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THE USE OF THE TRANSMISSION ELECTRON MICROSCOPE IN PATHOLOGY[1]

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SUMMARY: The history and development of the transmission electron microscope (TEM) are outlined. The use of the TEM in diagnostic pathology and research into animal and human diseases is also discussed with some examples relevant to studies in animals.

Key words: transmission electron microscope, pathology

INTRODUCTION

The use of the transmission electron microscope (TEM) in many fields of biomedical research and disease diagnosis has reached a stage where the instrument is providing one of the most powerful means of investigating the complex organization of tissues at the cellular and molecular level. This indispensable tool has contributed enormously to the understanding of the structural intricacies of normal and disordered cells and tissues. The TEM has played a significant role in the understanding of neoplastic processes by providing information on the ultrastructure of tumours and neoplastic cells. The diverse ways in which the TEM can be used to study structural organisations is illustrated by the fact that the TEM has been employed not only to study the ultrastructural detail of cells but also the microstructure of crystals, particularly those of metal. This paper is aimed at providing some information on the use of the TEM in pathology.

THE USE OF THE TRANSMISSION ELECTRON MICROSCOPE IN PATHOLOGY

The first electron microscope was built in 1931 by Knoll and Ruska in Germany (Ham and Cormack, 1979). However, it was not until 1939 that the transmission electron microscope (TEM) was first used successfully to study a biological specimen (tubercle bacilli) at the University of Toronto in Canada (Mandal and Wenzl, 1979). Two years later, in 1941, the first commercially available TEM in North America was produced.

The kidney was one of the earliest tissue studied with the TEM when it was used for a detailed investigation of the renal corpuscles and the renal tubules (Pearce and Baker, 1950). Since then a stream of information has flowed from electron microscopic studies of cells, opening up a new world of knowledge about the detailed structures of cells and tissues. In biological investigations, despite being limited to a small area of the whole specimen, TEM studies have helped in the better understanding of the fine microanatomical structures of many organs in the mammalian body. They have helped to resolve cellular constitutents such

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as the mitochondria, Golgi complexes and the endoplasmic reticulum as well as membrane structures like the cellular membranes, nuclear membranes and plasma membrane infoldings.

The pioneering studies of Low (1953) clearly demonstrated that the pulmonary alveoli were lined by a continuous layer of epithelium. The identification of specific cell types became possible when TEM studies on the lung of cattle resolved the morphology and identity of the type I and type II pneumonocytes in the alveolar lining cells at the blood-air barrier (Epling, 1964).

The pathogenesis and cytopathological changes in many viral diseases affecting the respiratory system has been investigated. The marked tropism of viruses for cell types, structures involved in damage subsequent to infection and the different exudative and regenerative phases following injury have been studied for acute viral pneumonia produced by feline calicivirus (Langloss *et al.*, 1978) and bovine parainfluenza 3-virus (Bryson *et al.*, 1983).

Enteric infection with resulting diarrhoea is an important problem especially in young farm animals. There are many causes of diarrhoea and many investigations including TEM studies have been conducted. The location and distribution of *Campylobacter* sp. in calves and lambs have been described (Terzolo *et al.*, 1987). Transmission electron microscopic studies in calves with spontaneous Cryptosporidium infection indicated a marked predilection of the protozoa for the follicular associated epithelium over the ileal Peyer's patches (Landsverk, 1987). The cellular events associated with the establishment and proliferation of trypanosomes in the skin have been reported using TEM studies. About 80% of *T. congolense* were observed between interstitial cells and bundles of interstitial fibres. The remaining 20% were found in collagen fibres. It is suggested that the skin might be a privileged site for the parasite (Dwinger *et al.*, 1988).

In the study of neoplasms, classification and histogenesis of the tumour are of paramount importance. Although most pathological diagnosis of tumours can be reasonably confirmed with light microscopy (LM), about one to eight percent do not conform to the general classification (Henderson *et al.*, 1986). In such circumstances, TEM can often contribute to the diagnosis by demonstrating the ultrastructure beyond the resolution of LM. Many studies on tumors have been directed towards the histogenesis and changes during the growth and development of the tumour. Imai *et al.* (1988) in their investigations on bronchogenic squamous cellcarcinomas, have classified the tumour cells in the basal layer into three types according to the electron density of the cytoplasm and the size of their intercellular space. In the identification of cell types in adenomatoid tumours, which affect the fallopian tubes, uterus and epididymis, many theories have been proposed with regard to their histogenesis. Endothelial, epithelial, mesonephric and mesothelial origins have been suggested. With a combination of ultrastructural, mucin histochemical and immunohistochemical studies, the mesothelial theory of histogenesis for adenomatoid tumours has been strongly accepted (Stephenson and Mills, 1988).

Transmission electron microscopic studies have also been useful in the identification of viral inclusions and viral replication in cells. Baskerville (1972) studied the ultrastructural changes in the pulmonary airways of pigs infected with Aujeszky's disease virus and noted that the characteristic herpesvirus inclusions were located in the nucleus of bronchial epithelial cells, nucleus of bronchiolar smooth muscle cells and in macrophages.

Vaccination is a common method by which animals may be protected against pathogens. In bacterial vaccine production, for example, it is often important to identify and localise the protective antigens on the cell walls. In this aspect, the TEM has been used extensively to study the composition and characteristics of many bacterial cell wall structures. Besides studying cell walls, it is also possible to determine the capsular materials associated with the bacterial cells by TEM (Pyliotis and Mukkur, 1981).

Studies with TEM on infectious diseases not only involve examining tissues from animals but also investigations on infected tissue culture preparations. Recently, by using such a system, Belanger *et al.* (1988) have identified well-defined bridges between the viral

particles of bovine respiratory syncytial virus, an important agent affecting the respiratory system in cattle. This is the first time such bridges have been reported and their significance has yet to be established.

Although used mainly in research, the TEM may be used helpfully for routine diagnosis of viral infections by demonstrating viruses in clinical material by negative staining electron microscopy e.g. contagious ecthyma poxvirus in sheep. The use of the TEM to assist in the rapid diagnosis of viral diseases of veterinary importance has been reviewed (Gibbs *et al.*, 1980). These authors concluded that a system of priority should be established for handling specimens as it would be unrealistic to examine all clinical samples by TEM. A high priority was considered essential in situations where a rapid laboratory diagnosis was required, as with exotic diseases, where enforcement and control measures have to be instituted immediately and no other quick methods of diagnosis are available. Although the TEM is expensive to install and operate, it may provide the only evidence for diagnosis in clinical samples containing viruses that are difficult to isolate. Examples are rotaviruses (Fenner *et al.*, 1987), mixed virus populations or viruses that do not grow in the generally available culture systems or do not produce obvious cytopathic effects. England and Reed (1980) showed that negative contrast electron microscopy (NCEM) can be used as a primary tool for the diagnosis of viral infections of veterinary significance, especially those viruses associated with enteric, respiratory and skin infections. Although it was suggested that the diagnosis should be confirmed wherever possible by viral isolation, immunofluorescence or serological procedures, their experience indicated that NCEM provided reliable results.

In conclusion, there is no doubt that the TEM has come to occupy a well established role in diagnostic pathology and in research into animal and human diseases. The growth in the number of TEM studies, especially in research, has been phenomenal in recent years and seems likely to continue in the future as new marker systems for cell and tissue structures are developed.

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RINGKASAN

KEGUNAAN MIKROSKOP TRANSMISI ELEKTRON DALAM PATOLOGI

Sejarah dan kemajuan dalam penggunaan mikroskop transmisi elektron telah diketahui umum. Kegunaan mikroskop transmisi elektron dalam diagnostik patologi dan penyelidikan keatas penyakit-penyakit haiwan dan manusia telah dibincangkan dengan contoh-contoh yang berkaitan dalam kajian-kajian pada haiwan.