of dental cysts was clearly associated with areas of mucous metaplasia with mucus secreting cells being CAM 5.2⁺. The reactivity of dentigerous cysts for CAM 5.2 often seemed to be within isolated single or groups of surface cells.

Our present observations, together with those obtained using frozen sections, suggest that it is not possible to differentiate between odontogenic cyst types on the basis of LP34 immunoreactivity. Although epithelial reactivity of odontogenic keratocyst linings with LP34 was subjectively less intense than that of dental and dentigerous cyst linings, the considerable differences between our results and those of MacDonald and Fletcher presumably indicate that LP34 reactivity in paraffin wax sections of odontogenic keratocysts is particularly laboratory or technique dependent. Indeed, the Dako data sheet concerning LP34 states that this antibody may not give "satisfactory" results on formalin fixed, paraffin wax sections unless large amounts of keratin proteins are present.

Finally, it is generally agreed that the greatest diagnostic difficulty in distinguishing between odontogenic cyst types occurs when the typical histological appearance of the epithelial lining is masked by inflammatory changes or metaplastic keratinisation. As both events are known to affect keratin expression it is unlikely that keratin profiles will aid the histological diagnosis of odontogenic cysts.
Statistics on microcomputers

The article on correlation and regression shows the correlation coefficient of \( r = 0.021 \) for the data in figure 1c, depicting a curvilinear relation between measurements A and B (page 5).

With all due respect, I believe that the correlation coefficient is inappropriate for a curvilinear relation of this sort; instead, an appropriate quadratic equation could be derived for the relation and the correlation calculated from that; alternatively, and more simply, the correlation ratio or eta coefficient.\(^2\)

The eta coefficient calculated for the measurements read (admittedly with some small inaccuracy) from the graph shown in figure 1c provides a value of 0.9154. In other words there is a very good curvilinear correlation shown, and this figure certainly fits better with the "eyeball" estimate of linear quality to the points on their graph.

The eta coefficient certainly does not seem to be widely used. It is indeed a little crude compared with deriving the proper quadratic equation, but the approximations are very close and rarely give rise to differences larger than the second decimal place.

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Drs Macdonald and Fletcher comment:

We are grateful to Matthews and Browne for their comments and we note the differences in staining pattern in odontogenic keratocyst linings using LP34 between their method and the method we described. The methods are different in several important respects. The tissue on which we based our observations had not been subjected to any form of decalcification. We used a normal rabbit serum pretreatment and incubated the section overnight with primary antibody (LP34; 1/200 dilution). This, we have found, virtually eliminates background staining while giving strong reliable reactivity. The method described by Matthews and Browne uses tissue which has been decalcified in 10% formic acid and pretreated with ovalbumin, and the incubation period and dilution of the primary antibody were different (LP34; 1/40-1/100 for 60 minutes).

Experienced immunohistochemists agree that to expect reproducible results using monoclonal antibodies, such as LP34, the pretreatment of the tissue and methods of staining used should be similar wherever possible.

We should like to take this opportunity to re-actify a typographical error and a detail from the material and methods section of our recent article. The antibody CAM 5.2 was supplied by Becton Dickinson and the secondary antibody was used was a peroxidase conjugated rabbit antimouse serum (Dako; 1/25 dilution).

AW MACDONALD
A FLETCHER

C-myc oncogene product p62 in ovarian mucinous neoplasms

In the article by Polacaro et al the oncogene protein product of c-myc was noted to differ in localisation in ovarian mucinous cystadenoma and cystadenocarcinoma. Such a difference can be explained by either compartmentalisation or artefactual localisation. The latter was considered to be unlikely because such a difference could be seen between two different epithelial types on the same tissue block in studies on colonic polypos. Such a conclusion is based on the assumption that the c-myc protein in different epithelia reacts similarly with fixatives and that the nuclear structure (especially the nuclear matrix), with respect to its ability to retain the c-myc protein, is in a similar state in both epithelia.

The first assumption is not always true. Abnormal c-myc protein is not unknown in malignancies (in Raji cell line) and these proteins in different tissues may behave differently when exposed to fixatives. This has been noted in mutated c-myc in vitro. Lee et al defined the regions in c-myc protein which are important but not necessary for nuclear localisation. In transfected cells expressing different mutants of c-myc, however, there is a discrepancy in the localisation of the protein when deletions of these regions occur in the mutant cells, depending on the method of processing.

If the protein is similarly mutated in malignant epithelium we might be seeing an artefact of protein displacement during tissue processing, but structural changes in protein are probably uncommon in malignancies. In fact, there is evidence that preservation of specific regions of the c-myc protein is important for its transforming ability and that these regions overlap with those which are most important for nuclear localisation. Whether there are contributory factors which may cause protein displacement during processing, such as structural changes in the nuclear matrix and the chromatin state, need to be investigated further.\(^3\)

Such an analysis does not exclude a change in compartmentalisation nor does it negate the importance of consistently finding cytoplasmic localisation of c-myc in certain neoplastic states. After all, what histology demands is only a reproducible artefact. On the other hand, there would be important implications for the function of the c-myc gene if we assume that cytoplasmic localisation is affected by targeting in malignant epithelium. We need to assess critically tissue processing.\(^4\)

We have shown previously that fixation and embedding do affect the quantitative and localisation of c-myc protein in cell lines and normal tissues.\(^5\) The optimal conditions for more reliable and consistent localisation of c-myc protein in frozen tissue have been defined. Purely cytoplasmic staining is very common, even in many normal tissues in paraffin wax blocks, and a complete nuclear localisation is seen in the corresponding frozen blocks if processed using the conditions we have defined. In a case of mucinous ovarian tumour of borderline malignancy, we found the same discrepancy between the paraffin wax and frozen blocks. Although it is hard for us to prove that it is the nuclear localisation that is real, it will probably be more difficult to argue that the cytoplasmic localisation is not artefactual.

For optimal immunostaining, the frozen sections are freshly cut immediately at 45°C for five to 30 minutes, air dried for one hour and fixed in 1% paraformaldehyde for 30 minutes. Periodate-lycine-paraformaldehyde gives similar results, while acetone or formaldehyde weaker staining with a tendency of more cytoplasmic than nuclear staining as the duration of fixation increases. The sections are then put in 0.1%. Triton-X-100 for 10 minutes. Standard peroxidase or APAAP techniques can then be applied.

In trying out these methods we have seen different artefacts, leading us to conclude that cytoplasmic localisation is easily simulated by manipulation of the tissue processing.

Finally, we might also need to look at the difference in nuclear matrix between benign and malignant epithelia. It is probably not too far-fetched to imagine that cytoplasmic localisation of c-myc protein in paraffin wax sections is related more to the chromatin pattern. If this were the case, maybe a more reasonable speculation on the function of c-myc is that it helps to maintain the chromatin in a state ready for cell division.

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Diagnostic importance of cytokeratin expression in linings of odontogenic cysts.

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