

Rapid electron microscopy in oncology

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SUMMARY The case is argued for wider use of electron microscopy as an aid to histological diagnosis in problem cases such as tumours of uncertain histogenesis. In practical terms, electron microscopy can produce results as 'immediate' as many special stains in regular use. The cost of providing an effective service can be favourably compared with that of various other diagnostic aids commonly called upon in normal clinical practice. It is suggested that exploitation of this growth area of morphological pathology will enhance the attractions of the discipline of histopathology to talented potential recruits.

The history of histopathology is a history of ever more precise identification of cells and their constituents by morphological and cytochemical means. This is of particular importance in tumour pathology. In general, the more precise the identification the more accurate the diagnosis: given experience of the behaviour of a newly isolated group of lesions, the more accurate the prognosis and the more appropriate the treatment. This does not mean that for a given patient at present a more precise diagnosis necessarily means a more accurate prognosis and better treatment; in general, however, this is likely to follow. The transmission electron microscope is a potent tool in accurate cellular diagnosis; this is well established in renal pathology and clinical virology. The purpose of this report is to draw attention to the growing role of electron microscopy in modern tumour diagnosis.

The principles

The value of electron microscopy in the diagnosis of tumours lies in trying to identify in the poorly differentiated neoplastic cell structural elements which might be characteristic of the cell line from which the tumour arose. In other words, the questions that can be answered with an electron microscope are those which require resolution of structural details beyond the reach of the light microscope. What is that cell? What is that inclusion? How are these cells arranged? The following are among the many clues which may point towards an answer to questions such as these.

(1) Secretory granules and other inclusions. These may suggest cellular identity. They include melanosomes, APUD granules, mucus, lipid, and glycogen.

(2) Mitochondrial size and shape. Steroid cells can be identified by their characteristic mitochondria.

(3) Lysosomes. Numerous lysosomes of characteristic structure occur in cells of macrophage lineage.

(4) Endoplasmic reticulum. Steroid cells tend to contain smooth, and protein secreting cells rough endoplasmic reticulum.

(5) Myofibrils. Tumours of smooth muscle can be identified by their content of actin-like fibrils and by characteristic dark bodies; rhabdomyosarcoma may be recognised by the presence of both thick and thin filaments, even when cross-striations are absent on light microscopy.

(6) Desmosomes. Epithelial cells often and mesenchymal cells rarely have desmosomes. Attached bundles of tonofilaments in squamous carcinoma are particularly prominent.

(7) Microvilli. Epithelial cells have prominent relatively straight microvilli. Intracellular clusters of microvilli are particularly characteristic of epithelial cells.

(8) Normal and abnormal interstitial matrix fibrils. Fibrosarcomas produce collagen; medullary carcinomas of thyroid produce amyloid. Both are recognisable ultrastructurally.

(9) Basement membrane arrangement. Epithelial cells often cluster in a single basket of basement membrane, whereas mesenchymal cells tend to lie independently, often without an identifiable basement membrane.

(10) Cell relationships in special circumstances.

Neoplasms of neurilemmal and meningeal origin have characteristic elongated interwoven processes.

Translating this into practical problem-oriented terms, the electron microscope integrates well into the everyday work of a routine surgical pathology service department in the diagnosis of anaplastic carcinoma from other spindle cell tumours of doubtful histogenesis; in the identification of amelanotic melanoma; in the precise identification of muscle and other mesenchymal and neural tumours; in the study of endocrine tumours and carcinoids; in the classification of lymphomas. Such rare granulomatous lesions as malakoplakia and Whipple's disease may be precisely identified by electron microscopy. Taking a wider view, many aspects of common and uncommon human tumours await precise ultrastructural documentation. Such work may have no immediate apparent diagnostic application but from it will emerge new criteria for the future refinement of tissue diagnosis and possibly new and previously unrecognised groups of neoplasms.

The practice

There are three essentials, apart from good technique, in the use of an electron microscope in tumour diagnosis—speed, reliability, and adequate specimens. The first essential is speed. Before a diagnostic tool can be taken seriously it must be as fast as others used in the field. Material can now be routinely fixed, blocked, sectioned, stained, and examined within 24 hours of receipt (Johannessen, 1973; Rowden and Lewis, 1974). Prints and a report can be routinely available within 48 hours and, where necessary, within 24 hours. In cases of urgency sections can be on the electron microscope for examination within three hours of the receipt of tissue. From our experience of service departments, this compares favourably with conventional histology and 'special stains'. The second essential is reliability; the electron microscope must be adequately maintained and promptly repaired when necessary. Highly trained specialist technical staff are needed whose function is the rapid production of good material. The third essential is actually obtaining materials for electron microscopic examination. This requires considerable persuasion and education of pathologists, clinicians, technicians, and others. It is reasonable to hope that all histopathologists will come to recognise the need for ultrastructural examination in a given case just as they currently recognise the need for reticulin or fat stains. In general, clinicians respond with enthusiasm if care is taken to provide a prompt electron microscopic report at the end of the exercise and to show micrographs regularly as part of oncological pathology seminars.

Ideally a small piece from every biopsy should be fixed in glutaraldehyde or buffered formaldehyde at the time of operation, held in buffer, and processed further should electron microscopy be required. Adequate micrographs, however, can be obtained from a routine formalin 'keep', or even by cutting fragments out of a paraffin block. Micrographs obtained by the latter device may not be pretty but can clinch a diagnosis.

All of this does not imply the impending obsolescence of the light microscope. It should be emphasised that an electron microscope can give grossly misleading information if applied to a heterogeneous structure like a neoplasm without great care as to localisation; the aim must be to get a complete picture of the lesion from gross specimen, through histological sections, to electron micrographs. One must be sure that the right area is examined with the electron microscope; because of the small size of the block involved, selection is vital. This is done either by carefully matching the electron microscope block with the material which goes into paraffin, or by selecting the appropriate area from the paraffin block. Finally, ultrastructural information relates poorly to proliferative and invasive behaviour: an electron microscope gives little information as to malignancy. By its very nature, ultrastructural technique will always remain a subsidiary component of histopathology.

The cost

If the full benefit of the ultrastructural dimension is to be realised in diagnosis, a completely professional approach to the provision of the service is essential. This must involve the establishment of electron microscope units or subunits wholly or mainly dedicated to a service function, with appropriate medical, technical, and ancillary staff. These units must be unembarrassed by the often quite different priorities of experimental research.

The setting up of diagnostic electron microscope units is now being taken very seriously in North America (Williams, 1975). Since 1966, the Veterans Administration has installed 40 high-resolution transmission electron microscopes in its hospitals and 15 more are planned in the next few years; it is suggested that a better diagnosis is obtained by ultrastructural studies in 4% (Williams, 1975) to 8% (Gyorkey *et al.*, 1975) of biopsies. Our own experience would support a figure in this range.

This will of course take money. American estimates of costs per specimen examined vary; the Veterans Administration Central Office supports electron microscope units on a basis of \$100 per specimen at an examination rate of 250 specimens

per annum. The average UK electron microscope unit, if used for routine purposes alone, could probably handle up to 500 cases per annum, perhaps servicing a population of about 500 000. The minimum effective staff for such a unit would be a chief technician with a junior and a laboratory aide.

The capital cost at 1976 prices is around £30 000 (less than some multichannel analysers). If this cost is written off over 10 years, and technical salaries, service contracts, consumables, and general expenses are added, a reasonable costing is about £20 per case. This commitment would absorb perhaps half of the time of a full-time consultant, adding a further £10 to the unit cost. For comparison, the current cost in the private sector for a diagnostic frozen section with paraffin confirmation is upwards of £16, for a barium enema £30, and for aortography £50. Electron microscopy cannot reasonably be regarded as an unusually expensive diagnostic aid.

The capital costs seem large, but it is our belief that there already exist, in many centres, adequate facilities in terms of space and capital equipment to provide the basis of an effective ultrastructural diagnostic service. These facilities are often under-used, due only to lack of staff or some specific simple instrumental bottleneck such as shortage of microtomes. The provision of extra staff specifically for routine diagnostic purposes would often be all that is needed to provide a nucleus for the growth of the service, with the desirable by-product of the more

efficient use of existing costly capital equipment.

The indirect results of investment in pathological ultrastructure are possibly even more important than the direct. It would be easy for histopathology, the earliest branch of the subject, to lose its attraction to talented recruits unless it exploits its own growing points. Pathological ultrastructure is a major growth point of histopathology; as Virchow saw with the light microscope, there is a whole new sphere of pattern recognition and identification to be explored in the interests of the patient. The advance of ultrastructural pathology will bring about not the obsolescence of classical histopathological skills but rather their enhancement. For in the end a diagnosis is reached by looking at the patient, the haematoxylin and eosin section, and the electron micrograph—in that order.

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