MICROANATOMY OF CELL AND TISSUE SURFACES

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MICROANATOMY OF CELL AND TISSUE SURFACES:

An Atlas of Scanning Electron Microscopy

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" . . . What is the use of a book
— thought Alice —
without pictures and conversations ".

(L. CARROL, *Alice in Wonderland*)

PREFACE

Progress in learning and understanding the anatomy of cell and tissues has depended very largely on the direct approach of microscopy. At first, the light microscope laid the foundations of the subject. More recently, the electron microscope applied to the study of thin sections has contributed enormously to our understanding of how cells and tissues are constructed. And now, most recently of all, the scanning electron microscope has opened for direct viewing the surfaces of cells and tissues.

This latest development coincides in time with a great increase in research and interest in cell surfaces. No longer are these surfaces shrouded in mystery; they are recognized as dynamic parts of the cell, constantly changing and constantly repairing. Once nothing more than a selective barrier to diffusion of small molecules, they are now known to engage in active transport of metabolites. Their molecular and microscopic structure varies from cell type to cell type as does their function. To be able to see these features at better and better resolutions has introduced totally new concepts about how the cell copes with and exchanges metabolites with its environment.

For these several reasons and to make the new information more generally available to the student and investigator we have put together this atlas on the structure of cells and tissue surfaces. Most of the micrographs have not heretofore been published and many of them depict features of cell and tissue surfaces that have not before been observed by this or any other approach. We have, therefore, been obliged in many instances to provide original interpretations in the descriptions that accompany the pictures. The text also includes some background information on the general properties and functions of the particular tissue under consideration. It is that through all these, the book will contribute to a better understanding of tissue surfaces and inspire a more active investigation of this important aspect of the living cell.

As is ever the case, books are not created without the involvement and generous help of many persons other than the authors. This has been no exception. Thus, we are pleased to acknowledge the loan of a scanning electron microscope (JSM-35) from JEOL, Inc., Medford, Mass., on which most of the micrographs were taken. For technical assistance, we are grateful to Robert McGrew and Virginia Fonte. Our secretary, Karen Bird, seemed never to tire of typing revised versions of the text. For many aspects of the photographic and art work we are pleased to thank Richard Carter and Cathy Verhulst. For literature searches and bibliographic work we have enjoyed the assistance of Leslie Frasier. And finally, what merits the manuscript has in terms of accuracy and English, we owe to the generous reading and editing by Dr. Mary Bonneville.

For some of the best micrographs in the collection, we are indebted to other microscopists among whom are the following: Juan Vial, M. K. Nemanic, D. R. Pitelka, E. Shelton, J. M. Orenstein, and R. H. Steinberg. H. R. Toben provided materials for the blood preparations.

Publishers are frequently the helpful people behind the scenes. In this instance, we want to thank G. F. Vallardi who has been most patient and generous with financial assistance. And not least, if last, to be thanked are our families for granting us so many hours with " the book ".

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April 1977

LIST OF ABBREVIATIONS AND SYMBOLS OF MICROGRAPHS

A Artery
AC Absorptive Cell
As Acrosome
B Bleb
BB Brush Border
BC Bile Canaliculus
BD Biliary Duct
BL Basal Lamina
BoC Bowman's Capsule
Br Bronchiole
BrC Brush Cell
BV Blood Vessel
C Cilium
Ca Capillary
CB Compact Bone
CC Cornified Cell
CF Collagen Fiber
CL Crypt of Lieberkühn
ClC Clara Cell
Co Cone (Retina)
Cr Crypt
D Dermis
DS Disse's Space
E Epidermis
Eb Erythroblast
Ec Erythrocyte
En Endoneurium
EP Enamel Prism
Et Endothelial Cell
F Fenestration (Endothelium)
Fb Fibroblast or Fibrocyte
FC Follicle Cell
Fl Flagellum
Fp Filopodium
FP Filiform Papilla
G Glomerulus
GC Goblet Cell
GE Gastric Epithelium
GG Gastric Gland
H Hepatocyte
HC Haversian Canal
HF Hair Follicle
HL Henle's Loop
HS Haversian System
Il Interstitial Lamella (Bone)
INL Inner Nuclear Layer (Retina)
K Kupffer Cell
L (Hepatic) Lamina
La Lacuna (Bone)
Lc Lymphocyte
LF Lens Fiber
Ll Lamella (Bone)
Lp Lamellipodium
LP Lamina Propria
M Mitosis (with phases of Cell Cycle: G_1, G_2, S, Tp, Ck)
Ma Matrix (Bone)
MA Mammary Alveolus
Mc Monocyte
MC Mucous Cell
Mf Myofibril
Mp Microplica
Mph Macrophage
MP Middle Piece of Sperm Flagellum
Mv Microvillus
MvC Microvillous Cell
N Nucleus
NF Nerve Fiber
NR Node of Ranvier
O Oocyte
Oll Outer Lamella (Bone)
ONL Outer Nuclear Layer (Retina)
OPL Outer Plexiform Layer (Retina)
P Papilla
Pc Podocyte
Pe Periostium
PP Podocyte Pedicle
PT Proximal Tubule
PV Portal Vein
RC Reticulum Cell
RF Reticular Fiber
Ro Rod (Retina)
RS Root Sheath (Hair)
S Sinusoid
SB Spongy Bone
Sc Stereocilium
SC Stratum Corneum
SCB Splenic Cord of Billroth
SD Stratum Disjunctum
Se Sebaceous Gland
SE Seminiferous Epithelium
SeC Septal Cell
SG Stratum Granulosum
SH Sperm Head
Sl Sarcolemma
SL Stratum Lucidum
Sm Sarcomere
Sp Spermatocyte
Sr Sarcoplasmic reticulum
Ss Sarcosome
SS Stratum Spinosum
StE Stratified Epithelium
Sw Sweat Gland
Sz Spermatozoon
UT Uriniferous Tubule
UrP Urinary Pole (Renal Corpuscle)
V Central Hepatic Vein
VaP Vascular Pole (Renal Corpuscle)
VP Vallus of Papilla (Tongue)
VS Venous Sinus (Spleen)
Z Z Band (Muscle)
ZP Zona Pellucida

TABLE OF CONTENTS

CELLS

CHAPTER

1

CELL TOPOGRAPHY

The surface, where the cell faces its immediate environment, is one of the most highly specialized parts of the cell. It consists of at least three layers: the bimolecular leaflet of phospholipid (or *unit membrane*), sandwiched between an outer layer rich in polysaccharide (the *glycocalyx*), and an inner layer representing the adjacent layers of the cytoplasmic cortex. This combination of surface layers is designed to transport actively certain components of the environment that can be used metabolically, and to exclude others. It is modified as well to facilitate transport by a variety of structural features that increase the surface area of the cell without increasing its volume. And finally this surface provides the cell with some protection against fluctuations in the composition, tonicity, etc. of the surroundings.

Some of these surface features are molecular in size and therefore not visible with the scanning microscope; others are relatively macroscopic. These latter fall essentially into three classes: microvilli, ruffles (or lamellipodia) and „ zeiotic blebs ". There are variations on these, especially in fully differentiated cells, but on free cells such as those of the blood and cells growing in culture, the larger topographic features are remarkably limited in the variation they show.

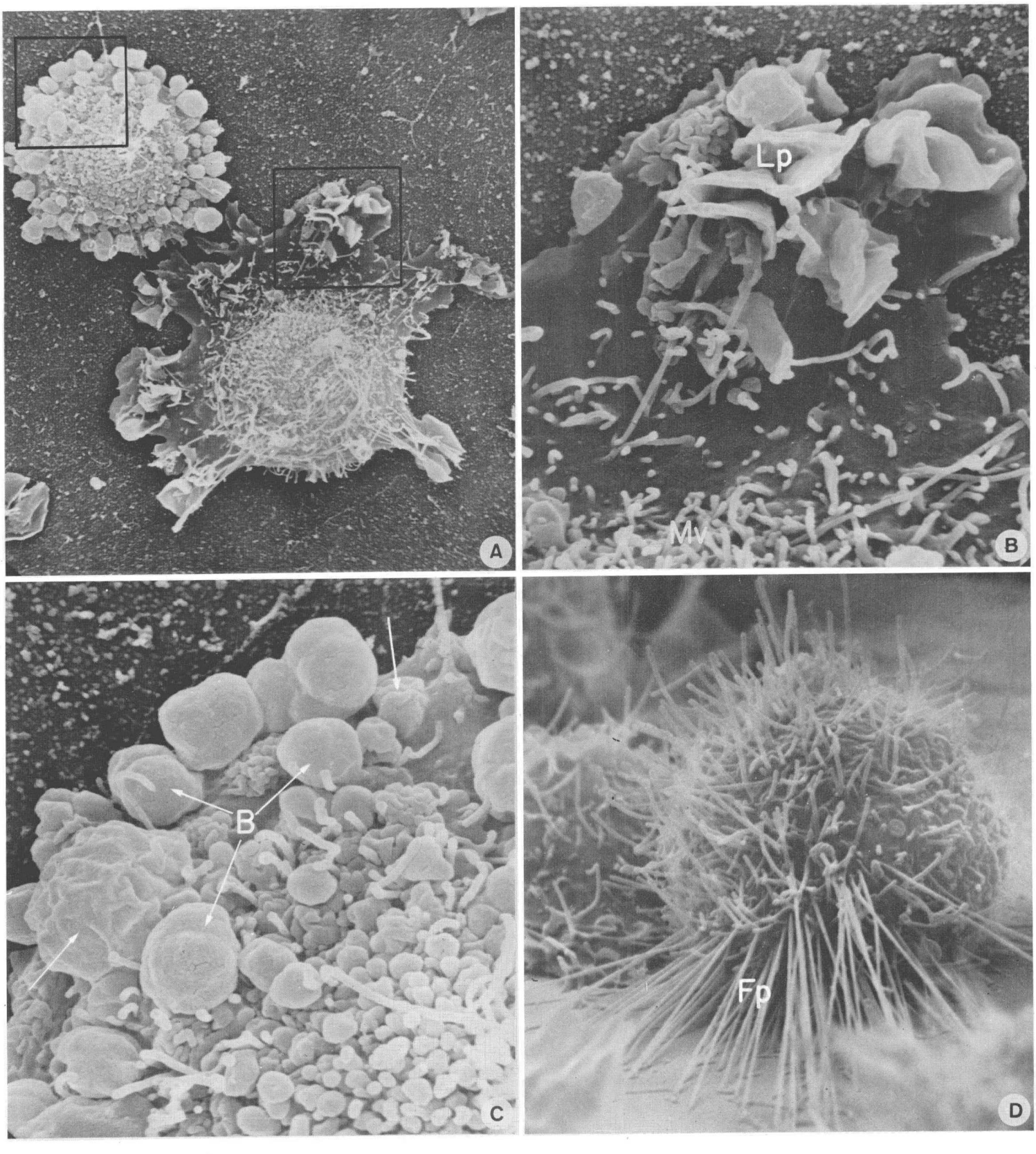
Plate 1

Fig. A: This micrograph includes images of two rat tumor cells in culture. They have recently divided and are beginning to spread out to adopt a form they will retain during the interphase of the cell cycle. The cell at the upper left still shows on its surface numerous blebs which are characteristic of the G1 phase of the cell cycle. The other cell is more advanced in the cycle and has a wealth of ruffles or lamellipodia around its margins. The central regions of both cells are covered with microvilli. The areas within the outlines are depicted at higher magnifications in Figs. **B** and **C** (× 2,500; rat sarcoma cell).

Fig. B: A part of the lower cell in Fig. A shown at higher magnification and resolution to portray the details of ruffling. These structures (lamellipodia, **Lp**) characteristically project vertically from the cell surface sometimes as much as several micrometers. They are usually 0.1 μm thick. They form in a few minutes and appear to migrate from the margin as they engulf quanta of the liquid environment. Microvilli (**Mv**) with a typically uniform diameter of 0.1 μm are included at the lower part of the image (× 6,000; cell from rat sarcoma).

Fig. C: A marginal area of the upper cell in Fig. **A** is included to show the phenomenon of blebbing that is common during certain phases of mitosis and early G1 of the cell cycle. Blebs (**B** →) vary greatly in size from 2 μm down to a small fraction of a μm. Presumably the smaller ones are early stages in the development of the larger ones. Blebs with wrinkled surfaces are assumed to be collapsing (**arrows**). Toward the cell center at the lower right a few microvilli are interspersed with the blebs (× 8,500; cell from rat sarcoma).

Fig. D: The central image here represents a cultured neuroblastoma cell in metaphase of mitosis. The upper surface of the cell is covered with microvilli of which several stand up erectly from the cell surface. The other slender extensions of the surface which reach out and attach the cell to the substrate are called filopodia (**Fp**). These are more slender than the microvilli and appear on the cell principally at this phase in the cell cycle, when they seem to be essential for the cell to be fastened to a substrate (× 6,000; cell of mouse neuroblastoma). From V. G. FONTE, N. WELLER, and K. R. PORTER. 31st Annual Proceedings, Electron Microscopy Society of America. P. 608, 1973.


A
Lp
Mv
B
B
C
Fp
D

Microvilli

Microvilli are slender cylindrical extensions which tend to project vertically from the cell's surface. Their length varies greatly from a fraction of one to several micrometers, but their diameter is normally uniform at 0.1 μm. Some cells show many microvilli, others very few, and in cultured cells such as that in Plate I, Fig. A, they are most numerous over the thicker central part of the cell. Microvilli are generally regarded as a device for increasing the total surface area and thus for facilitating metabolite transport. Individually, in the living cell, microvilli change length quite actively, sometimes elongating and sometimes shortening. In both their number and their length they are probably responsive to the metabolic activity of the cell. It is not surprising, therefore, that tumor cells, with a larger appetite for glucose and amino acids, commonly possess a greater number of microvilli.

Ruffles

Ruffles (or *lamellipodia*) are different from microvilli. These are flat, sail-like extensions which may project several micrometers above the cell surface. They vary in height and breadth but are uniform in thickness at 0.1 μm. In this latter respect only, they resemble the microvilli (Plate 1, Figs. A and B; Plate 2, Fig. A). Ruffles appear most prominently at the cell's margins where, in living cells, they have been observed to develop to their full height in a few minutes. Once formed they tend to migrate from the margin toward the cell center and to surround and engulf quanta of the liquid environment as they move. This phenomenon, called pinocytosis, is particularly common in cells that are rapidly growing and appears to represent an additional device the cell has for incorporating metabolites. Ruffles are particularly common on macrophages which have a phagocytic role to perform (Plate 2, Fig. A).

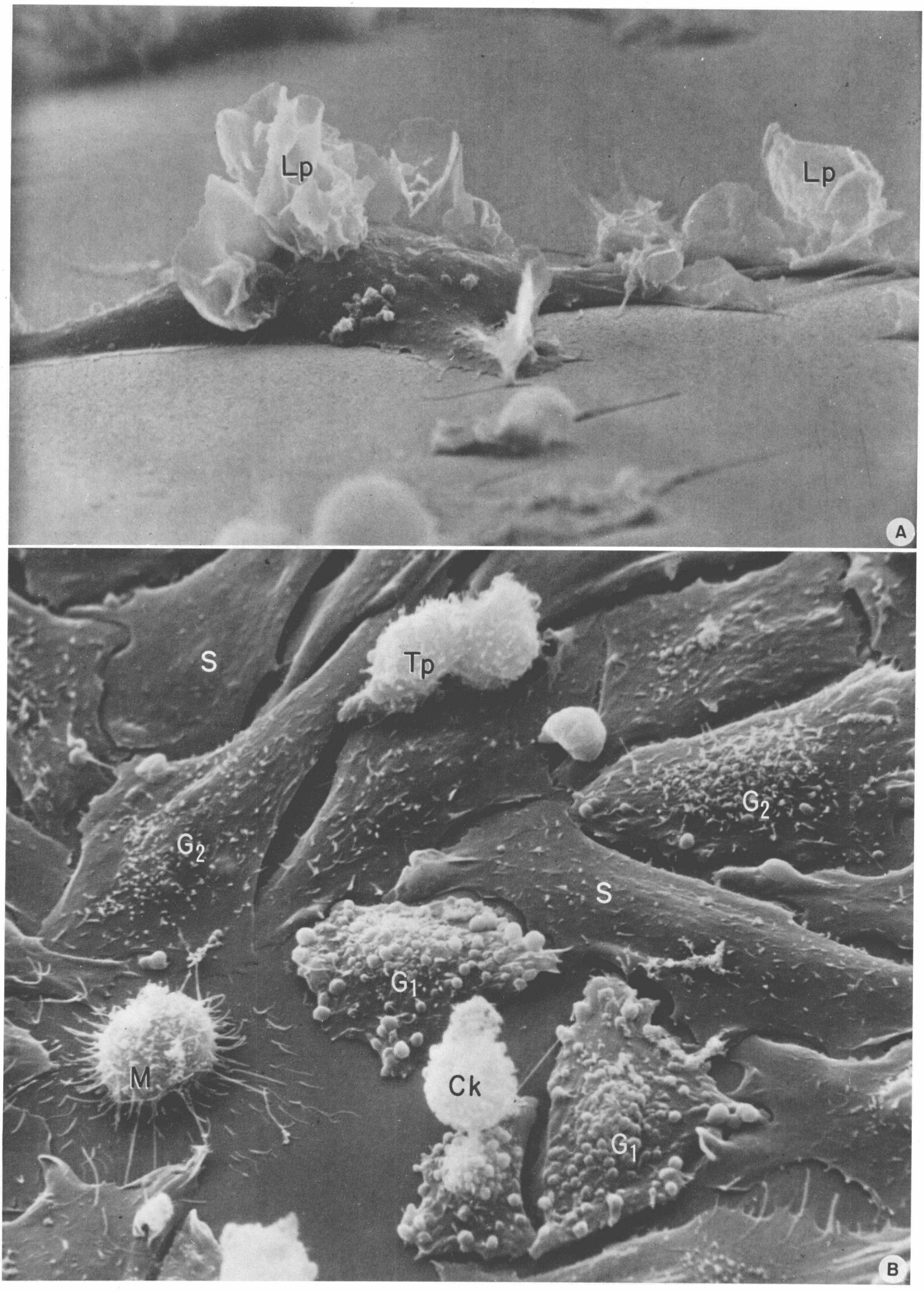
Blebs

Blebs are spherical excrescences from the surface which develop and then recede in the living cell as if the surface were boiling. (Plate 1, Figs. A and C). Their diameters vary from a fraction of a micrometer to several micrometers. They occur over the cell's entire surface but tend to be larger toward the margins. After reaching a maximum size they may shrink to a wrinkled remnant of their former diameter (arrows, Plate 1, Fig. C). Their function is not known. As noted again below, blebs are especially common on cell surfaces during the late phases of mitosis and during the early stages of the cell cycle.

Plate 2

Fig. A: This image offers a lateral view of a macrophage fixed in migration over a flat surface. Its margins and central area show multiple ruffles (**Lp**). These extend like thin sails several μm above the cell surface. They are thought to be important to the pinocytotic and phagocytotic activity of this type of cell ($\times$ 3,500; from body cavity of mouse). From V. G. Fonte, N. Weller, and K. R. Porter. 31st Annual Proceedings, Electron Microscope Society of America. P. 608, 1973.

Fig. B: This micrograph depicts a population of Chinese hamster ovary cells (CHO) growing in tissue culture. The cell at **M** is in mitosis and shows a characteristic population of filopodia extending from the cell to the substrate. Cells marked $\mathbf{G_1}$ are in that phase of the cell cycle. It is typical for their surfaces to show many blebs. Cells in this phase (**S**) of the cycle tend to have smooth surfaces, whereas those in $\mathbf{G_2}$ are larger and require numerous microvilli on their surfaces. Those cells in the image marked **Tp** and **Ck** are in telophase and a late stage of cytokinesis respectively ($\times$ 2,000; CHO cells). From K. R. Porter, T. T. Puck, A. W. Hsie, and D. Kelley, 1974. Cell 2:145-162.


Lp
Lp
A
S
Tp
G2
G2
S
G1
M
Ck
G1
B

CHANGES IN TOPOGRAPHY DURING THE CELL CYCLE

It has been observed that the topography of living cells changes constantly, and this is particularly true for cells that are growing and multiplying. Where, under *in vitro* conditions, such cells can be synchronized so that the majority of a population are all in mitosis or some other phase of the growth cycle, it is possible to identify a topography with each of the separate phases. Thus when cells are in metaphase of mitosis, it is customary for them to attach to the substrate by slender filopodia (Plate 1, Fig. D; Plate 2, Fig. B). These filopodia differ from microvilli in being more slender and generally longer; their distal tips are modified attachment. Later the microvilli that are present on cells in early mitosis are replaced by blebs. These persist into the early phases of G1 (the first growth phase). When, after 8 or 10 hours, the cell moves on into S the replication of its DNA, it tends to stretch out and to lose its surface excrescences (Plate 2, Fig B). The relatively smooth appearance of S cells is followed by a return of microvilli as the cells enter G2 and prepare to divide.

SURFACE FEATURES OF DIFFERENTIATED CELLS

Cells commonly differentiate to perform specific functions, and these may depend on the development of special surface structures. Among these, microvilli are not very different from those features just described as occurring on certain free and relatively undifferentiated cells. Others, however, are unique. It is the purpose of this section to look at all of these surface features as they occur on differentiated cells.

Plate 3

Fig. A: Cilia (**C**) are among the most prominent and interesting of cell surface extensions. Except in certain sensory epithelia where they are modified to receive stimuli, they are, as here, motile. With a diameter of 0.2 μm they are easily visible in scanning electron micrographs. In this instance a few mucus cells (**MC**) are visible through the cilia (**C**) (× 9,000; from a surface in the nasal cavity of a rat).

Fig. B: Stereocilia (**Sc**) are common on several tissue surfaces and especially in the male reproductive tract. They are really very long microvilli. Unlike cilia, they are non-motile (× 11.000; from the epididymis of the rat).