improvement, a computed tomography scan indicated the possibility of a faint abnormality in the right frontal lobe. The cerebrospinal fluid cell count was now $110 \times 10^6/l$; 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.⁵ Although the test has subsequently been shown to be quite reliable in distinguishing tuberculous meningitis from acute viral meningitis,² its predictive value⁶ 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.⁷ Perhaps measurement of interferon-alpha in the cerebrospinal fluid would be a better bet.⁸

GW SMITH

Department of Medical Microbiology,
The Royal Free Hospital,
London NW3 2QG.

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Matters arising

Neutrophil elastase and elastic tissue in emphysema

We are pleased that the results presented in the paper by Fox *et al.*¹ 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 no gold-labelled antibody binding to the 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.*,² 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 antiserum 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 to isolated elastin when incubated in vitro, a clearly fallacious conclusion. Indeed, it 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 emphysematous but abnormal (232-320 microns)³ and which we describe as mild emphysema (220-330 microns). MLI of normal lungs were reported by Dunnill⁴ to be in the range of 130 microns. Saetta *et al.*⁵ used a destructive index as a method of quantifying parenchymal destruction and found a 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 8, 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.

VV DAMIANO

The Graduate Hospital,
One Graduate Plaza,
Philadelphia, USA, 19146.

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Matters arising

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Dr Fox *et al* comment:

Dr Damiano points out that our classification of emphysema was inadequate. We felt, however, that it was important to measure emphysema in the same tissue sections used for immunogold analysis, thus the tissue we examined for localisation of human neutrophil elastase (HNE) was that on which we had measured transect lengths. To eliminate the high background staining that we observed with formalin, we fixed the tissue for electron microscopy by a modified Karnovsky method. We then went on to measure transect lengths¹; this is the distance between alveolar septa as measured on random lines by computer image analysis; it is *not* the same as the mean linear intercept (MLI) described by Dunnill² and used by Damiano *et al.*³ Thus both the preparation of the tissue and the method by which the tissue was measured were different to the methods used by Damiano *et al.* When we compare the degree of emphysema, however, (fig 1)¹ with that shown by Damiano *et al* (fig 7)³ as severe emphysema, our cases seem comparable with those described by Damiano *et al.* Therefore, we do not think that we have worked with tissue showing minimal emphysema, and furthermore, in measuring transect lengths and immunogold label in the same section, we are confident that the tissue we labelled with immunogold was emphysematous. We remain interested to know how Damiano *et al* knew that the sections they labelled with immunogold were emphysematous.

We did not describe in detail the conditions under which we were able to get positive binding of gold-labelled antibody to elastin in the absence of primary antibody; this entailed longer incubation times, possibly reflecting a change in conformation or overall shape of the IgG molecule due to binding with the gold particle, which could conceivably affect access of the gold-labelled IgG to the elastin. We found that under the conditions we used to detect HNE in polymorphonuclear neutrophil granules, our control using no primary antibody with goat serum blocking agent gave negative results.

As Damiano correctly points out, there is non-specific binding of immunogold when formalin is used to fix the sections. For this reason we used another method of fixation which resulted in virtually zero background levels, and correction for background labelling was not necessary. Although, as Damiano says, there was no label observed

over elastic tissue for either the control or emphysematous lung tissue, this could not have been due to the blocking of specific antigenic sites by normal goat serum as he suggests, as polymorphonuclear granules in the same sections were positively stained (we did not assess sections if polymorphonuclear granules were absent, as these were used as the internal control). We did, in fact, use a range of dilutions of primary and secondary antisera and blocking agents, from 1/10 to 1/10 000. The dilutions used were those in which there was the largest signal to noise ratio which, by coincidence, was the same dilution as that used by Damiano *et al.* We do think that HNE binds to elastin to degrade the substrate, and there are data to suggest that HNE binds tightly to elastin. There are, to our knowledge, however, no data to suggest that the reaction is irreversible. It is unusual in nature for an enzyme, which is essentially a catalyst, to bind irreversibly to its natural substrate. In doing so, it would presumably not be able to proteolyse its substrate or to have access to other substrate molecules, a self-defeating process which is more likely to result in *less* substrate degradation. We therefore believe that the enzyme degrades the elastin and is released at the same time as the molecule is cleaved. The exact kinetics that occur *in vivo*, particularly in the presence of endogenous inhibitors of the enzyme, are not understood. In our *in vitro* study, however, the elastin-enzyme preparation was washed in buffer to remove unbound enzyme and may have removed residually "bound" HNE; this explains our observation of the absence of specific binding of HNE to elastin *in vitro*.

In summary, we have developed an immunogold labelling technique that will specifically identify HNE when we know it to be present; thus we found it in polymorphonuclear neutrophil granules in human lung and in rat lung after intratracheal instillation. The fact that we could not find HNE in association with elastic tissue in the same sections of emphysematous human lung leads us to believe that it does not bind irreversibly to elastic tissue. We think that the principal methodological difference between the two studies³ is our use of goat serum as a blocking agent. We wonder if Damiano *et al* used a blocking agent on their tissue sections which was homologous to the second antibody. This is a well established technique.⁴

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Tributyrin hydrolysis for identifying *Branhamella catarrhalis*

The Tributyrin test (LAB M) as described by Richards¹ has been in use in this laboratory for over two years. Initially it was used as a final confirmatory test along with a DNase test (after screening by colonial appearance/Gram film/positive oxidase test/negative sugar utilisation tests (Oxoid, *Neisseria* identification discs), and it was always found to correlate directly with a positive DNase test. It is a rapid and very convenient test and was never found to be positive with any other species erroneously "picked" as potential *Branhamella catarrhalis*. Accordingly, the Tributyrin test was then used as a sole confirmatory test (after screening by appropriate colonial appearance/Gram film/positive oxidase test) for routine respiratory and conjunctival isolates. Systemic isolates were identified using all the tests in combination.

Ahmad *et al* made no reference to the Tributyrin test in the identification of *B catarrhalis* and advocated the use of the Superoxol test.² He found that the National Collection of Type Culture strains of *B catarrhalis* and the strains designated *Branhamella* species identified as *B catarrhalis* (both DNase and Superoxol positive). Strain code number NC03623, however, is atypical on several counts; it produces smaller colonies and is both Tributyrin and DNase negative and produces a "slower" Superoxol reaction. The negative DNase test has been confirmed by the NCTC and this strain is no longer regarded as a typical *B catarrhalis*. The fact that the Tributyrin test readily picked up this anomalous strain whereas the Superoxol test did not would suggest that it is a more discriminating test for the identification of *B catarrhalis*.

PT MANNION

Brighton Public Health Laboratory,
Royal Sussex County Hospital,
Brighton, East Sussex.

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