The Use of Enzyme-labeled Antibodies to Increase the Sensitivity of Immunocapillarymigration *

CRISTINA GLAD** and
ANDERS O. GRUBB

Department of Clinical Chemistry,
University of Lund, Malmö General
Hospital, S-214 01 Malmö, Sweden

A novel procedure for immunochemical quantitation termed immunocapillarymigration has recently been described.[1,2] It was based upon capillarymigration of antigen solutions along strips of a porous material to which antibodies were attached. Migration of antigen was specifically delayed in comparison with other constituents contained in the solution and was related to antigen concentration. In the initial study fluorescein-labeled antibodies were used to expose antigen-covered areas on the immune strips. The sensitivity of the procedure was limited to 40 mg/l. In the present study we demonstrate that the sensitivity of immunocapillarymigration can be increased 100-fold with the use of enzyme-labeled antibodies, obviating the need for ultraviolet light in the procedure.

The immunoglobulin fraction of the antiserum was prepared by ammonium sulfate precipitation and covalently linked to CNBr-activated cellulose acetate sheets (Sartorius, Göttingen, W. Germany).[3] The sheets were dried, cut into strips and capillarymigration of samples in the strips was performed as described previously.[2] Following migration of samples the antigen-covered areas were exposed in two steps. Firstly, the strips were incubated for 3 min in a solution of horseradish peroxidase-labeled antibodies, prepared according to Avrameas *et. al.*,[4] and excess antibodies washed from the strips by use of running tap water. Secondly, the strips were incubated in a peroxidase-substrate solution (20 mg 3-amino-9-ethylcarbazone-2.5 ml dimethylformamide made up to 50 ml with 0.05 mol/l acetate-buffer pH 5.0 containing 25 μl 30 % H_2O_2). Dense coloured areas developed within 5 min. The heights of these areas were measured and compared with corresponding heights produced by serial dilutions of a standard serum (Fig. 1).

When the antibody activity of the immunoglobulin fraction linked to the strips was decreased by addition of non-antibody immunoglobulins, lower concentrations of antigens in the samples could be measured. Concentrations of transferrin and C-reactive protein of 0.3 mg/l could be clearly differentiated from zero. This meant an improvement in sensitivity of 100 times compared to the previously described technique using fluorescein-labeled antibodies.[2]

A comparison of immunocapillarymigration and electroimmunoassay[5] for the quantitation of C-reactive protein in 19 human plasma samples provided a correlation coefficient of 0.92 (Fig. 2). The standard deviation of the immunocapillarymigration assay calculated from duplicate determinations was 15 % of the mean. This comparatively low precision for the procedure may be related to the rather low enzyme activity of the peroxidase-labeled antibodies. Consequently a relatively long incubation in the substrate solution was required resulting in a diffuse demarcation on the immune strips probably caused by diffusion of

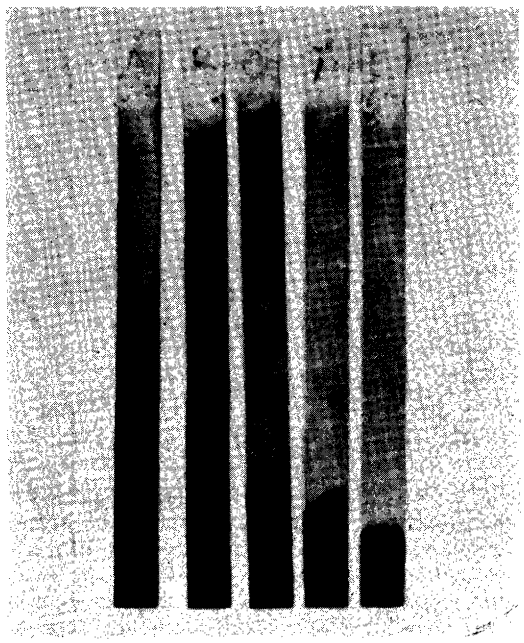

Fig. 1. Stained immunocapillarymigration strips. Antibodies against human C-reactive protein were coupled to the strips and serial dilutions of a standard plasma were allowed to migrate along the strips and the C-reactive protein covered areas were exposed by use of peroxidase-labeled antibodies against C-reactive protein. The concentrations of C-reactive protein used were 4.8, 2.4, 1.2, 0.6 and 0.3 mg/l.

* Communication at the Meeting of the Swedish Biochemical Society in Lund, 5–6th June, 1980.

** To whom correspondence should be sent.

0302-4369/80/060449-02$02.50

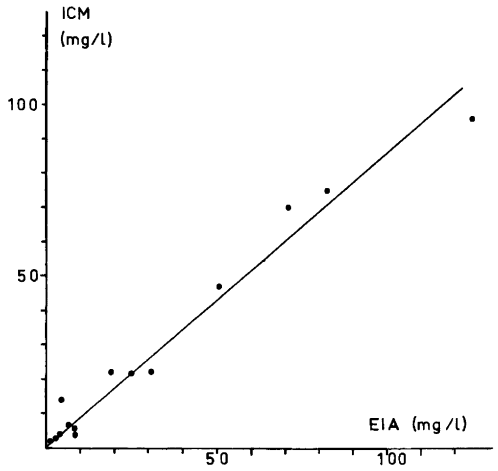

Fig. 2. Correlation between the determinations of the C-reactive protein concentration in 19 human plasma samples by electroimmunoassey (EIA) and by immunocapillarymigration (ICM). The equation $y=0.86x+0.15$, where y is the estimation by ICM, gives the mathematical relation between the estimations as calculated by the method of least squares.

the coloured product. Preliminary experiments, using conjugated antibodies with higher enzymatic activity indicate that it is possible to improve the precision of the method.

In conclusion, use of enzyme-labeled antibodies increases the sensitivity of immunolapillarymigration to such an extent that the procedure allows rapid determination of the plasma concentration of C-reactive protein. Since an increase in the plasma level of this protein is a very early sign of inflammatory diseases, immunocapillarymigration might be used for the early detection of such diseases.

Acknowledgements. This work was supported by grants from AB KabiVitrum, Stockholm, Sweden, *Kungliga Fysiografiska Sällskapet i Lund*, The Medical Faculty, University of Lund and The Swedish Medical Research Council (Project No. B 81-13X-05196-04B).

1. Glad, C. and Grubb, A. O. *Biochem. Soc. Trans. 5* (1977) 712.
2. Glad, C. and Grubb, A. O. *Anal. Biochem. 85* (1978) 180.
3. Nishikawa, A. H. and Bailon, P. *Anal. Biochem. 64* (1975) 268.
4. Avrameas, S. and Ternynck, T. *Immunochemistry 8* (1971) 1175.
5. Laurell, C.-B. *Anal. Biochem. 15* (1966) 45.

Received May 29, 1980.