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The role of electron microscopy in the diagnosis of tumours of the head and neck

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Abstract

A four-year study was undertaken to determine the value of electron microscopy in the diagnosis of head and neck tumours. During this period 80 samples were submitted for examination, of which 69 contained assessable tumour. Electron microscopy made a major contribution to the diagnosis in 25 cases (36 per cent). Areas in which ultrastructural examination was of diagnostic significance included the precise categorization of apparently undifferentiated carcinoma and the identification of melanomas. Little diagnostic benefit was gained from electron microscopic examination of thyroid, lymphoid or salivary gland neoplasms. The value of electron microscopy in relation to immunohistochemistry is discussed.

Introduction

Many different tumour types occur within the head and neck region. The most frequent is squamous carcinoma, but lymphoma, salivary and thyroid neoplasms, melanoma, soft tissue sarcoma and metastatic disease from outside the head and neck may be seen. With such a variety of tumour types and sites of origin the true nature of the lesion may be difficult to determine using routine histopathology.

Electron microscopy is widely available to histopathologists and is used, with the more recent technique of immunohistochemistry, in the identification of tumours. We have previously assessed the contribution of electron microscopy to general tumour diagnosis in this institution (Fisher *et al.*, 1985). Arising out of this larger study we have examined the role of electron microscopy when applied to the specific field of head and neck oncology and here present our results. This study attempts to identify the diagnostic problems in which electron microscopy is of value and to assess its role in relation to that of immunohistochemistry.

Material and methods

Source of material

Over a four-year period material was collected from all suspected head and neck tumours biopsied in the Department of Otolaryngology, University College Hospital, London. A suitably sized portion was placed into glutaraldehyde in the operating theatre at the same time as the main specimen was fixed in 10 per cent formol-saline in the usual manner. On occasion a further portion of tissue was snap-frozen in liquid nitrogen for immunohistochemical studies where antigens were not preserved by formalin fixation and paraffin processing.

Processing and examination

Specimens were fixed for 3–4 hours in 4 per cent glutaraldehyde in 0.125M phosphate buffer (pH 7.3) as soon as possible after surgical removal (Chambers *et al.*, 1968). They were then washed in buffer and stored at 4°C until the routine histology was viewed. If it was decided to proceed to electron microscopy the samples were post-fixed in phosphate-buffered 1 per cent osmium tetroxide, dehydrated through graded ethanol, and embedded in Araldite. A rapid processing method (Carr and Toner, 1978) was used for urgent cases. Semi-thin sections (0.5 *um*) were cut and stained with 0.25 per cent toluidine blue in borax, allowing selection of a suitable area for EM examination. Subsequent ultra-thin sections (60–90 *nm*) were cut, mounted on rhodium-plated copper grids and stained with uranyl acetate and lead citrate, and examined in the electron microscope. Immunohistochemical staining was carried out on paraffin sections (and occasionally on frozen sections) by a standard indirect peroxidase method.

Assessment of the value of electron microscopy

The contribution of electron microscopy was assessed as previously described (Fisher *et al.*, 1985). In brief, three diagnostic categories were used:

1. Cases in which electron microscopy was judged to be significantly contributory to the final diagnosis.
2. Those cases in which electron microscopy was of no diagnostic value.
3. Cases in which the electron microscopic findings were confirmatory or supportive of the light microscopical diagnosis.

Results

In this four-year period, 159 specimens of presumed

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TABLE I
CASES NOT PROCESSED FOR ELECTRON MICROSCOPY

Final diagnosis	Number of cases	
No viable tumour present	26	
Squamous carcinoma	44	
Lymphoma	3	
—1 centroblastic-centrocytic		
—1 lymphocytic (CLL)		
—1 myeloma		
Laryngeal nodule	2	
Follicular adenoma of thyroid	2	
Lingual thyroid	1	
Nodular goitre	1	
Total	79	(49.6%)

head and neck tumour were received for light and electron microscopy. The former provided the definitive diagnosis or excluded neoplasia in 79 cases (50 per cent). The majority of these were squamous cell carcinomas (Table I), although several contained no neoplastic tissue. The remaining 80 were submitted for ultrastructural examination. All the cases were sent either in glutaraldehyde or as fresh tissue so there was no need to retrieve material from paraffin blocks. Because of degenerative and necrotic changes or sampling error, 11 cases contained no assessable tumour, leaving 69 cases for analysis. Table II shows the overall contribution of electron microscopy in these cases, according to tumour type. Of 69 specimens examined, electron microscopy made a significant contribution in 25 (36 per cent), confirmed or supported the light microscopical diagnosis in a further 25 (36 per cent) and made no diagnostic contribution in 19 (27 per cent). The 25 cases in which ultrastructural examination provided a definite diagnosis label or narrowed the differential diagnosis are shown in Table III.

TABLE II
OVERALL CONTRIBUTION OF ELECTRON MICROSCOPY

Type of tumour	Final diagnosis	No. of cases	Role of electron microscopy Category 1	2	3
Spindle cell sarcomas	Osteosarcoma	1			1
	Fibrosarcoma	1			1
	Chondrosarcoma	1	1		1
	Rhabdomyosarcoma	1			
	Total	4	1		3
Salivary gland neoplasms	Pleomorphic adenoma	1			1
	Adenocarcinoma	1			1
	Acinic cell carcinoma	2	2		
	Lymphoma	1		1	
	Total	5	2	1	2
Undifferentiated neoplasms	*Squamous carcinoma*	12	12		
	Adenocarcinoma	2	2		
	Adenosquamous carcinoma	1	1		
	Anaplastic carcinoma	8		8	
	Malignant melanoma	2	2		
	Uncharacterized	2		2	
	Total	27	17	10	
Lymphoreticular neoplasms	Hodgkin's disease	4		4	
	Non-Hodgkins's lymphoma	8	1		7
	Histiocytosis X	1	1		
	Total	13	2	4	7
Thyroid lesions	Adenoma	3			3
	Carcinoma	5			5
	Thyroiditis	1			1
	Nodular goitre	1			1
	Total	10			10
Miscellaneous	Schwannoma	1			1
	Fibrous dysplasia	1		1	
	Granular cell tumour	1			1
	Chordoma	2	2		
	Ewing's sarcoma	1	1		
	Basal cell papilloma	1		1	
	Haemangioma	1		1	
	Leukaemic infiltrate	1		1	
	Amyloid	1			1
	Total	10	3	4	3
	Total	10	3	4	3
Overall total		69	25	19	25

Discussion

The pioneering work of Friedmann demonstrated that electron microscopy is of value in resolving several diagnostic difficulties in head and neck oncology (Friedmann, 1961; 1971). The most frequent problem in which electron microscopy played a role was in the precise identification of a carcinoma which appeared undifferentiated by light microscopy (Fig. 1). Of 15 such tumours, 12 were found to be squamous cell carcinomas, two adenocarinomas and one an adenosqaumous carcinoma (Glossop *et al.*, 1984). In the head and neck region it is not uncommon to detect metastatic carcinoma in a lymph node and subsequent investigation and management depend upon the type of carcinoma identified: a squamous carcinoma may have a primary site in the paranasal sinuses, nasopharynx, or upper aerodigestive tract, whereas an adenocarcinoma is more likely to have arisen in the lung or gastrointestinal tract. Utilization of immunohistochemistry in cases of undifferentiated tumour may lead to the identification of carcinoma by cytokeratin expression (Bosq *et al.*, 1985; Gatter *et al.*, 1985), but further categorization into squamous, glandular or mixed carcinoma types is not yet possible by this method. Fifteen examples (19 per cent of those examined) were further classified in this study. However, since most adenocarcinomas have common

TABLE III
CASES IN WHICH ELECTRON MICROSCOPY SIGNIFICANTLY ADVANCED THE DIAGNOSIS

Nature of problem	Number of cases	Final diagnosis	
Undifferentiated carcinoma	15	Squamous cell carcinoma	(12)
		Adenocarcinoma	(2)
		Adenosquamous carcinoma	(1)
Anaplastic round cell tumour	2	Malignant melanoma	(2)
Lymphoma vs carcinoma	1	Undifferentiated carcinoma	
Small round cell tumour			
? lymophoma ? Ewing's	1	Ewing's sarcoma	
Adenocarcinoma *vs* chordoma	2	Chordoma	(2)
Spindle cell neoplasm	1	Rhabdomyosarcoma	
Round cell neoplasm in salivary gland	2	Acinic cell carcinoma	(2)
Total	25		

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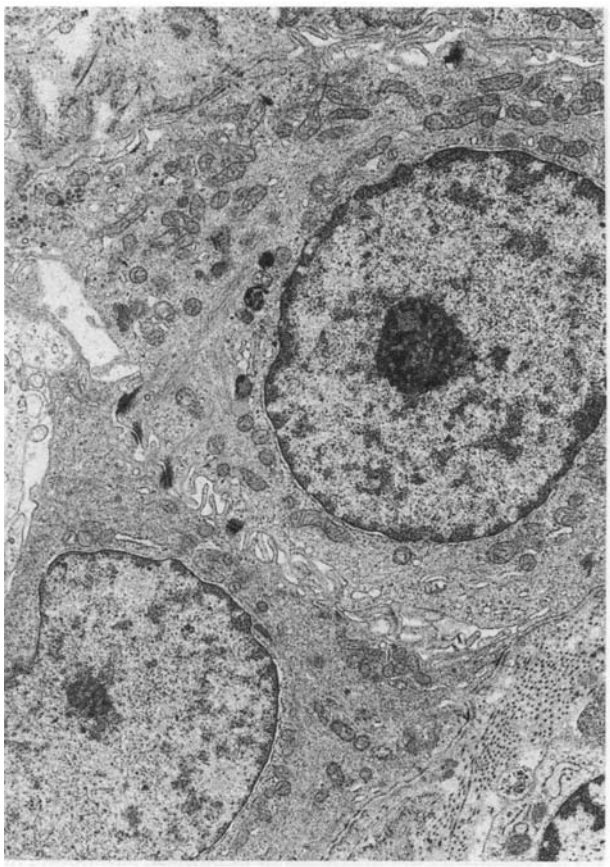

FIG. 1

Squamous cell carcinoma, moderately differentiated. A group of cells with surface projections is bounded by a continuous external lamina (bottom right). Bundles of cytoplasmic filaments insert into desmosome-like intercellular junctions (×7,000, ×10,400).

ultrastructural features, the primary site can rarely be identified by electron microscopy (Fisher *et al.*, 1985). Our results are in agreement with those of Diebold *et al.* (1984), who looked at 40 lymph node tumours where light microscopy was inconclusive. Of these, 26 (65 per cent) were further categorized by electron microscopy, with 10 adenocarcinomas, three squamous carcinomas, and eight remaining undifferentiated. The distinction of chordoma and adenocarcinoma may be difficult at both light and electron microscope levels and requires careful study (Erlandson *et al.*, 1968). Immunohistochemically, both lesions are reported to express cytokeratins, but chordomas also show S100 protein positivity (Abenoza and Sibley, 1986), which is less common in adenocarcinomas. Characteristically electron microscopy of chordoma demonstrates vacuolization of the cytoplasm such that some cells seem 'to consist of an envelope and nucleus only' (Friedmann, 1963).

Traditionally electron microscopy has been used for the distinction of anaplastic polygonal cell tumours, separating carcinoma, lymphoma and melanoma (Ghadially, 1980). However, immunohistochemical studies on paraffin section using a panel of antibodies, including those against leucocyte common antigens, cytokeratins, epithelial membrane antigen and S100 protein (Bosq *et al.*, 1985; Gatter *et al.*, 1985) are now also used to make this distinction. Detection of lymphoma by immunological methods is both straightforward and reliable (Pizzolo *et al.*, 1981; Warnke *et al.*, 1984), although some are marker-negative. Electron microscopy of lymphoma cells may, however, demonstrate intracellular vacuoles and inclusion bodies which contain viral particles (Friedmann, 1971) and contribute to understanding of aetiology.

Friedman (1963) described the characteristic ultrastructural features in five cases of malignant melanoma of the nose and especially the complex structure of the intracellular premelanosome. In our series, electron microscopy detected two nasal melanomas, both of which had been diagnosed as anaplastic carcinoma by light microscopy. Standard histological stains for melanin were negative in these lesions. Immunohistochemically, both the tumours were initially S100 protein negative, although repeat staining performed following the results of electron microscopy (Fig. 2) detected some positivity in one case. Not all melanomas are S100 positive and lesions other than melanoma may be positive with this antibody (Kahn *et al.*, 1983) so that electron microscopy remains of value in the diagnosis of melanoma.

In the diagnosis of lymphoreticular neoplasms, electron microscopy has been largely superseded by immunohistochemistry. Of the non-Hodgkin's lymphomas in the study, electron microscopy was of significant value in a single case in which carcinoma could not be excluded at the light microscopic level. In the remaining cases examined, electron microscopy confirmed lymphoma, but provided no further diagnostic information. Similarly, Hodgkin's disease remains a light microscopic diagnosis. Electron microscopy was of value in a case of Langerhans cell histiocytosis (histiocytosis X) where the typical Birbeck granules (Favara *et al.*, 1983) were seen (Fig. 3). Immunohistochemically, S100 protein is useful for identi-

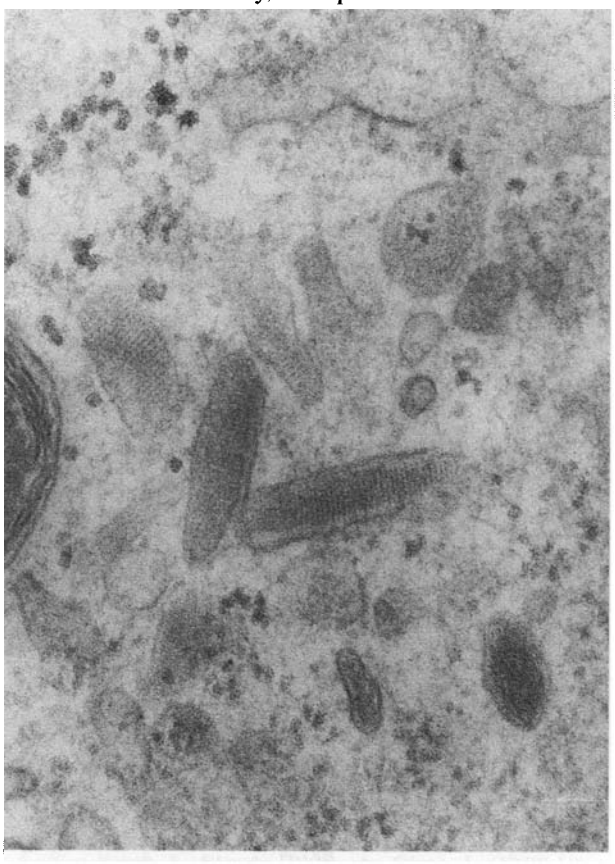

FIG. 2

Malignant melanoma. Melanosomes are present in the cytoplasm. (×64,750, ×92,500).

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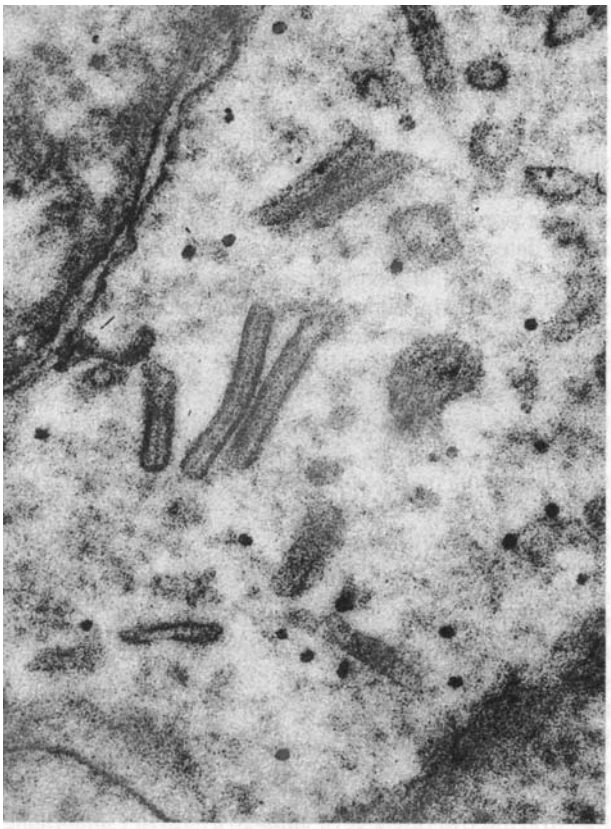

FIG. 3

Langerhans Cell Histiocytosis (Histiocytosis X). Birbeck granules display their characteristic structure. (×58,000, ×69,500).

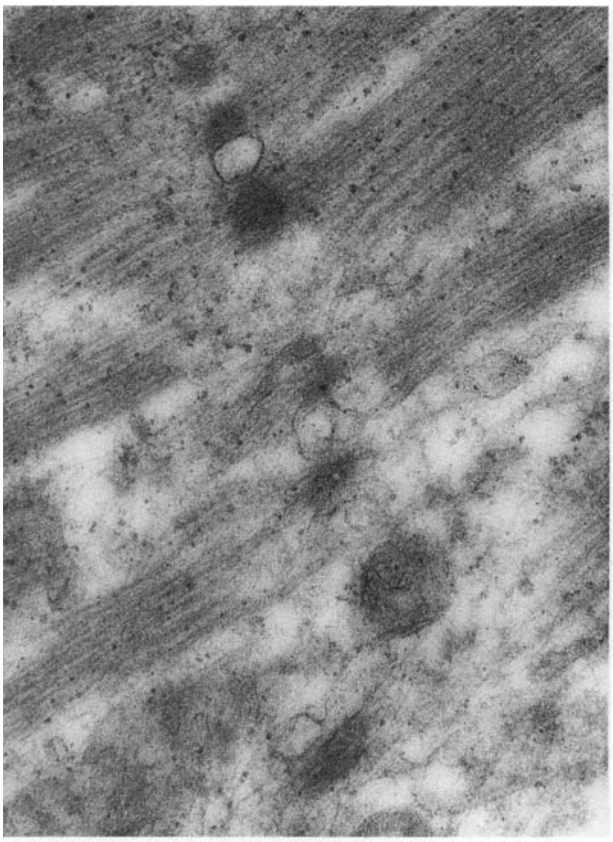

FIG. 4

Rhabdomyosarcoma. Interdigitating thick and thin filaments with Z-band formation and scattered glycogen granules. (×56,700, ×81,000).

fying Langerhans cells and Mierau and Favara (1984) suggest that both electron microscopy and immunological methods should be used to make this diagnosis.

Four sarcomas were examined in this study. In three, electron microscopy confirmed the light microscopical impression, but in one the diagnosis of rhabdomyosarcoma was made by the detection of the typical features of thick and thin filaments with Z-band formation (Fig. 4) (Horvat *et al.*, 1970). This tumour was desmin-positive but myoglobin-negative. Although a panel of immunohistochemical markers is a useful diagnostic aid for spindle cell sarcomas, none is completely specific or sensitive; desmin positivity has been reported in an increasing variety of sarcoma sub-types (Truong *et al.*, 1990) and electron microscopy has an important role in this area, particularly for marker-negative tumours (Fisher, 1990). Among round cell tumours, electron microscopy was of value in diagnosing Ewing's sarcoma, which lacked immunohistochemical markers. In the former case, a negative leucocyte common antigen immunostain suggested Ewing's sarcoma, but electron microscopy showed features (Llombart-Bosch *et al.*, 1978) consistent with this diagnosis and, quite importantly, excluded features of other tumour types.

Thyroid gland tumours were diagnosed mainly by light microscopy with electron microscopy being confirmatory. Immunohistochemical staining with antithyroglobulin may reveal the thyroid origin of an otherwise undifferentiated adenocarcinoma. Electron microscopy can be useful in supporting the diagnosis of medullary carcinoma of

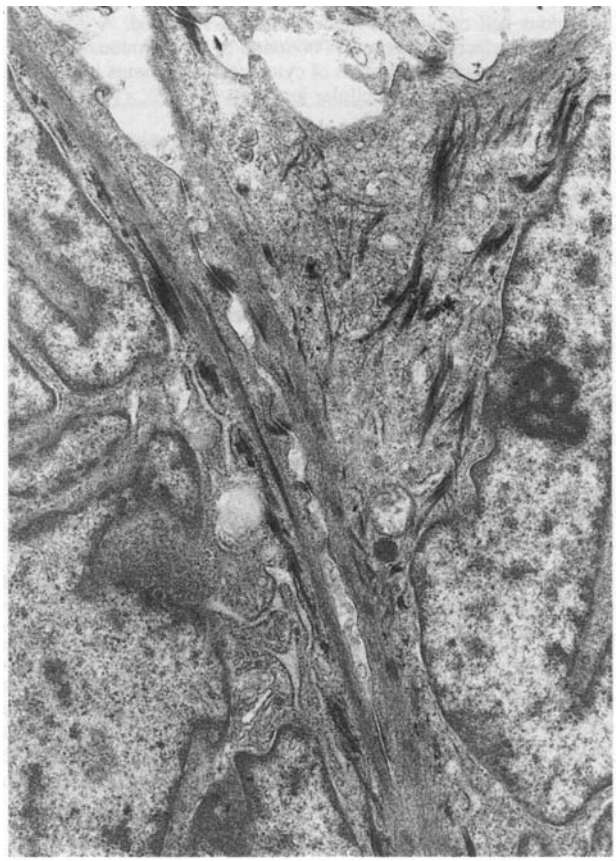

FIG. 5

Myoepithelial cells. There are intercellular junctions, bundles of myofilaments and coarser, paranuclear tonofilament-like aggregates. (×8,250, ×30,200).

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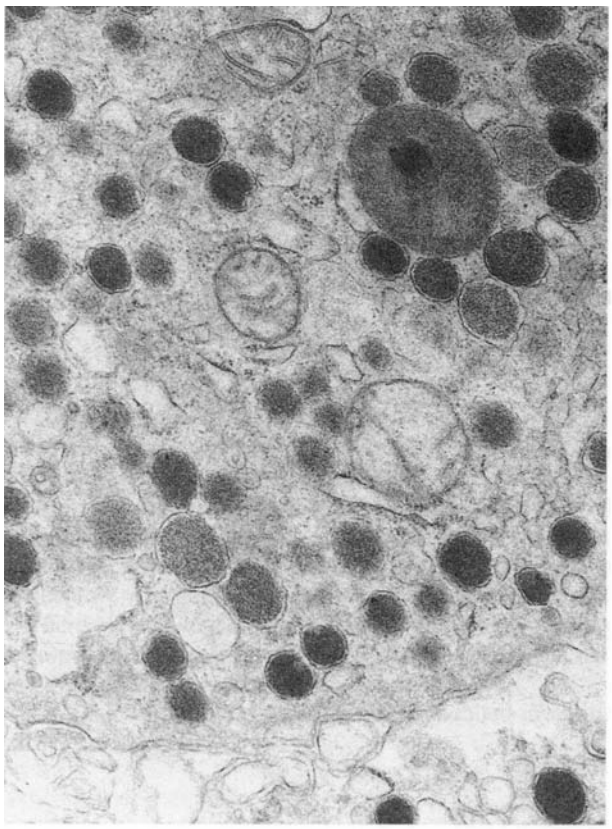

FIG. 6

Medullary thyroid carcinoma. Membrane bound dense core (neurosecretory) granules of varying size are abundant. (×23,400, ×34,000).

the thyroid (Ghadially, 1980) by demonstrating characteristic neuroendocrine granules (Fig. 5), but immunohistochemical positivity for neuroendocrine markers and calcitonin is usually present. In the salivary gland, electron microscopy was of significant value in the diagnosis of acinic cell carcinoma, ultrastructural examination revealing the typical cytoplasmic granules that were not apparent by light microscopy (Erlandson and Tander, 1972). However, in most cases, ultrastructural studies of salivary gland neoplasms have provided information as to the possible histogenesis of tumours (Erlandson *et al.*, 1968) by, for example, demonstrating myoepithelial cells (Fig. 6) but are not useful diagnostically.

In head and neck oncology, therefore, electron microscopy is of value for certain specific diagnostic problems. Although immunological staining methods have modified the traditional role of electron microscopy, ultrastructural examination can still be used for sub-categorizing undifferentiated carcinoma, in the detection of melanoma, in soft tissue sarcoma diagnosis, and in the diagnosis of certain specific neoplasms by identifying sub-microscopic features indicative of the cells involved. Within the latter group are Ewing's sarcoma, Langerhans cell histiocytosis and neuroendocrine tumours including medullary carcinoma of the thryoid. Electron microscopy is of relatively little value in the diagnosis of lymphoid, thyroid and salivary gland neoplasms.

In a previous study (Fisher *et al.*, 1985) we discussed the cost of electron microscopy, pointing out that greater selectivity of cases submitted for this investigation will lead to its more economic and efficient use. We hope that this study involving a specific anatomical region will encourage this, and recommend that both immunohistochemical methods and electron microscopy be used together in a complementary fashion.

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References

Abenoza, P., Sibley, R. K. (1986) Chordoma: an immunohistologic study. *Human Pathology,* **17:** 744–747.

Bosq, J., Gatter, K. C., Micheau, C., Mason, D. Y. (1985) Role of immunohistochemistry in diagnosis of nasopharyngeal tumours. *Journal of Clinical Pathology,* **38:** 845–848.

Carr, I., Toner, P. G. (1978) Rapid electron microscopy in oncology. *Journal of Clinical Pathology,* **30:** 13–18.

Chambers, R. W., Bowling, M. C., Grimley, P. M. (1968) Glutaraldehyde fixation in routine histopathology. *Archives of Pathology,* **85:** 18–29.

Diebold, J., Reynes, M., Tricottet, V. (1984) Electron microscopy in the diagnosis of malignant adenopathies. Paper given at the International Academy of Pathology Electron Microscopy Symposium: differential diagnosis of tumours. Brussels.

Erlandson, R. A., Tander, B., Liberman, P. H., Higinbotham, N. L. (1968) Ultrastructure of human chordoma. *Cancer Research,* **28:** 2115–2125.

Erlandson, R. A., Tander, B. (1972) Ultrastructure of the acinic cell carcinoma of the parotid gland. *Archives of Pathology,* **93:** 130–140.

Favara, B. E., McCarthy, R. C., Mierau, G. W. (1983) Issues in pathology: Histiocytosis X. *Human Pathology,* **14:** 663–676.

Fisher, C. (1990) The value of electron microscopy and immunohistochemistry in the diagnosis of soft tissue sarcomas. A study of 200 cases. *Histopathology,* **16:** 441–454.

Fisher, C., Ramsay, A. D., Griffiths, M., McDougal, J. (1985) An assessment of the value of electron microscopy in tumour diagnosis. *Journal of Clinical Pathology,* **38:** 403–408.

Friedmann, I. (1961) Electron microscopy of human biopsy material. *Proceedings of the Royal Society of Medicine,* **151:** 1064–1071.

Friedmann, I. (1963) Electron microscopy of rare diseases of the nose. *Transactions of the American Academy of Ophthalmology and Otolaryngology,* (May–June): 261–280.

Friedmann, I. (1971) Electron microscopy in head and neck oncology. *Acta Otolaryngologica,* **71:** 115–122.

Gatter, K. C., Abdulaziz, Z., Beverley, P. (1982) Use of monoclonal antibodies for the histopathological diagnosis of human malignancy. *Journal of Clinical Pathology,* **35:** 1253–1267.

Gatter, K. C., Alcock, C., Heryet, A., Mason, D. Y. (1985) Clinical importance of analyzing malignant tumours of uncertain origin with immunohistological techniques. *Lancet,* **i:** 1302–1305.

Ghadially, F. N. (1980) *Diagnostic electron microscopy of tumours.* London: Butterworth and Co.

Glossop, L. P., Griffiths, M., Grant, H. R. (1984) Combined adenocarcinoma and squamous carcinoma of the hypopharynx. *Journal of Laryngology and Otology,* **98:** 1161–1166.

Horvat, B. L., Caines, M., Fisher, E. R. (1970) The ultrastructure of rhabdomyosarcoma. *American Journal of Clinical Pathology,* **53:** 555–564.

Kahn, H. J., Marks, A., Thom, R. T., Baumal, R. (1983) Role of antibody to S100 protein in diagnostic pathology. *American Journal of Clinical Pathology,* **79:** 341–347.

Llombart-Bosch, A., Blanche, R., Peydro-Olaya, A. (1978) Ultrastructural study of 28 cases of Ewing's sarcoma: typical and atypical forms. *Cancer,* **41:** 1362–1373.

Mierau, G. W., Favara, B. E. (1984) Histiocytosis X: EM or S100?

Poster presented at the International Academy of Pathology Electron Microscopy Symposium: differential diagnosis of tumours. Brussels.

Pizzolo, G., Sloane, J., Beverley, P. (1981) Differential diagnosis of malignant lymphoma and non-lymphoid tumours using monoclonal antileucocyte antibody. *Cancer*, **46:** 2640–2647.

Truong, L. D., Rangdaeng, S., Cagle, P., Ro, J. Y., Hawkins, H., Font, R. L. (1990) The utility of desmin. A study of 584 cases and review of the literature. *American Journal of Clinical Pathology*, **93:** 305–314.

Warnke, R. A., Gatter, K. C., Falini, B. (1984) The diagnosis of human lymphoma using monoclonal antileucocyte antibodies. *New England Journal of Medicine*, **309:** 1275–1281.

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