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*Dr Doussis et al comment:*
Dr Metze makes some interesting observations on the histochemistry and ultrastructure of osteoclast-like giant cells. Like many of our own observations, they appear to lead to the conclusion that giant cells in bone, be they osteoclasts or macrophage polykaryons, are part of the mononuclear phagocyte system. We have not noted a diminution of CD68 reaction in larger osteoclasts, foreign body macrophage polykaryons, or osteoclast-like giant cells in giant cell lesions of bone in soft tissue, and we are surprised by this interpretation of figs 2A and 3A. We are not certain whether any cytochemical or immunocytochemical marker can reliably reflect the physiological activity of giant cells, but would agree that the tissue matrix (as well as cellular and hormonal factors) are likely to be important in determining the phenotype of these cells, and some of our recent results strongly suggest this is the case.[1]

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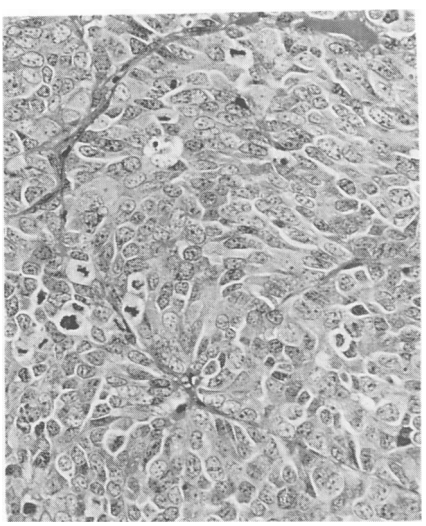
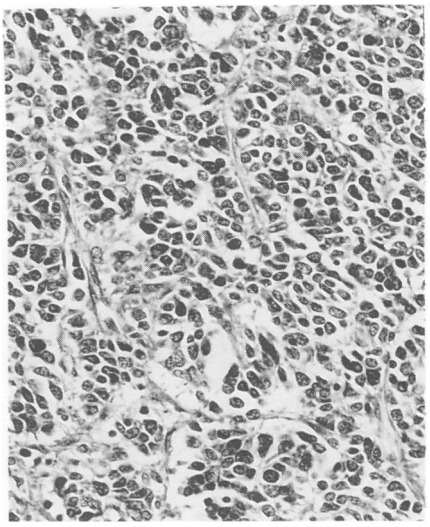
## Value of adequate fixation for accurate histological interpretation

I read with interest Start *et al's* article,[1] which emphasises the importance of proper fixation for accurate histological interpretation. Here, I present a case in which delayed fixation caused diagnostically important phenotypic changes.

An operation for small cell carcinoma (SCC) of the gall bladder was carried out in a 51 year old woman. A small piece of the tumour, submitted for intraoperative diagnosis, was immediately fixed in 20% formalin, the standard fixative in our laboratory. Several hours later the resected tumourous gall bladder was submitted and fixed in the same way. Histologically, the former was the intermediate cell type of SCC with good tissue preservation (fig 1), while the latter was the oat cell type of SCC and had an autolytic nature (fig 2). Both were positive for the Grimelius stain and for neuron specific enolase (NSE) immunohistochemical stain, and had neurosecretory granules observable by electron microscopy, despite the differences in cellular features.





*Intraoperative specimen (fig 1) and postoperative specimen (fig 2).*

It has been noted that the frequency of the diagnosis of the oat cell type of SCC is strikingly high in postmortem compared with biopsy specimens,[2] and also that there are no significant clinical, biological, or ultrastructural differences between the two types.[3–5] Based on these observations, the recent proposal[6] that the terms "oat cells" and "intermediate cells" should be deleted from the subtypings of SCC seems quite reasonable in light of evidence suggesting that oat cells may be the result of autolysis of intermediate cells of SCC.

Autolysis prevents proper fixation and interpretation. Larger surgical specimens, as Start *et al* suggest,[1] may have varying degrees of autolysis before their arrival at the pathology laboratory. I would therefore recommend that with larger surgical specimens, intraoperative samples should be obtained for subsequent proper fixation and interpretation whenever possible.

Incidentally, properly fixed, well preserved specimens could eventually eliminate certain descriptive terms, such as clear cell variant, often used in various tumour classifications, because such phenotypic variations may be attributable to differences in the quality of tissue preservation, as in SCC.

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*Dr Start comments:*
Dr Kudo describes an interesting example of how inadequate primary fixation may compromise histological interpretation. Prompt fixation should prevent autolysis and bacterial contamination but it is important to remember that changes in tissue volume and a variety of artefacts may still occur.[1] Delayed fixation affects the number of observable mitotic figures in tissues,[2] and so may influence systems of mitosis counting that are used in the diagnosis of malignancy in uterine smooth muscle tumours[3] and to provide prognostic indices in other tumours.[4 5] Fixatives may also directly influence the immunoreactivity of tissue antigens.[6 7] Such observations show that accurate histological interpretation may come to depend on detailed knowledge of tissue fixation and preparation.

Dr Kudo's suggestion that intraoperative biopsy specimens should always be taken from larger specimens should be strongly discouraged in the absence of a definite clinical or diagnostic indication. In addition to producing unnecessary specimens, sampling errors may arise and more importantly any manipulation of specimens may create distortion and complicate or compromise the subsequent pathological assessment. In our experience the quality of fixation is best improved by better education of all relevant staff including clinicians, when combined with the rapid transfer of specimens to the laboratory where fixation can be optimised. Proper fixation is important.

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