appropriate treatment.[4] This method for histological grade has been adopted by the Royal College of Pathologists' Working Group for use in the NHS Breast Screening Programme.[5]

Parham and colleagues have concluded from a small series of cases (105) that mitotic counts and semiquantitative assessment of tumour necrosis are the most significant factors. Unfortunately, despite their criticism of the Bloom and Richardson method, the authors appear to have fallen foul of exactly the same imprecision which they eschewed. Although they have followed us in defining the field area for mitotic counting, they do not state in their paper how many mitoses per field are allocated for each point scored. Their evaluation of tumour necrosis also lacks clarity. It is admirable to define the dimensions of an area of necrosis but there is surely a flaw in the assessment of *multiple* foci if only the largest focus is counted. On this basis a tumour could have several foci of necrosis each of which might score 1 or 2 points, but this only qualifies it for an overall score of 2, less than a tumour with a single focus scoring 3. The relative lack of numerical data in this paper is also surprising and we are not told the number of cases in each necrosis group. For these reasons we must conclude that not only are there doubts about the reproducibility of this new method but fear that for lack of an adequate description no one else will actually be able to use it.

A number of other points are pertinent. The study is confined to tumours of no special type which seriously reduces its utility, since, as we have shown recently, only 50% of cases of invasive breast carcinoma fall into this category.[6] It is remarkable that no reference is made in this paper to lymph node stage, widely regarded as one of the most powerful prognostic factors available in breast cancer, especially as Fisher and colleagues have shown a close correlation between tumour necrosis and nodal status.[7] Finally, any method which divides patients into four rather than three groups will appear to be more discriminating. We would refer the authors to our paper confirming the utility of the Nottingham Prognostic Index.[5] Using the integer scores five groups of patients are identified with an annual mortality ranging from 1·5 to 32%. In practice, however, prognosis must be related to the available treatment options; in our experience the use of more than three groups serves no useful purpose.

CW ELSTON
IO ELLIS
Nottingham City Hospital, Hucknall Road, Nottingham NG5 1PB.

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Dr Parham comments:

Drs Elston and Ellis express surprise that in our paper proposing a simplified method of grading breast cancer[1] we do not cite their recent publication on histological grading.[2] I must confess that while myself and my co-authors may have some favourable attributes, we are not clairvoyants. Our paper was accepted for publication, in its submitted form, on the 1 November 1991 (indicated in the bottom left hand corner of the first page). Their paper was not published until later the same month (8/11/91).

The aim of our study was to produce a simple method of grading breast tumours. The measurement of multiple areas of necrosis, while commendable, would make the method complex and probably less reproducible. For this reason, the largest dimension of necrosis was utilised. For clarity, the scoring of mitotic counts in our paper is the same for both, the new grading method, and the conventional Bloom and Richardson grading method.

Drs Elston and Ellis comment that breast tumours of no special histological type account for only 50% of breast cancers and that this limits the utility of our new grading method. My experience and the findings of others suggest that the figure is nearer 70–75%.[3 4] The remaining tumours, apart from infiltrating lobular carcinomas (accounting for approximately 10% of cases), have special histological features which tend to place them into favourable prognostic groups.

No mention of lymph node stage is made in our preliminary paper, as we concentrated on presenting the prognostic information that can be obtained from the primary tumour. We do, however, state that the combination of the new grading method, with tumour stage and hence lymph node status, may provide further prognostic information. These aspects are currently being investigated.

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Immunophenotype of multinucleated cells in giant cell lesions

I read the interesting paper by Dr Doussis and colleagues[1] and discuss it here in the light of our own results.

In our investigation enzyme histochemistry was applied to cryostat sections of unfixed and undecalcified specimens of 101 different tumours or tumour-like lesions of bone.[2] In all cases the osteoclast-like giant cells showed the same pattern of reactions, which was identical with that of osteoclasts but different from that of the multinucleated neoplastic cells: a lack of demonstrable alkaline phosphatase, but clearly detectable activity of tartrate-resistant acid phophatase (TRACPase) activity; non-specific acid esterase, leucinamino-peptidase, and NADH-tetrazolium oxido-reductase activity. Microdensitometry of the enzyme reaction product[3 4] in giant cells of varying sizes in six different bone tumours exhibited the same trend in all cases: a continuous decline of the relative activities of non-specific esterase and NADH-tetrazolium oxido-reductase, but an increase in the TRACPase activity with increasing cell size. Among the very large giant cells, however, there were cells with both high and very low TRACPase activities. Additional electron microscopic examination showed swollen mitochondria with cristolysis, fragmentation, and swelling of cisternae of endoplasmic reticulum and the nculear envelope, more and larger digestive vacuoles with myelin-like material, and many vacuoles of variable size scattered throughout an electron dense cytoplasm.[2 3] This pattern differed from that seen in the smaller giant cells. Thus we hypothesised that with an increase in cell size osteoclast-like giant cells changed their physiological activities and that at least some of the very large cells degenerated.

It is interesting to note that in the study by Doussis *et al* the pattern of reactivity for anti-CD 68 was quite similar to that of non-specific esterase and NADH-tetrazolium oxido-reductase, because the giant cells with larger diameters clearly showed a lower density of the immunoperoxidase reaction product than the smaller ones (figs 2A and 3A of the paper by Doussis *et al*). We think that these photographs confirm our theory. A microdensitometric examination[4] of these sections would certainly demonstrate a size dependent pattern of the anti-CD68 reaction product comparable with that obtained in the study of the above mentioned two enzymes.

Doussis *et al* show that giant cells of giant cell tumours can be distinguished from other giant cell containing bone tumours by the absence or paucity of the HLA-DR reaction.[1] The authors mention, as one of the possible explanations, that this phenomenon might be due to differences in the nature of the giant cells. But our study of enzyme physiology and ultrastructure of osteoclast-like giant cells in various bone lesions does not support this hypothesis. Furthermore, despite some differences, osteoclast-like giant cells of both giant cell tumours and other giant cell containing tumours or bone lesions share many antigens in common.[1 5–7] Bearing in mind the observation that lymphokines modulate the expression of HLA-DR in human monocytes and macrophages,[8] we suggest that this is also the case for the osteoclast-like giant cells. Therefore, we favour the alternative explanation given by Doussis *et al*, that the differing HLA-DR expression may reflect variations in the tissue matrix or in the immunological response to the neoplasm among the various bone tumours or tumour-like lesions.

K METZE
Department of Pathology, Faculty of Medicine, POB 6111, State University of Campinas, BR 13081 Campinas-SP, Brazil

1 Doussis IA, Puddle B, Athanasou NA. Immunophenotype of multinucleated and mononuclear cells in giant cell lesions of bone and soft tissue. *J Clin Pathol* 1992;45:398–404.

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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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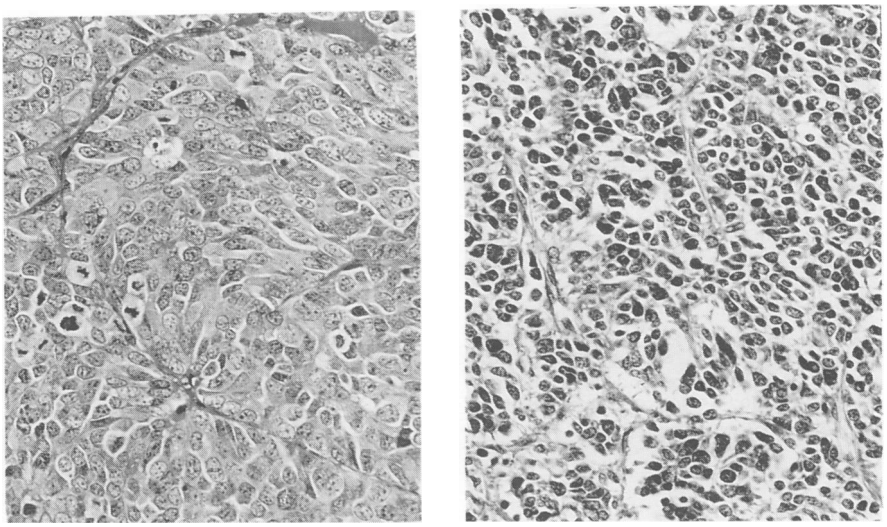
Value of adequate fixation for accurate histological interpretation

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.

M KUDO
Department of Pathology,
Toho University School of Medicine,
Ota-ku, Tokyo 143, Japan

1 Start RD, Cross SS, Smith JHF. Assessment of specimen fixation in a surgical pathology service. *J Clin Pathol* 1992;**45**:546–7.
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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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