LETTERS TO THE EDITOR

Elastase- α_1 proteinase inhibitor assay

Sir: We would like to comment on a recent paper in the *Annals*¹ because the published results and objections to our elastase- α_1 proteinase inhibitor assay do not agree with all previously published data. Many studies have been successfully performed during the past 10 years using our elastase assay systems.

The paper published in the *Annals* compared our two hour elastase assay with an 'in-house elastase assay' by measuring human plasma samples of patients with either rheumatoid arthritis or seronegative spondylarthritides and peripheral joint synovitis. The authors obtained much higher values when measuring plasma samples of the group with rheumatoid arthritis by our assay. The authors concluded from these results and from results obtained with fast protein liquid chromatography (Superose 12) that our assay may suffer seriously from interference by rheumatoid factor. Neumeier *et al.*, however, in 1982 showed that our assay is not influenced by free haemoglobin, lipids, high concentrations of bilirubin, rheumatoid factor (even purified), α_1 proteinase inhibitor, and α_2 macroglobulin.² Influence by rheumatoid factor would in this case mean the binding of immunoglobulin (rheumatoid factor) to antibodies of two different animal species, which is rather unlikely.

There are several points we would like to make about the published results of Banks *et al.* Firstly, samples for the in-house assay were prediluted 50 times with dilution/wash buffer, which was phosphate buffered saline containing 0.5 M NaCl, 0.1% Tween 20, pH 7.2. Our dilution buffer contains a protein matrix, which is essential in our system. Secondly, the 'in-house assay' needed one additional incubation step for the peroxidase labelled swine antirabbit antibody. This additional incubation step certainly would not increase the sensitivity or specificity of the assay, but more probably led to a decrease of sensitivity. Thirdly, we wonder whether the coating capacity was sufficient to detect high levels of elastase with the in-house assay? Finally, the described chromatography is of no value as the Superose 12 HR 10/30 column used has a maximum capacity (source Pharmacia and own experiments) of 5 to 30 mg total protein (optimum 5-10 mg, volume $\leq$ 200 μ l). The 500 μ l serum sample used contained about 40-50 mg protein, which means that the authors overloaded the column. This leads to non-specific and non-reproducible aggregation of proteins and, possibly, the results obtained by the authors. We recommend the injection of 50 mg marker proteins in 500 μ l phosphate buffered saline, and then the authors will see the poor resolution of proteins obtained by their fast protein liquid chromatography gel filtration. In the paper the authors did not mention the protein concentration of the fractions (elution pattern 280 nm not shown) and whether fractions from fast protein liquid chromatography were further diluted (if so: Which buffer was used?). The chromatogram showed a void volume of about 2 ml for the Superose column, whereas in our

experiments and those of Pharmacia void volumes of between 7 and 9 ml were obtained.

We hope that this letter corrects the wrong impression readers might have received of our elastase assay.

ARNULF HEUBNER
BÄRBEL UTZ
E Merck
Postfach 4119
6100 Darmstadt 1
Frankfurter Strasse 250
Germany

- 1 Banks R E, Evans S W, Taylor K F, Bird H A, Whicher J T. Measurement of plasma concentrations of polymorphonuclear elastase- α_1 proteinase inhibitor (elastase- α_1 trypsin) in patients with rheumatoid arthritis: interference by rheumatoid factor. *Ann Rheum Dis* 1990; 49: 18-21.
- 2 Neumeier D, Fateh-Moghadam A, Menzel G. Humane granulocytelastase. I. Zur Methodik einer enzymimmunologischen Bestimmung des Elastase- α_1 -Proteinase-inhibitor-Komplexes. *Z Anal Chem* 1982; 311: 389-90.

Sir: We would like to point out that in November 1988, before our paper¹ was submitted for publication, a draft of our results was sent to BDH Ltd who market the Merck assay kit in the United Kingdom. The above letter to the *Annals of the Rheumatic Diseases* is the first response that we have received from Merck, however.

We do not dispute that many studies have been successfully performed within the past 10 years using the Merck polymorphonuclear elastase kit. The only reservation that we have is when the assay is used with samples containing rheumatoid factor. Indeed, as described in our paper, we found no significant difference between results obtained with the Merck assay and those obtained with our 'in-house' assay for control samples and samples from patients with seronegative spondylarthritides. The only other published report which we have found which has investigated the possible interference of rheumatoid factor in the Merck elastase assay is that quoted in the above letter from Merck—namely, the paper by Neumeier, Fateh-Moghadam, and Menzel.² As scant methodological detail is provided in that paper, however, it is impossible for the reader to make any assessment about the statement that rheumatoid factor does not interfere with the assay.

The interference of rheumatoid factor in immunoassays is well reported, and rheumatoid factors have been shown to bind to immunoglobulins from many species, including rabbit and sheep. As stated in the letter from Drs Heubner and Utz (Merck), the interference of rheumatoid factor in their assay would occur as the result of crosslinking antibodies from two different species—that is, the coating antibody and the labelled detection antibody. However, contrary to their claim that this is 'rather unlikely', Elkon *et al.* have shown that rheumatoid factor can bind both immobilised rabbit IgG and radiolabelled sheep IgG in the same assay.

The appropriate dilution buffers were used for each assay, and we did not find a protein matrix to be essential in our system. We do not claim that the additional incubation step which we performed in the 'in-house' assay led to any increase in its sensitivity. The coating capacity in the 'in-house' assay was sufficient to produce a standard curve of similar range to that obtained with the Merck assay, and after appropriate dilution synovial fluid samples containing elastase- α_1 proteinase inhibitor at

concentrations greater than 10 000 μ g/l were successfully measured. In a larger series of samples from patients with rheumatoid arthritis we have now found an increased range of plasma concentrations than in our previous report but still found that many measurements (about half) lie within the normal range.

We would dispute the claim made by Merck that the described fast protein liquid chromatography is of no value. Although we loaded more total protein than is recommended by Pharmacia (the manufacturer) for optimal high resolution with the particular column, we obtained reproducible results. We did note a decreased resolution as reflected by the relatively large number of fractions needed to collect the peaks of interest and the narrow interval between the peaks. Owing to the large differences in molecular weight between the elastase complex and rheumatoid factor, however, we were still able to resolve these substances, as shown in our paper. This decrease in resolution, but still satisfactory separation of substances of these molecular weights, would be expected with this column under these conditions (source: Pharmacia). The void volumes of between 7 and 9 ml for this column obtained by Pharmacia and Merck were also obtained in our experiments and not 2 ml as stated in the above letter. This misconception is entirely our fault as we omitted to state in the paper that the fraction collector was not started until after the first 5 ml of each run to avoid needlessly collecting the bulk of the void volume. Fractions from the fast protein liquid chromatography separation were assayed in each assay system usually after being diluted 50-fold with the appropriate dilution buffer in each case.

We remain confident that the evidence presented in our paper indicates a probable interference of rheumatoid factor in the Merck elastase assay, despite the criticisms levelled at our study by Drs Heubner and Utz of Merck and which we hope that we have replied to satisfactorily. Additionally, we find it very difficult to believe that the excellent correlation we obtained between rheumatoid factor levels and the discrepancy between results obtained with the two assays is purely coincidental and that the same is true for the fast protein liquid chromatography data.

R E BANKS
S W EVANS
J T WHICHER
Department of Chemical Pathology and Immunology
University of Leeds

K F TAYLOR
Department of Immunology
General Infirmary at Leeds

H A BIRD
Rheumatology and Rehabilitation Unit
Department of Medicine
University of Leeds

- 1 Banks R E, Evans S W, Taylor K F, Bird H A, Whicher J T. Measurement of plasma concentrations of polymorphonuclear elastase- α_1 proteinase inhibitor (elastase- α_1 trypsin) in patients with rheumatoid arthritis: interference by rheumatoid factor. *Ann Rheum Dis* 1990; 49: 18-21.
- 2 Neumeier D, Fateh-Moghadam A, Menzel G. Humane granulocytelastase. I. Zur Methodik einer enzymimmunologischen Bestimmung des Elastase- α_1 -Proteinase-inhibitor-Komplexes. *Z Anal Chem* 1982; 311: 389-90.
- 3 Elkon K B, Caeiro F, Gharavi A E, Patel B M, Ferjencik P P, Hughes G R V. Radioimmunoassay profile of antiglobulins in connective tissue diseases: elevated level of IgA antglobulin in systemic sicca syndrome. *Clin Exp Immunol* 1981; 46: 547-56.