# AN AUTOMATED IMMUNOPRECIPITIN ASSAY OF PROTHROMBIN FOR THE CONTROL OF ANTI-COAGULANT THERAPY

A.B. Watson (1), C.N.Wood (2), J.M.Lavergne (3) and J.F. Davidson (1), (1) Department of Haematology, Royal Infirmary, Glasgow; (2) Unit of Forensic Science, Department of Pharmaceutical Chemistry, University of Strathclyde, Glasgow; (3) Hôpital de Bicêtre, Paris

Coumarin anti-coagulant therapy is almost universally regulated by the estimation of the combined biological activities of Factors II, VII, IX and X. More efficient control may be achieved by the specific determination of one of these Factors - Prothrombin, particularly if some form of automation could be applied.

The Technicon Automated Immunoprecipitin (AIP) system (Larson & others, 1970), developed for the estimation of serum proteins, was further modified and applied to the determination of prothrombin in plasma. Initial experiments confirmed that the basic AIP methodology produced insufficient sensitivity despite alteration to the various reaction parameters. The low concentration of prothrombin in plasma necessitates the use of low plasma dilution in the system. Consequently, the resulting concentration of the light scattering β lipoprotein produces excessive background, thereby preventing satisfactory differentiation between test and blank results.

A modification of a standard method for the isolation of β lipoprotein in serum (Burstein & others, 1970) was developed and applied to the test plasmas and antiserum, prior to AIP assay. The incorporation of the delipidation process significantly reduced sample background and permitted a satisfactory increase in sensitivity. The removal of β lipoprotein was however, accompanied by the loss of prothrombin from the test plasma. The loss, as estimated by the Laurell electro-immunodiffusion assay, was found to be proportional to the original plasma prothrombin concentration. 30 samples with prothrombin levels ranging between 42% and 102% of normal pooled plasma concentration, gave a mean loss of 29.7% of original activity.

Reproducibility of the delipidation process was studied by the Laurell technique and the Coefficient of Variation (C.V.) was calculated to be 7.8% (C.V.for the Laurell assay itself was estimated to be 6.7%). Reproducibility of our 'lipid-poor' AIP assay was also studied and a C.V. of 4.8% obtained.

50 non-pathological plasma samples were estimated for prothrombin by the Laurell technique and the 'lipid-poor' AIP assay. Although excellent correlation between the two assays was obtained (Correlation Coefficient 0.88), an unacceptable AIP intercept of 46.7% and Regression Coefficient of 0.49 suggested possible non-specificity of the antiserum used. Re-estimation of a number of the original samples by the 'lipid-poor' AIP assay using a more specific prothrombin antiserum produced improved statistical values (AIP intercept 29.6%, Regression Coefficient 0.64).

The delipidation process may allow the estimation of a number of other plasma/serum proteins present in low concentration, which are otherwise unmeasurable by the standard AIP methodology.

Burstein,M., Scholnick,H.R. & Morfin,R. (1970). J.Lipid Res., 11, 583 - 595.
Larson,C., Orenstein,P. & Ritchie,R.F. (1970). Advances in Automated Analysis, Technicon International Congress.