Letters to the Editor

Rehydration of air-dried smears: application in body cavity fluid cytology

Two types of smears are commonly used for cytological examination of body cavity fluids: (i) wet alcohol-fixed. Papanicolaou stained, or haematoxylon and eosin stained smears; and (ii) air-dried Romanowsky stained smears. We discuss our improvement on the alcohol-fixed smear technique.

Wet-fixed Papanicolaou stained smears have some disadvantages. Floating of the cells off the slide is a not uncommon occurrence. Albumination of the slides to prevent this, however, gives the smears a heavy green background. The nuclei are sometimes, particularly in adenocarcinoma with prominent morula formation, stained rather dark which obscures detailed nuclear morphology (fig 1a). The central or thicker areas of a cell cluster are often artefactually stained orange rather than green. Air-drying artefacts are quite common.

In our institute we use the air-dried rehydration technique for fine needle aspiration cytology smears.1 In view of the fact that body cavity fluids are good nutrients, and that the cells suspended in the fluid should be viable just like aspirated tissue we tried the same technique on fluid specimens.

Centrifuged, concentrated cell suspensions were spread on to albuminised glass slides. The slides were dried at room temperature. As soon as they were dry they were rehydrated for 30 seconds in 0.9% sodium chloride solution and finally fixed in 95% ethyl alcohol. They were then stained with haematoxylin and eosin. Two control smears were prepared for each case, one air-dried and rehydrated as above and stained with Papanicolaou stain, and another wet-fixed and stained with Papanicolaou stain as usual.

Of 300 cases examined over three months, haematoxylin and eosin stained rehydrated air-dried smears offered several advantages: the nuclear morphology was better than wet-fixed Papanicolaou-stained smears; the nuclei were crisper, the chromatin pattern clearer, and nucleoli more conspicuous. In thick cell clusters staining was still uniform and cells could be "seen through" (fig 1b). The background was very clear; unexpectedly the albumin did not take up haematoxylin and eosin.

On the whole, these slides were more pleasant to look at. The only disadvantage of which we are aware up to now is the extra time needed for air drying and rehydration.

Rehydrated air-dried Papanicolaou-stained slides were better than wet-fixed smears but not as good as those stained with haematoxylin and eosin.

We feel that the availability of a crisp chromatin pattern for examination in difficult cases may help in deciding whether the lesion is a malignant or a reactive process, and in our institute, rehydrated air-dried smears are used routinely to complement wet-fixed and air-dried smears.

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References


Figure (a) Cluster of adenocarcinoma cells from a pleural fluid smear fixed wet and stained with Papanicolaou.
(b) Another cluster of adenocarcinoma cells from the same case. The smear was air-dried, rehydrated, and stained with haematoxylin and eosin.


False positive bromide partition test in lymphomatous meningitis

The bromide partition test is still advocated for the diagnosis of tuberculous meningitis.1 The reliability of this test has been established in comparative studies of patients with tuberculous meningitis and acute viral lymphocytic meningitis. A low (<1-6) cerebrospinal fluid (CSF) ratio has good predictive value for tuberculous meningitis, especially in countries where there is a relatively high incidence.2

Many cases of chronic meningitis in patients without AIDS do not have an infectious aetiology, but tuberculous meningitis must be included in the differential diagnosis.3 The usefulness of the bromide partition test in this clinical setting has not been reported. I have recently seen a case in which the result was misleading.

Case report

A 62 year old woman with a four week history of depression, anorexia, and weight loss was admitted to hospital following the onset of agitation, confusion, and dizziness. She had photophobia and papilloedema with non-specific electroencephalogram abnormalities but a normal computed tomography scan. Her cerebrospinal fluid cell count was 58 x 10⁶/l (predominantly lymphocytes) and the protein concentration was 1-0 g/l. Cerebrospinal fluid glucose was 30% of the blood concentration. Tests for acid fast bacilli, cryptococcal antigen, and antibodies to syphilis, borreliae, and toxoplasma were negative. There were no clinical or haematological pointers to neoplastic disease; the erythrocyte sedimentation rate was 8 mm and a Mantoux test was negative with 1:1000 PPD.

The patient remained confused and febrile (up to 38°C) for the next three days without any specific treatment being given. Examination of a second cerebrospinal fluid sample gave essentially the same results; the cytological appearance of the lymphocytes did not suggest that any of them was malignant. Treatment with acyclovir was started but there was no clinical response after a week; herpes simplex antibodies were not detectable by radioimmunoassay. Anti-tuberculous chemotherapy (rifampicin, isoniazid, and pyrazinamide) was therefore started.

One week later, with no clinical...
improvement, a computed tomography scan indicated the possibility of a faint abnormality in the right frontal lobe. The cerebrospinal fluid cell count was now $110 \times 10^6$; 80% were lymphocytes, with immunological markers indicating that this was a polyclonal "reactive" lymphocytosis. The protein and glucose concentrations were unchanged but the bromide partition ratio was low (1.5). After a further two weeks' antituberculous treatment, however, the patient's condition had deteriorated, without further change in the cerebrospinal fluid. Five days' treatment with high dose steroids also failed to produce any clinical improvement. A brain biopsy specimen finally showed cerebral infiltration by a high grade B cell lymphoma. The patient responded initially to whole brain radiotherapy but she died six months later.

False positive results with the bromide partition test are not a new occurrence.

"From the point of view of diagnosis we do not for one moment suggest that a low bromide ratio is pathognomonic of tuberculous meningitis," wrote the authors of an early study, even though they found only two false positive results in 33 patients without tuberculous meningitis. The test has subsequently been shown to be quite reliable in distinguishing tuberculous meningitis from acute viral meningitis, its predictive value is quite unknown in the context of chronic meningitis. Until we have this information I would caution against using the test for clinical decision making. Perhaps measurement of interferon-alpha in the cerebrospinal fluid would be a better bet.

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References


Matters arising

Neutrophil elastase and elastic tissue in emphysema

We are pleased that the results presented in the paper by Fox et al., although seriously incomplete, are not contrary to and do not disprove our findings nor do they disprove the hypothesis that elastase, released by neutrophils in the lungs of emphysematous patients, binds to interstitial elastin.

Fox et al investigated the immunolocalisation of neutrophil elastase (HNE) in normal and emphysematous lungs using mean linear intercept ranges of 82–108 microns which they defined as normal, and 117–177 which they defined as emphysematous. When normal goat serum was diluted 1/5 with phosphate buffered saline containing bovine serum albumin, the authors state that they were able to block effectively the hydrophobic or electrostatic sites present on interstitial elastin and thus observed a gold labelled antibody binding to elastin of emphysematous lungs; they did observe a positive reaction on the granules of the in situ neutrophils.

Using BSA blocking, described in the method of Damiano et al., they obtained a positive binding of gold-labelled antibody on interstitial elastin, which they attributed to the binding of IgG to hydrophobic sites on the elastin. It is surprising that these authors found gold label on elastin when the primary antibody was omitted and only the gold-conjugated goat anti-rabbit IgG secondary antibody was used. We do not obtain gold binding to elastin when the primary antibody is omitted or when the primary antibody, affinity adsorbed with excess antigen, is used in place of the primary antibody.

It is thus not unexpected that Fox et al observed gold-labelled antibody binding when preimmune, HNE adsorbed anti-HNE, anti-factor VIII, or anti-HNE sera were used. The control sera, mentioned above, should be used to establish the non-specific binding background levels which, if quantified, can be subtracted from the total label obtained with the specific antibody. The difference between specific and non-specific can then be attributed to the labelling of interstitial elastase. As Fox et al did not quantify their data it is not possible to determine if there was any label above background. The fact that no label was observed for either the controls or the specific antibody when using normal goat serum as the blocking reagent suggests that the normal goat serum may be blocking specific antigenic sites and that either the concentration of the primary anti-HNE antiseraum should be increased or the concentration of the normal goat serum should be decreased until a positive specific reaction is observed.

We find it strange that the authors did not venture to try lower dilutions of the primary antibody when they obtained a negative result, at 1/1000 dilution, on isolated human elastin after incubation with HNE in vitro. One would interpret such an observation as indicating that HNE does not bind the isolated elastin when incubated in vitro, a clearly fallacious conclusion. Indeed, there would have been valuable to have a titre on how effective their antibody was against HNE, perhaps by using an ELISA.

If the authors had reviewed our paper, more carefully they would have found that their mean linear intercept (MLI) values of 117–177 microns for emphysematous patients are considerably lower than the values which Thurlbeck defines as normal (232–324 microns) and which we describe as mild emphysema (220–330 microns). MLI of normal lungs were reported by Dummii to be in the range of 130 microns. Saetta et al. described a destructive index as a method of quantifying parenchymal destruction and found no correlation between MLI and destructive index associated with the lungs of smokers having MLIs in the range of 243 to 460 microns. As we reported in our figure 1A, many of the sections with mild emphysema had MLIs of 200 to 300 and showed very little or no label associated with elastase binding to interstitial elastin. Sections which exhibited the highest label had MLIs in the range of 400 to 600 microns.

We appreciate the interest and concern that Fox et al have shown for our work, but we are disappointed that they worked with lungs showing only minimal emphysema. We hope that the apparent inconsistencies arising from their work can be resolved in the future.

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References
