INTRODUCTION

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ELECTRON MICROSCOPY

History and Purpose of Electron Microscopy

This work is a technique covering standard methods of the preparation of biological materials in transmission and scanning electron microscopy. The transmission electron microscope (TEM) is similar in function to a light microscope and requires thin specimens that allow electron penetration. The scanning electron microscope (SEM) is used to study specimen surfaces, similar to a dissecting microscope, and requires specimen conductivity and resistance to the electron beam. Both instruments require special preparation of biological material and the goal of this text is to present the theory and procedures for such preparation. The instruments are new and the possibilities for use in biology are immeasurable. The development of electron microscopy represents one of the most rapid advances of a research tool in the history of science. In comparison, the development of the light microscope took about 370 years while the present day instruments utilizing electrons are the result of less than 50 years development.

The importance of the electron microscope to biology can be seen in a comparison of resolution capabilities (Table 1 - 2). The light microscope extended man's visual observations to particles as small as 0.2 Um, whereas the transmission electron microscope is capable of viewing particles (or rather molecules) the size of 0.1 to 0.2 nm, the scanning electron microscope can view surface features to 5 to 10 nm. The traditional units of measurements are given in table 1-1 as well as the international or SI units which will be used here.

Since 1950, the TEM has been used for direct examination of cell surface down to the level of micromolecules. Thus, the instrument has bridged the gap between direct examination by light microscopy (resolution ca 0.2 um; table 1-2) and determination of molecular structure of X-ray diffraction and birefringement microscopy. The SEM came into use in the late 1960s allowing accurate three dimensional viewing of fine surface structure and determination (electron probe) of cellular constituents thereby connecting surface structure with chemical constituents.

The findings resulting from electron microscopy have greatly influenced modern biology. A modern text in biology refers frequently to the concepts of viral structure as well as

prokaryotic and eukaryotic cell structure. Elegant scanning electron micrographs demonstrate the three dimensional structure of pollen grains, spores, and animal surfaces. With regard to viral fine structure, the electron microscope quickly yielded a large amount of new information. First, three organisms, being too small to be seen individually under a microscope (10 to 500 nm), were found to have a number of characteristic shapes and sizes. From this information, certain malignant types could then be identified in tumor tissue. Secondly, the instrument provided an accurate method for counting virus particles. Finally, the electron microscope permitted the investigator to determine the substructure of the protein coat and the process of bacterial infection by phage type virus. A number of Nobel Prizes have been awarded in associated areas of research, especially in regard to the transfer of genetic information and structure-function of cell organelles.

Probably the greatest contribution to biology was the use of the transmission electron microscope in fine structural studies of cells of higher organisms. The chief difference between light and electron microscopic images of cell lies in the far greater amount of detail visible, using the electron microscope. One of the important generalizations to emerge from the explosive

advances in knowledge of cell fine structure concerns the ubiquity of complex membrane systems. The various organelles such as mitochondria plastids, and Golgi bodies appear as sharply defined objects with intricate internal structure.

A number of previously unknown organelles have also been found (lysosomes). This detail, of course, is present because of the one thousand fold increase of resolving power of the electron microscope. The fine detail has permitted biochemists as well as biologists to postulate how various physiological processes could be carried out in the respective organelle. That is, where the enzymatic systems might reside and how these enzymatic complexes might be structured. Being a very new tool, the amount of basic information still to come from such an instrument is probably enormous.

History

The first transmission electron microscope with electromagnetic lenses was built by Knoll and Ruska in the years 1930 to 1933. Commercial electron microscopes were made by Siemens in Germany in 1939 and by the American firm, RCA, in 1941. The first electron micrograph or photograph taken with the electron microscope of biological material apparently was published in

1934 (a bacterial cell, whole mount). However, the main breakthrough in the study of biological specimens did not come until 1952 and 1953. At this time ultrathin sections were obtained using a modified histological microtome, good fixation with osmium tetroxide, and plastic embedding. The most successful techniques available up to this time, had been whole mount preparations and replication.

In 1935, Knoll suggested that a focused electron beam scanning a specimen would cause a current to be emitted that could be used to record the surface. Von Ardene in 1938, built the first scanning electron microscope using two magnetic lenses and a recording drum. In 1942 Zworykin, Hillier and Snyder built an SEM using two electrostatic lenses. The first commercial SEM was produced at Cambridge, England in 1965 after almost 15 years of intensive effort and its resolution was around 30 nm. Since the SEM does not require a major amount of specimen preparation, studies on biological specimens were rapid after 1965. The first studies usually used metal coated material to insure electron emission and more recently uncoated, fresh materials, as well as frozen specimens have been used. The dramatic and crisp three dimensional micrographs of fine structure of specimen surfaces are probably one of the major contributions of the SEM in

biology.

Today, the TEM finds many fields of application such as structural analysis in engineering, study of prism surfaces in physics, study and analysis of minerals in geology, detection and study of tumor causing virus in medicine and localization of enzyme systems in biology. The SEM is specially useful in studies of circuitry and transistors, analysis of materials using the electron probe, and photography of fine surface structures of organisms. A number of firms throughout the world manufacture TEM and SEM instruments. (JELCO, Hitachi, Phillips, AEI, Materials and Analysis corp., Ziess, ETEC, as well as others shown in appendix I). A number of new generation electron microscopes (high voltage (TEM, SEM, STEM) are now coming into being and are used in biological studies (Beeston, 1973; Cosslett, 1974; Parson et al., 1974).