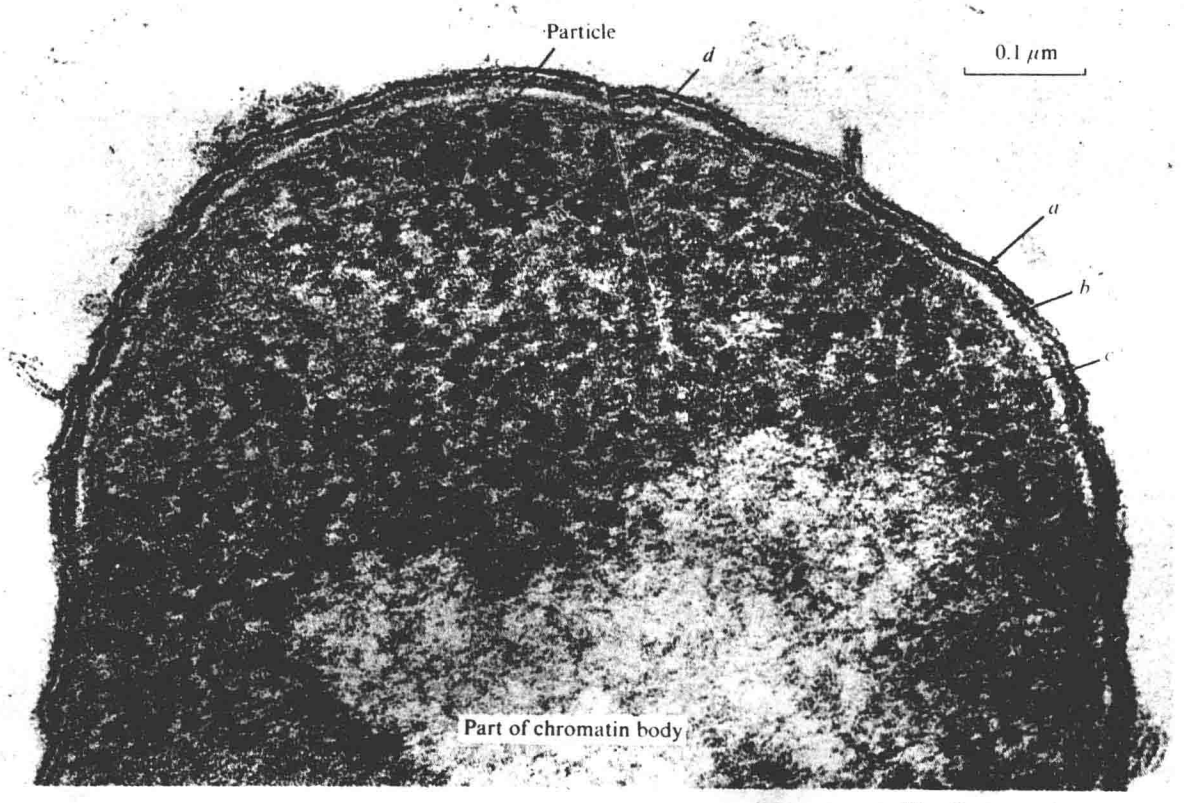

Fig. 5

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13 The limiting boundary referred to in frame 12, together with the clear region inside it, constitute the cell wall complex of this bacterial cell. The poorly defined limiting membrane also referred to above, has an actual thickness of about 7–8 nm. This membrane, which is characteristically present in all living cells, is often referred to as a *unit membrane*.

You can see that the bacterial cell has a protective cell wall complex, which may also provide a means of structural support for maintaining the cell's shape.

What function then can you suggest for the inner limiting unit membrane?

It may have the function of selective permeability; i.e. it will allow some substances to enter or leave the body of the cell, but will prevent others from doing so.

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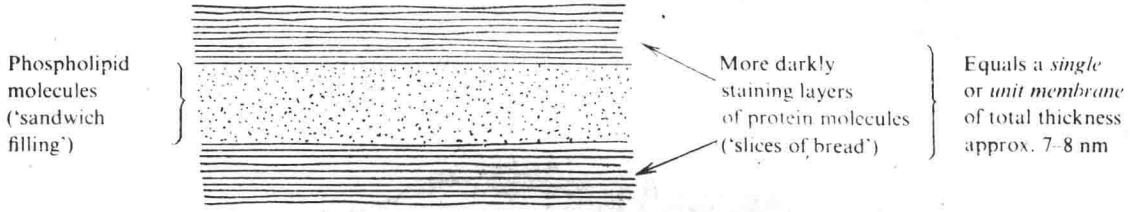
14 One simple interpretation of the unit membrane structure as seen from electron micrographs suggests that it is a three-layered structure, comprising of two layers of protein molecules sandwiching between them a layer of phospholipid molecules as shown:



In fig. 6 are four electron micrographs of cell boundaries from different cells showing a portion of the limiting membrane. From the information given, state whether you can see one or more than one unit membrane present in each case, and give your reasons.