An Introduction to Electron Microscopy

'Yes, I have a pair of eyes,' replied Sam, 'and that's just it. If they wos a pair o' patent double million magnifyin' gas microscopes of hextra power, p'raps I might be able to see through a flight o' stairs and a deal door; but bein' only eyes, you see, my wisdom's limited.'

Sam Weller in"Pickwick Papers". Charles Dickens.

By Dr. C. R. Leeson and Dr. L. T. Threadgold.

Until the early 1940's conventional methods of histology and cytology involved the use of the light microscope. Relatively early in its history, this instrument was made to preform to its optical limits. Advances in light microscopy, therefore, have been mainly in the application of physical techniques, such as improvements in microtomes, the use of ultra-violet absorption or the phase contrast microscope, or of chemical methods, such as histochemistry.

The major limiting factor of the light microscope is its resolution (the ability to distinguish as separate two points lying in close proximity). Resolution depends upon the wave length of the light source and the numerical aperture of the lens and therefore, with an optical microscope, details which are smaller than about one half the wave length of visible light are impossible to distinguish. As the numerical aperture is fixed at about 1.4, the only hope for higher resolution depends upon using an illuminating source of shorter wave length than visible light. Hence the development of the ultraviolet microscope, the X-ray microscope, and the electron microscope.

The structure of light and electron microscope is in many ways analogous, see diagram. In the latter instrument, the illuminating source is a beam of high velocity electrons, accelerated in a vacuum, the beam being passed through the material to be examined and focussed upon a fluorescent screen by means of a series of electromagnetic lenses. The images of the specimen can then be viewed upon the screen or a photographic plate substituted if a permanent record is desired. The wave length of the electrons depends upon the acceleration voltage. At 50,000 volts, the wave length is 0.0535 Angstrom units (one Angstrom is one ten millionth of a millimetre). This can be compared with the wave length of monochromatic light which is 4,000 Angstroms. Therefore, the theoretical limit of the electron microscope is approximately 4 Angstroms compared to about 1700 Angstroms for the light microscope.

However, the electron microscope itself has inherent disadvantages in that the numerical aperture must be small, due to the abberations of magnetic lenses and, in biological research, the general resolution attained is about 20 Angstroms. Furthermore, electrons have poor penetration and the system must operate in a vacuum. Because of these two facts the electron microscope, although commercially available for two decades, has only recently been employed in biological research.

Due to the high magnifications involved and the poor penetration by electrons, it is essential that tissue structure is preserved in as great a detail as possible and that ultrathin sections are cut. Essentially, the preparation of material for electron microscopy follows the three main stages of preparation perfected by light microscopists: (1) the fixation of living material in such a way that changes to the structure are minimal, (2) sectioning of the "fixed" material, (3) selective staining in order that certain parts of the cell are emphasised.

It is the second stage which is of prime importance in electron microscopy. Sections between 200-400 Angstroms in thickness are required in order that the majority of electrons can pass through the tissue without being scattered, and can energise the fluorescent screen. With too thick a section, the majority of electrons is scattered and the fluorescent screen will show no image, whereas if the section is extremely thin, too many electrons pass through it and contrast is so low that structures are indistinguishable from each other. The problem of thin sectioning has been solved by the use of a hard embedding material—a synthetic resin such as methacrylate—instead of the paraffin wax used by light microscopists and by the perfection of special or ultra-microtomes. Important in ultramicrotomy has been the development of knives made of either glass or commercial diamonds. The latter are in use in the Anatomy Department of Dalhousie University.

It is essential that the cutting edge of the knife is perfectly smooth, has no mininature ridges or grooves, as these destroy structure during the sectioning procedure.

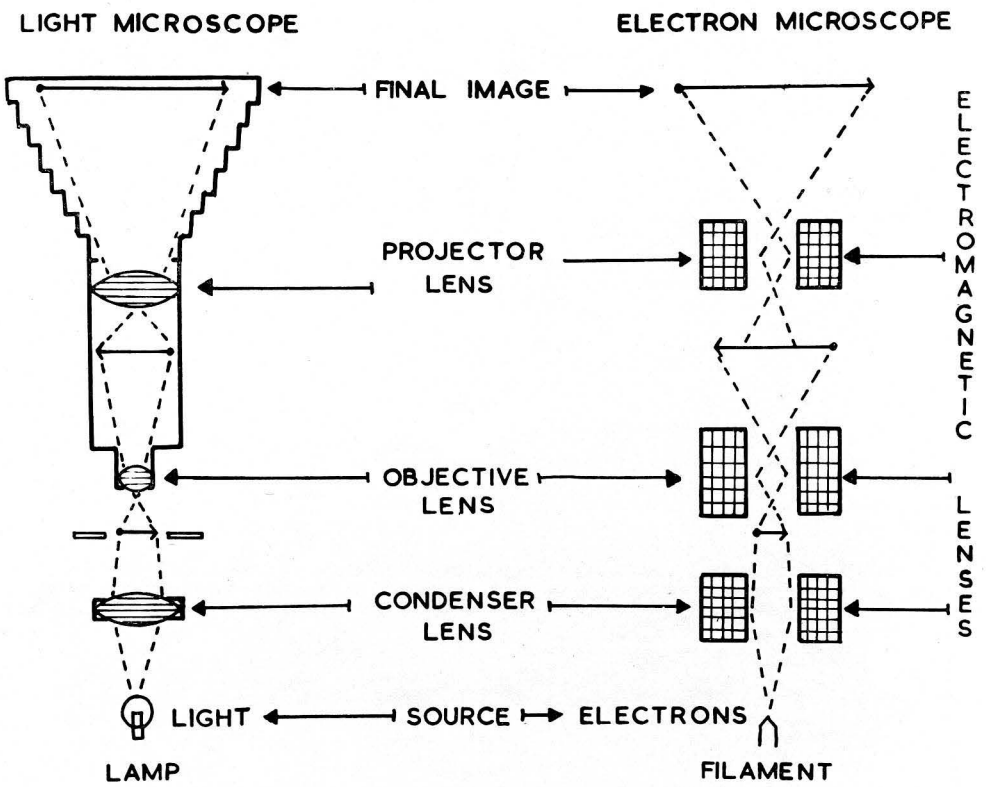
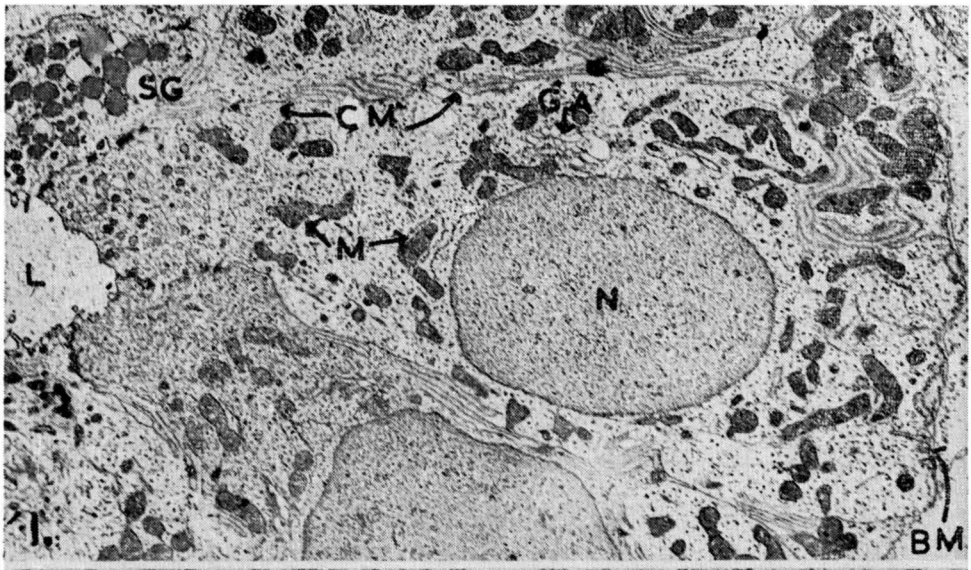


Diagram illustrating the essential features of the light and electron microscope. The column of the electron microscope has been inverted for ease of comparison.

The third stage of preparation—of staining the tissue so that some parts of the cell will obstruct the electron beam more than others and so become visible upon the fluorescent screen—has been achieved in most instances by combining it with the first stage, fixation. Osmium tetroxide, used commonly as the fixative, is at the same time taken up selectively by certain parts of the cell, and these are thus rendered more 'opaque' to the electron beam because they scatter electrons more than other areas. Before a section or other specimen can be observed in the microscope, it must be supported upon a close-meshed copper grid (diam. 1/8 inch: mesh 200 per inch) which has been covered with a plastic film.

What are the Potentials of the Electron Microscope in Biology and Medicine?

The potential of the electron microscope is best shown by a description of the ultrastructure of a "typical cell". With the use of very thin sections, however, problems of interpretation arise. Since the magnification with the electron microscope is so much greater than that of the light microscope, details which cannot be resolved with the ordinary microscope—the so-called"ultrastructure"—are visible. This led to much criticism of the early workers in this field, since it appeared likely that many of these details were artefacts introduced during specimen preparation. However, different cells from a wide variety of organisms show similar details even when prepared with fixatives other than osmium tetroxide, and such ultrastructure is now generally accepted as a reality.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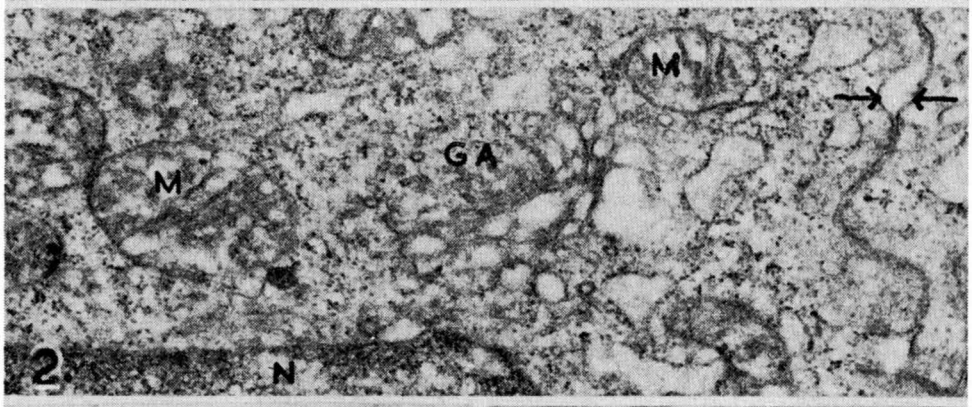
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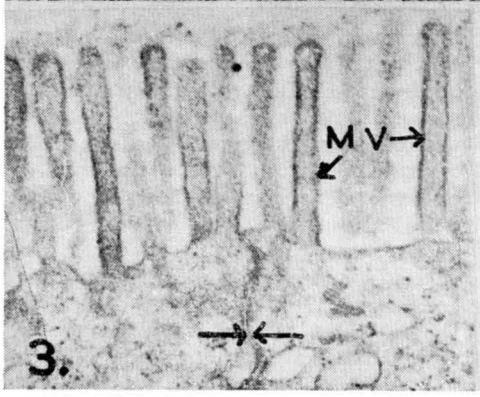
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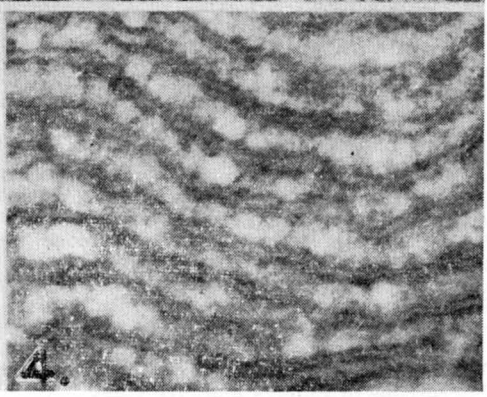
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The cell consists of the nucleus and cytoplasm, with the principle components of the latter being the mitochondria, Golgi complex, centrioles, chromidial substance, secretory granules and stored materials such as fat and glycogen, and the cell membrane.

The Nucleus.

The nucleus usually presents a fairly homogeneous, granular appearance but in some cells, denser regions, which probably correspond to the chromatin granules observed in light microscopy, may be seen, particularly towards the periphery of the nucleus. The nucleolus, when seen in thin sections, is composed of a system of coiled filaments which are made up of small dense granules. The nucleus is bounded by a membrane which is a double layered structure of a total thickness of 300-400 Angstroms, the outer layer of which is more tenuoos than the inner layer. Recently a number of observers have noted the presence of pores (about 500 Angstroms in diameter) in the unclear membrane. At the pores, the outer and inner layers are continuous, and their occurence would appear to indicate that there is free communication between the nucleus and the surrounding cytoplasm. The electron microscope has therefore proved what has long been suspected from the evidenve of light microscopy. The outer layer of the nuclear membrane has numerous granules attached to its cytoplasmic surface and in places shows small projections, which appear to be continuous with the cytoplasmic membranes described below. Although the nuclear membrane is included as part of the nucleus, there appears to be good reasons for including it with the general cytoplasmic membranes.

Mitochondria.

These cytoplasmic organelles contain several complex enzyme systems but no details of internal structure are visible with the light microscope. With the electron microscope complex internal structure is revealed. Mitochondria are bounded by two membranes, the outer of which is smooth, while the inner is thrown into narrow folds or cristae, which project into the interior of the organelle. Although the number and position of these internal membranes vary widely, the general pattern appears constant. There is a variation in arrangement of cristae from cell type to cell type and also in the same type of cell under different physiological conditions. Variations in pattern are even more striking between normal cells and those undergoing metaplasia. In normal liver cells, for example, the cristae are widely separated, but in those of hepatomas, the cristae are closely packed and more highly developed. This can probably be related to the increased metabolic activity.

Golgi Complex.

The Golgi complex or apparatus has been the subject of controversy among cytologists ever since it was described by Golgi in 1898. Much of the confusion was due to the fact that the majority of components of the complex was below the resolution of the light microscope. However, it has long been considered to be the site of elaboration of secretions, for example, the acrosome of the maturing spermatozoon. In electron microscopy, the Golgi complex consists of three fairly distinct components: 1. a series of vacuoles 2. layers of closely packed membranes in relation to the vacuoles, and 3. many small vesicles in the surrounding cytoplasm. The fundamental structure appears to be the membranous layers, really very flattened and elongated vesicles, which have no associated granules.

Centrioles.

In light microscope preparations, one can occasionally see one or two dark dots, the centrioles, within the Golgi region. These small particles are rarely seen in the thin sections used for electron microscopy but when seen, appear to have a constant mor-

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Plate:

Fig. 1. Low power electron micrograph to illustrate the features of a "typical" cell. Shown is an epithelial cell lining the duct of a salivary gland. Notice the nucleus (N), the basement membrane (BM), the lumen of the duct (L), the Golgi apparatus (GA), mitochondria (M), the cell membrane (CM), with numerous infoldings, and secretory granules (SG). x8,000.

Fig. 2. High power electron micrograph of the paranuclear region of an epithelial cell lining the gut. Notice the nucleus (N), mitochondria (M), Golgi apparatus (GA), and the opposing cell membranes of two cells (arrowed). x50,000

Fig. 3. Microvilli (MV) on the apical border of an epithelial cell lining the gut. Notice again the opposing cell membranes at the interphase between cells arrowed. x50,000

Fig. 4. Endoplasmic reticulum in an acinar cell of the submandibular salivary gland. The granular component (RNA) of the double membranes is clearly shown. x55,000

phology. They are cylinders whose walls are composed of a number of pairs of fibrils arranged around a central lumen. They are thought to play a role in the organisation of fibrillar material, for instance the formation of cilia, whose fine structure has many similarities to that of the centrioles. During cell division, they are associated with the formation of the spindle and astral rays.

Chromidial Substance.

In the cytoplasm of some cells, there are regions which stain with basic dyes, i.e. they stain in a similar manner to the chromatin of the nucleus. Due to this characteristic, the material in these regions is known to light microscopists as the chromidial or basophilic substance. In recent years, this substance has been shown to be rich in ribonucleic acid (RNA). Previous to electron microscope studies, little was known about this component, but it has now been revealed as consisting of a number of fibrils or lamellae, often lying close together. More recent work has shown that the fibrils or lamellae are, in fact, sections of very flattened membranous vesicles, with small granules attached to their outer surfaces. The term "endoplasmic reticulum" has been applied to these vesicles or membranes, together with their associated granules, which are composed of ribonucleoprotein. The degree of development and and arrangement of the endoplasmic reticulum varies widely in different cell types. In strongly basophilic cells, such as the acinar cells of pancreas and some salivary glands, and plasma cells, development of the membranes is striking. In some cancer cells, the membranes are poorly developed but there are excessive numbers of associated granules (of RNA), indicating a rapid protein synthesis.

Other Cytoplasmic Constituents.

Certain cell types contain other cytoplasmic contituents, which will be mentioned only briefly. They are of three types. The first type includes stored material, such as glycogen or fat, which usually appears distinct and homogeneous on electron micrographs. The second type is material elaborated by the cell, such as secretory granules in salivary glands or crystalline inclusions in the interstitial cells of the testis. Finally, fine fibrils are present in the cytoplasm of some cells and are particularly well developed in all types of muscle cell.

The Cell Membrane and its Specializations.

In electron micrographs, the cell (or plasma) membrane appears as a single line. At the interphase between cells, the membrane is usually regular in outline but on the free surface it often appears folded. A good example of the latter is seen in the epithelial cells lining the small intestine, where the striated border of these cells can be resolved into numerous finger-like projections or microvilli, bounded by a continuous cell membrane. A similar specialization is seen in the proximal convoluted tubule of the kidney, where the 'brush' border is seen to be due to the presence of numerous, irregular microvilli. Other complex foldings of the cell membrane are present in the basal regions of some epithelial cells, where they adjoin the basement membrane, for instance, in ducts of salivary glands, the epithelium of the choroid plexus, and certain epithelia of the kidney. Functionally, these infoldings increase the effective cell surface and are related to a fast transport of water and ions into the cells from surrounding blood vessels.

The Significance of Electron Microscopy in Medicine.

Here, the word medicine is used in its broadest sense, to include physiology, biochemistery, anatomy, embryology, pathology, and bacteriology. At present, the electron microscope is a research tool used mainly in the study of morphology. It must be made clear at the outset, however, that a study of ultrastructure is not an end in itself. Any advances in the study of ultrastructure must be related to function—to chemical and physical reactions—in order to achieve a complete understanding of biological phenomena. Therefore the study of ultrastructure is a field where the cytologist meets with the biochemist and biophysicist. It is a field which must be shared if the biological significance of details is to be understood and appreciated. A good example of this is the brillant work of Huxley on the ultrastructure of muscle, which has led to a satisfactory 'structural' explanation of the phenomenon of muscle contraction. As a result of his work, Huxley has proposed a "two filament" theory of contraction. Within the muscle myofibrils, there are two types of myofilaments, the thicker composed of myosin and the thinner composed mainly of actin. Short, hook-like bridges span the gap from the thick filaments to the thin. During muscle contraction, these bridges, due to energy release at their tips, act as 'ratchets' to draw the thin filaments in between the thicker ones, thus shortening the overall length of each myofibril.

A close correlation between structure and function is also possible in studies of problems of growth, cell differentiation and development. This is a field which occupies the attention of the present authors. One

problem under investigation at the present time is the development and differentiation of the notochord in various species. Aspects of this problem are, the formation of an external bounding sheath, whether the notochordal cells form a syncytium at any stage and the intra—or extra—cellular position of the large vacuoles which typify the notochord. All of these can be related to its role as a primitive supporting structure. Other problems in this field, at present under investigation here and elsewhere, include studies of the origin, from undifferentiated mesenchyme cell, of muscle cells and their myofibrils and of the cilia in the epithelial lining of the respiratory tract. The ultrastructure of normal cells throughout their life and physiological cycles, and the biophysical and biochemical processes with which they are associated, must be understood before any deviations from that normal can be evaluated.

It is possible to hint at only a few applications of the electron microscope to clinical problems. Already electron microscopy has opened up a wide field of study with regard to the structure and nature of bacteria and viruses. Here, additional techniques of specimen preparation are utilized, chief amongst them being the use of replicas, or casts, of whole organisms. The relationship between viruses and experimentally induced cancer has already been studied extensively. With regard to cancer in general, there is a growing appreciation of ultrastructural details which appear to characterise certain cancer cells. A few have been mentioned briefly in this article, for instance, the abnormal appearance of the cristae in mitochondria and the alteration in proportion of the components of the endoplasmic reticulum in hepatoma cells.

Future development in the field of electron microscopy will embrace technical improvements in the electron microscope itself, resulting in better resolution, and improvements in specimen preparation, such as the application of histochemical and cytochemical methods. These will, it is hoped, allow study of the cell and of its constituents at a molecular level. With knowledge of the normal ultrastructure of cells established, the way will then be open for studies upon experimentally altered cells. Of immediate interest here is the nature of changes in cell morphology and chemistry induced by the administration of drugs and hormones. Again, future research with the electron microscope will undoubtedly add greatly to our knowledge of nuclear-cytoplasmic interaction and of the mechanism of self perpetuation of the cell. Tremendous advances have been made in the brief decade which has followed the acceptance of the electron microscope as a research tool in the biological sciences. The next decade may well see an extension of its use to problems of routine diagnosis, prognosis and treatment evaluation.

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Suggested Reading.

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2. C. E. Hall. Introduction to Electron Microscopy. McGraw-Hill. 1953.
3. H. E. Huxley. The contraction of muscle. In Scientific American, vol. 199. Nov. 1958.
4. F. S. Sjostrand. The ultrastructure of cells as revealed by the electron microscope. International Review of Cytology, vol. 5. 1956
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6. V. K. Zworykin et al. Electron Optics and the Electron Microscope. John Wiley & Sons. 1948.

BOOK PRIZES

The Dalhousie Medical Students' Society awarded the following prizes for the year 1959-1960, on the basis of academic standing, financial need and participation in Student Affairs:

Second Year—W. B. Kingston
Third Year—M. A. MacKinnon
Fourth Year—Edward MacCarron
Fifth Year—Yale Kanter