Basic Biology Course

3 Dynamic Aspects of Cells

BASIC BIOLOGY COURSE
UNIT 1
MICROSCOPY AND ITS APPLICATION TO BIOLOGY

BOOK 3

Dynamic Aspects of Cells

MICHAEL A. TRIBE, IRWIN TALLAN, 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

First published 1976

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

Library of Congress Cataloguing in Publication Data
Main entry under title:
Dynamic aspects of cells.
(Basic biology course; book 3: Unit 1, Microscopy and its application to biology)
Bibliography: p.
1. Cytology – Programmed instruction. I. Tribe, Michael A. II. Series: Basic biology course; book 3.
QH581.2.D93 574.8'76 75-46198
ISBN 0 521 21175 1 hard covers
ISBN 0 521 21176 X limp covers

Foreword

This book is part of a Basic Biology Course for undergraduates written by the Inter University Biology Teaching Project Team at Sussex. The final version has been extensively revised in conjunction with Dr I. Tallan on leave from the University of Toronto, 1974–5.

The main aim of this book is to give you an insight into some of the dynamic activities going on inside cells by observing a number of different cells in culture. It is to be hoped that as a result of this study you will have a better appreciation of the great activity shown by the major cell organelles in the metabolic, genetic and locomotory behaviour of cells. You will see how remarkably well organized cells are, and that organelles such as mitochondria, for example, show considerable plasticity of structure, which was not evident from your observations in Books 1 and 2. The main feature of the book with its accompanying film loops, however, is the prominence which it gives to the unique ways in which living cells, and thereby whole organisms, grow and reproduce. Two remarkable processes occur in living systems – mitosis and meiosis. These mechanisms are discussed at length and the genetic consequences of the two mechanisms provide an important conclusion to the book.

Brighton, Sussex, 1975

M.A. Tribe
I. Tallan
M.R. Eraut
R.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:

Dr J.R.S. Whittle for reading the manuscript;

Dr J. Heaysman for her help and co-operation in the first section of this book;

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 and Mrs S. Collier project secretaries;

Mr C. Atherton for photographic assistance.

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

M. Abercrombie, J. Heaysman & S. Pegrum, Dept of Zoology, University College, London (figs. 1, 2 & 3)

B.R. Brinkley, Anderson Hospital, University of Texas (figs. 9 & 10)

E.J. DuPraw, Stanford University School of Medicine (figs. 5 & 6)

Philip Harris Biological Ltd, Weston-super-Mare, Somerset (fig. 8)

J. Kezer, Dept of Biology, University of Oregon, Eugene, Oregon (figs. 25 & 28)

F.J. Longo & E. Anderson, Dept of Zoology, University of Massachusetts, Amherst (fig. 21)

P. Moens, Dept of Biology, York University, Toronto (fig. 23*a*)

J.D. Pickett-Heaps, Dept of Botany, University of Colorado (fig. 7)

H.G. Schwarzacher, Histologisch-Embryologisches Institut, University of Vienna (fig. 16)

G.W. Shaw, Lancing College, Sussex (fig. 12)

M.J. Tegner & D. Epel, Marine Biology Research Division, Scripps Institute of Oceanography, La Jolla, California (fig. 19)

Contents

3.0. Introduction

The first two books of this series dealt primarily with the use of the light and electron microscopes in studying the structure of cells. Many of the preparative methods used with these techniques, while greatly increasing the resolution of detailed structure, kill the cells and hence reveal only static inert structures. However, phase-contrast microscopy allows us to see at least some of the details in living cells and permits a better appreciation of how vital and dynamic cells really are. In association with this book, we would like you to view some time-lapse filming of live cells so that you can see some examples of cellular activity. We begin, therefore, by looking at the structure, behaviour and locomotory activity of cells in culture, but then concentrate on cell division because this basic activity is one of the fundamental characteristics of living systems.

You might question why growth of an organism is accompanied by an increase in the number of cells rather than their size. One reason is that the functional efficiency of a multicellular organism is much greater than that of a single-celled organism. In a human society it is more efficient for the people in it to specialize in particular jobs which society requires, rather than each person trying to do every job. The same is true of multicellular organisms. Here different cells are specialized to perform specific functions, e.g. muscle cells for movement, nerve cells for conducting information, various secretory cells for producing digestive enzymes.

As we shall see in this book, all the cells comprising a multicellular organism arise from a single fertilized egg cell. Therefore, both cell division and cell differentiation are necessary if cells of different shape (morphology) and function are to be found in the same organism.

Another reason for cell division is that extensive increase in size of the organism would be difficult without it, since there is a limit to the size to which any cell can grow. This is determined by the problems of transporting essential substances (e.g. food and oxygen) along a diffusion pathway. Diffusion is only effective over very short distances and consequently the surface area to volume ratio of cells needs to be high (i.e. cells must be small; this will be discussed further in Book 5). If the volume to surface area ratio were to become larger, as it would do if a cell increased in size, activity would be seriously curtailed. Furthermore, even after the organism attains its full size, cell division remains important as a means of replacing old or damaged cells by new ones. Yet the capacity for repairing cells or replacing old cells with new ones gradually declines with increasing age of the individual organism. The phenomenon is referred to as ageing and it imposes on all organisms a finite life span. The ageing process is also reflected in the limited lifetime of human cell strains which have been isolated from the donor organism and placed in culture solution. In no instance has it been possible to subculture these cells indefinitely. However, if the cells are 'altered' in some way (e.g. by agencies such as viruses) their behaviour changes and their potential life span in culture seems to be infinite. Such cells are referred to as 'cell lines' to distinguish them from normal diploid* cell cultures, which are referred to as 'cell strains'. The former have many behavioural characteristics analogous to tumour tissue (cancerous tissue) transplanted into suitable hosts, where once again subcultivation of the tumour tissue in successive hosts may go on indefinitely.

*Diploid cells have two sets of chromosomes, one set derived from the mother and the other complementary set from the father.

3.0.1. Objectives

At the end of this book you should show some appreciation of the dynamic nature of cells by being able to:

(1) Describe the phenomenon of contact inhibition and briefly explain its significance in the early development of an organism.
(2) Distinguish and explain some of the differences between a 'cell strain' and a 'cell line'.
(3) Explain how the distribution of chromosomes is made in cell division compared to that of the cytoplasmic inclusions.
(4) Present and interpret evidence that DNA replication occurs during interphase.
(5) Describe gametogenesis and fertilization.
(6) Contrast the events and consequences of mitosis and meiosis.
(7) Place meiosis within its context of the life cycle of the organism.
(8) Describe synapsis of chromosomes and its significance.
(9) Relate the transmission of genes to meiotic events, particularly:
   (*a*) the segregation of alleles;
   (*b*) the independent assortment of some genes in contrast to the linkage shown by others; and
   (*c*) the occurrence of recombination among linked genes.

3.0.2. Preknowledge requirements

A familiarity with the structure of cells as given in Books 1 and 2, e.g. cell membrane, cytoplasm, mitochondria, ribosomes, nucleus, nuclear membrane, nucleolus, chromosomes.

Elementary knowledge of chemical elements and isotopes.

3.0.3. Instructions on working through programmed text

First check that you have the four film loops which accompany this book; (A) Locomotion and Contact Inhibition, (B) Mitosis, (C) Fertilization and Cleavage and (D) Meiosis. The layout of the text is in programmed form with questions and answers arranged sequentially down the page. You are provided with a masking card and probably a student response sheet or booklet. 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. Then *check* your answer with the answer given. If your answer is correct move the masking card down the page to the next double line, and so on. If any of your answers are 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

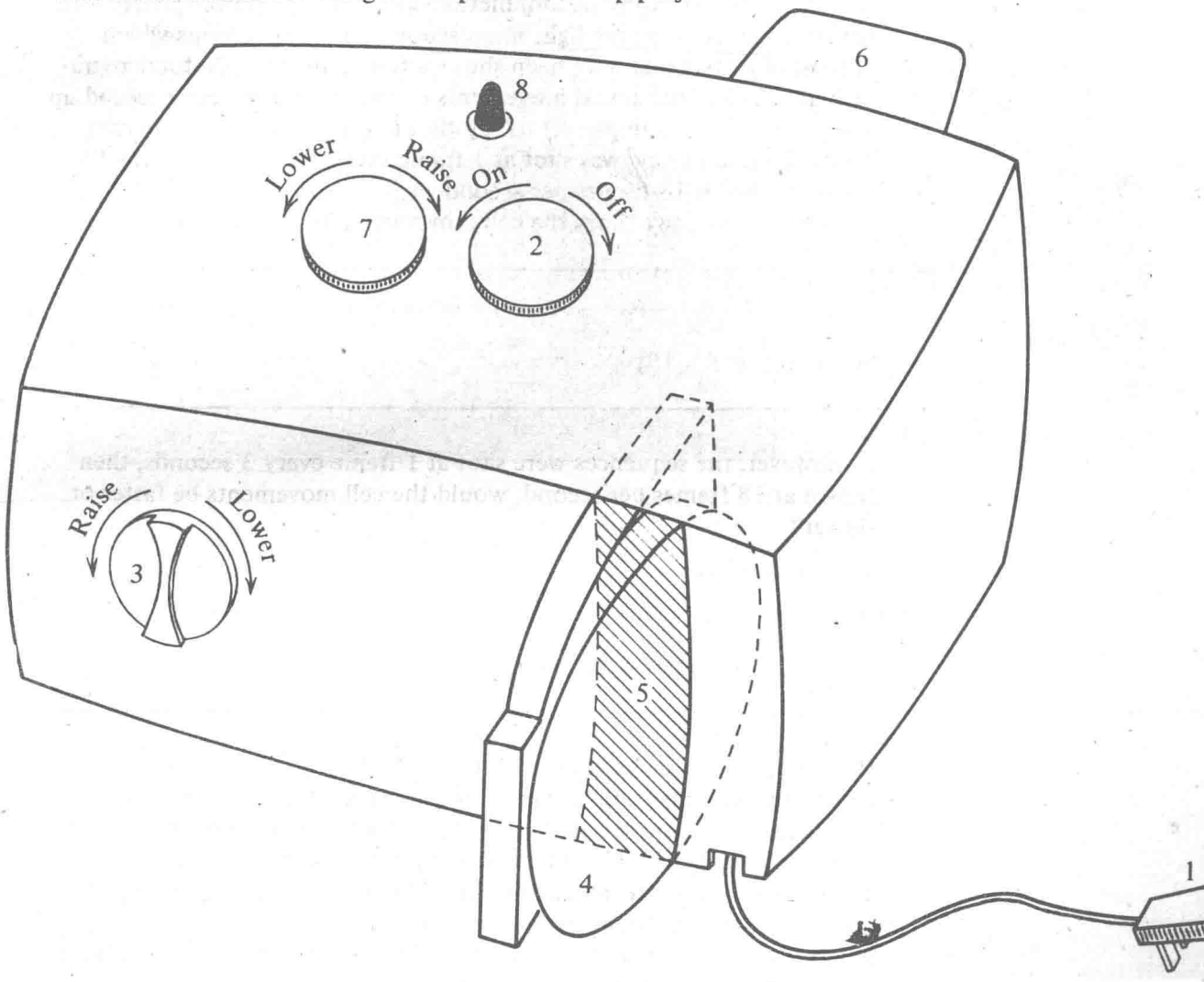
---

is a demarcation between one frame and the next. 'Intermissions' in this book are convenient stopping and starting points in the programme, since it

is unlikely that you will have time to read through the whole book in one session. Always read the appropriate intermission again before going on to a new section. These stopping points are marked by bold double lines.

The Appendixes in this book are complementary to the main text. Appendices 1 and 2 are not programmed, but they are written in the form of structured exercises with intermittent questions which are designed to challenge and involve you in the subject matter. Appendix 3 gives you some suggestions for practical work.

3.0.4. Instructions for using the Super 8 film loop projector



Read the following operational instructions through carefully first. Refer to the diagram and give special attention to F, G, H and I.

A. Plug (1) into mains. Switch on the projector by turning (2).
B. Centre the projector on the screen by moving the *screen* appropriately, and by adjusting (3).
C. Switch off the projector by turning (2).
D. Plug the film loop (4) into the slot (5) as shown in the diagram.
E. Switch on by turning (2).
F. *Immediately* focus the image by turning (6) appropriately.
G. *If necessary*, adjust the frame position by turning (7).
H. Press (8) to hold the film on a chosen frame; press again to restart.
I. Turn (2) to switch off the projector.

3.1. Locomotion and contact inhibition (Film loop A)

1 The previous books in this Basic Biology Course (Books 1 and 2) have given you an insight into the structure of cells as seen both by the light and electron microscopes. Most of the cells which you observed were static and usually fixed and killed so that they could be stained, or stained and sectioned, for the light microscope or the electron microscope. Living cells, however, are very dynamic structures with intrinsic behaviour patterns, and this aspect is more readily appreciated by using time-lapse photography in conjunction with the high power phase-contrast objective on the light microscope. All the film loops which accompany this book have been shot by time-lapse cinephotomicrography. In all cases the actual movements of the cells have been speeded up many times. For example, let us say that in one instance each picture under the microscope was shot at 1 frame every 5 seconds and the film viewing speed is 18 frames per second.
Calculate how many times the cell movements have been speeded up.

90 times (i.e. 5 × 18)

2 If, however, the sequences were shot at 1 frame every 3 seconds, then shown at 18 frames per second, would the cell movements be faster or slower?

Slower (only 54 times the normal speed)

3 Having emphasized the value of time-lapse photography in observing living cells and shown the method of calculating the rate at which cellular activities are speeded up by this process, let us begin our observations of living cells by examining the normal behaviour of some chick and mouse fibroblasts in culture. Fibroblasts in this case refer to certain cells which were obtained from the embryonic heart or muscle. Once obtained, the cells were placed in a special shallow glass dish containing suitable culture medium and incubated at 37 °C; i.e. in conditions permitting growth.
Before you look at film loop A, first examine an individual chick fibroblast (fig. 1), which is identical to those shown in many of the sequences of the film loop.

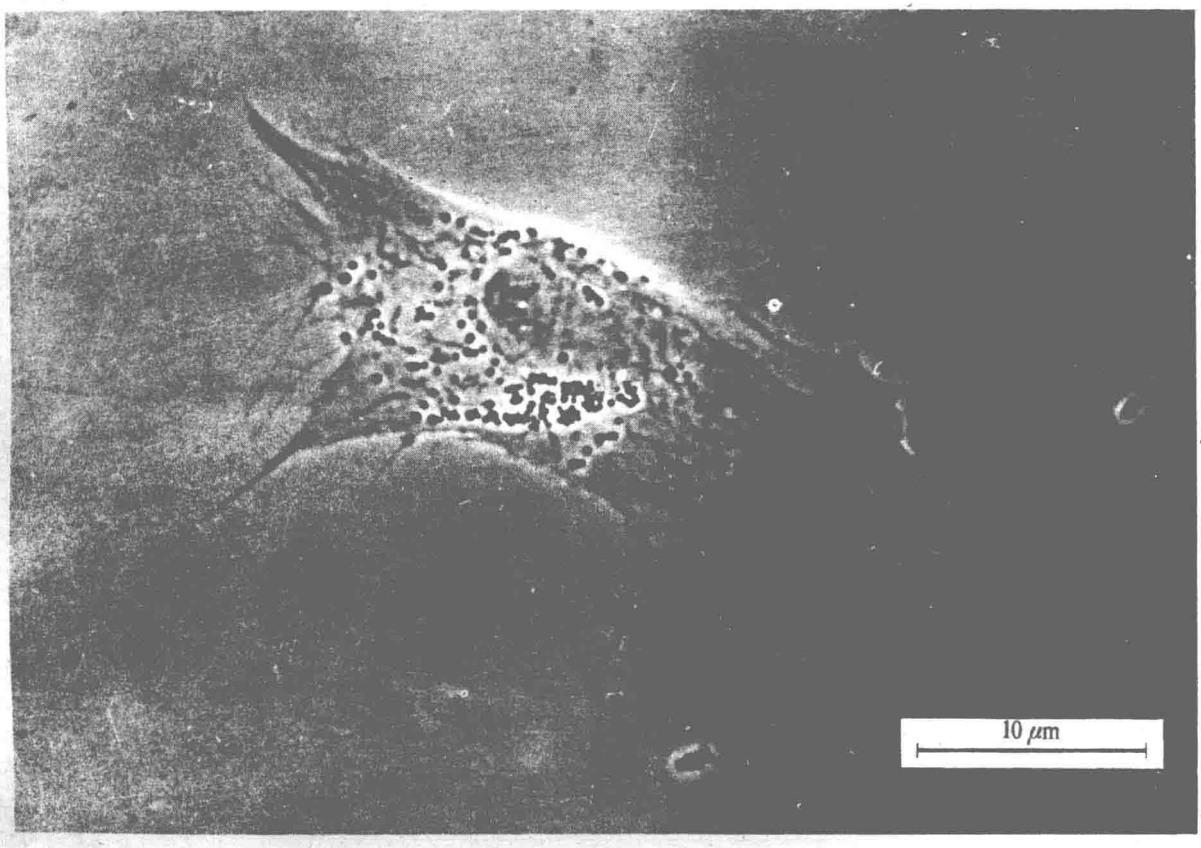


Fig. 1. A chick fibroblast.

Identify and describe as many structures as you can.

The cell has a limiting boundary (we cannot actually see the plasma membrane), which gives rise to fine extensions in some parts of the cell (see the left-hand side of the picture). Towards the right-hand side of the picture the boundary appears 'thicker' in places.
A nucleus can be resolved.
Many dark spherical granules can be seen internally.
Several thread-like bodies (actually mitochondria – the name means 'thread granule') can be observed internally.

[*Note*: conditions for observing the cells are such that the cells adhere to the surface of the glass coverslip (mounted in a specially designed chamber) and thus appear more or less flat.]

4 If the long axis of the cell is approximately 30 μm, (i) what is the longest dimension of the nucleus, and (ii) how many times has the photograph been magnified?
(Your answers should only be approximate.)

Fig. 2. Electron micrograph of a chick fibroblast. From Abercrombie *et al.* (1971), *Experimental Cell Research*, **67**, 359–67.

(i) Between 5 and 6 μm
(ii) 3670 times

---

5 Fig. 2 is an electron micrograph of a similar cell. It is a montage, the picture having been constructed from five overlapping electron micrographs which have been carefully cut and put together to make a composite picture of the whole cell.
Identify the structures labelled A–H.

A	Nucleus	E	Ribosomes
B	Nuclear membrane	F	Endoplasmic reticulum
C	Nucleolus	G	A 'clear' vacuole
D	Mitochondrion	H	A darkly staining inclusion

---

6 The greater resolving power of the electron microscope enables you to calculate the long axis of the nucleus with much greater accuracy than with the light micrograph.
What is the length of the nucleus in μm?

---

10 μm (about twice the apparent size obtained from the light micrograph)

---

7 Can you suggest reasons for the discrepancy between the two measurements, assuming that the cells are of comparable size (i.e. approximately 30 μm long)?

(i) The difficulty in resolving the nuclear membrane in the light micrograph, coupled with the 'halo' effect at edges of objects.
(ii) Although these are the same cell *types* they are nevertheless different cells with, perhaps, variation in nuclear size.
(iii) At the time of fixation and sectioning for the electron microscope, the nucleus (perhaps the whole cell) was in a more flattened state. Cellular structures are quite flexible and capable of changing shape. Look particularly at the way in which mitochondria change their shape when you view film loop A.

---

8 Now with reference to the instructions in section 3.0.4, look at the film loop A (Locomotion and Contact Inhibition). When you have looked at the whole film, concentrate again on sequences 1 and 2, which are of chick and mouse fibroblasts respectively. Pay particular attention to the

behaviour of one cell in the presence of others. When you have done this turn off the loop projector.
How would you describe the movement at the edge of the cell?

Undulating or, better, 'ruffling'. Ruffles arise at the edge of the cell and move back. (Look again at sequences 1 and 2 respectively.)
You can also see details of the 'ruffling' edge in fig. 2.

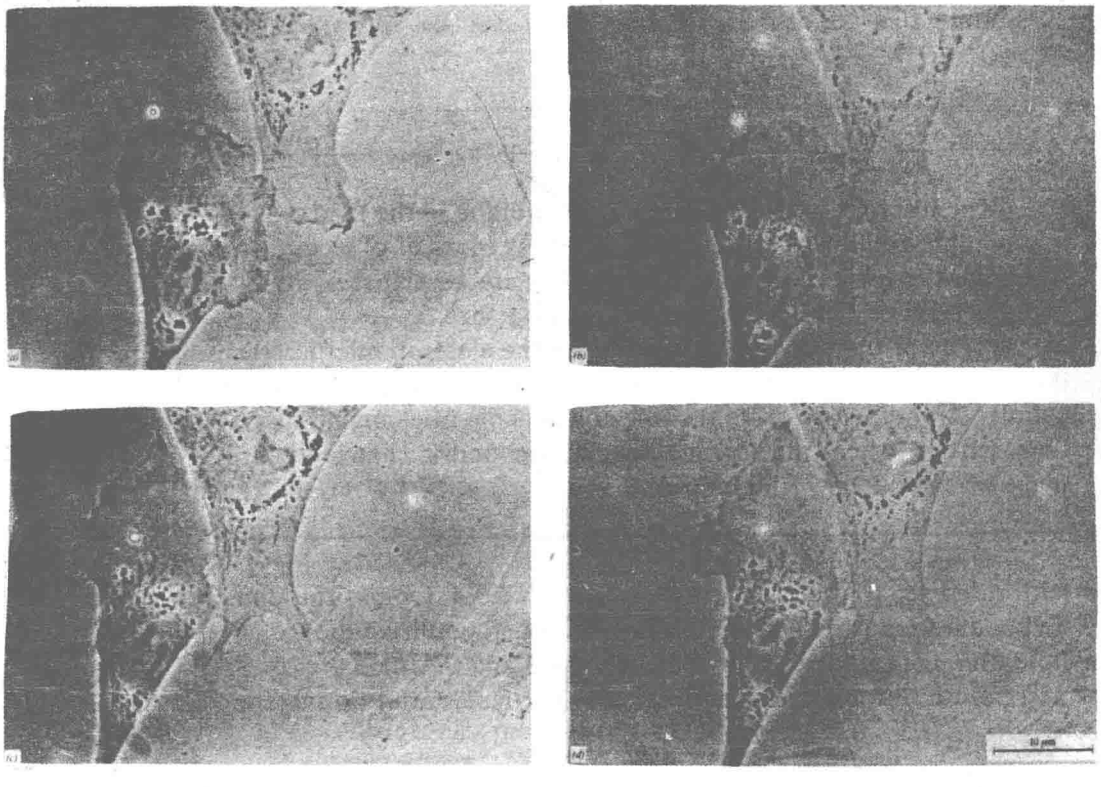
9 Is this movement most vigorous at the leading, trailing or side edge of the cells?

Leading

10 Can you suggest a mechanism by which these cells might move?

(i) The cell might move forward by the undulating movement of the leading edge producing waves of adhesive contact with the surface.

Fig. 3



(ii) The leading edge might extend and make firm contact with the surface, then contract to draw the rest of the cell forward.

---

11 With particular reference to sequence 3 of the film loop, showing chick fibroblasts, which of the following interactions occurs when two cells meet?

(1) One cell passes over the other.
(2) The cells avoid one another without touching.
(3) One cell pushes past the other.
(4) The cells stick together and then move apart.

---

(4) The cells stick together and then move apart.
Contact between cells can be seen more clearly from the still pictures in fig. 3.

---

12 This interaction is called CONTACT INHIBITION. What indicates the new direction to be taken by a cell?

A new, rapidly undulating leading edge is formed
[*Note*: fig. 3 also reveals the cell nuclei with their nucleoli far more clearly than fig. 1.]

---

13 Both sequences 3 and 4 in the film loop show contact inhibition between a few cells. Characteristically there is intensive ruffling of the leading edges of cell membranes as cells move and change direction. However, the sequence 4 of mouse fibroblasts shows a further activity, which is not apparent in the first three sequences. Look at the film loop again and see whether you can spot it – and if you can describe it.

Periodically small, transparent (white) vesicles appear at the rapidly ruffling surfaces of some cells. These white vesicles then move quite quickly into the internal regions of the cells and eventually disappear. (If you did not spot this activity, look at the film loop again.)

---

14 This phenomenon is called PINOCYTOSIS or 'cell-drinking' and the vesicles which you see are pinocytotic vesicles. We shall encounter pinocytosis again in Book 5 (*Cell Membranes*), where it will be discussed in more detail. For the present you should know that pinocytosis enables cells to take up large molecules in solution, which would not otherwise be able to diffuse across the plasma membrane.

---

15 As a result of contact inhibition the movement of cells is strongly channelled into the direction of cell-free spaces, as can be seen in sequence 5 where chick fibroblasts can be seen moving from an explant* into cell-free areas.
Run the film loop again and pay particular attention to this and the last sequence where you can observe what happens when two groups (monolayers)† of mouse muscle fibroblasts meet.
What happens when the available surface space is filled?

*An explant is a cluster of like cells (see Glossary for full explanation).
†A monolayer is a single layer of cells (see Glossary for full explanation).

Cells cannot move without immediately coming into contact with a neighbour, restricting cell movement to little more than oscillations.

16 At this point, when cells cover the available surface, cell division decreases dramatically. This observation led to the conclusion that there was a CONTACT INHIBITION OF DIVISION.
Can you suggest an alternative to space as the possible limiting factor to growth?

Exhaustion of the culture medium

17 Can you suggest some experimental tests of whether cell division has ceased because the medium in the culture is exhausted?

(i) Determine whether medium removed from contact-inhibited cultures will support the growth of non-confluent cells. (If it does it shows that the medium is not exhausted.)
(ii) Remove a section of cells from the edge and then determine whether the cells that move into the vacated space will divide. (If they do, it shows that the medium is not exhausted.)
(iii) Determine whether the concentration of serum in the cultures or more frequent changes of culture medium affect the saturation density attained by the cells. (If they do it suggests that the medium is a limiting factor – seemingly contrary to (i) and (ii).)

18 Recently an alternative suggestion has been made that there is a 'localized' exhaustion of the medium, created by an insufficient exchange (via diffusion) between the layer of medium in close contact with the cells and the bulk of the culture medium.

How can this hypothesis be tested?

By determining the effect of increasing the velocity of medium flow across the cell layer. Stoker (1974) showed that contact-inhibited cells can be induced to divide without changing the medium by increased agitation (shaking) of the culture.

19 Intermission

The role of cell–cell contact in the regulation of cell division of normal and cancer cells remains a disputed point, still under intensive investigation. Indeed, such studies of the growth of cells in culture are only one approach to the broader questions of the migration and differentiation of cells during the embryonic development of an individual. Nevertheless, observations on the behaviour of cells in culture can give us insight into a number of problems. One interesting observation is the differences in survival between cell 'strains' and cell 'lines'.

> A *cell strain* is a population of identical cells derived from animal tissue (usually embryonic tissue), capable of subcultivation more than once *in vitro*, but lacking the property of indefinite serial passage while preserving the chromosomal karyotype* characterizing the tissue of origin. Conversely, a *cell line* is a population of cells derived from animal tissue and grown *in vitro* by serial subcultivations for indefinite periods of time with a departure from the chromosome number characterizing its source. (Hayflick, 1961.)

Experiments have shown that even with the most careful and improved culture conditions using embryonic tissue as the source of the cell strain, the life span of the culture never exceeds about forty to fifty cultivations, i.e. the strain lives, multiplies, ages and dies within a period of 12 months, as shown in fig. 4.

*See Appendix 1 and Glossary for explanation of karyotype.