

also found to be a stabilizing agent, but its use made plate preparation unnecessarily complicated.

#### PREPARATION OF PLATES FOR ASSAY OF PROTEOLYSIS

Heated plates are used frequently to assay proteolysis, but their sensitivity is greatly reduced. As an alternative, the addition of AMCHA to the plate was studied. AMCHA plates were also found to have a reduced sensitivity to proteolysis but were marginally easier to prepare.

#### ACCURACY OF THE METHOD

A number of urokinase solutions of known strength were assayed on the fibrin plate and by a standard tube clot lysis method. The methods were found to be comparable in accuracy although at low activator concentrations the plate became less reproducible.

The original fibrin plate was found to be a convenient and reliable method for assaying plasminogen activators. The only useful modification was the addition of calcium chloride for fibrin stabilization. For immediate use, the unmodified plate was quite acceptable.

#### Comparison between a Direct and an Indirect Method for Determining Plasminogen

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An immunochemical method for the determination of plasminogen has been available since 1968 (Ganrot and Niléhn, 1968). As this assay is quite simple and rapid and thus would be of advantage in laboratory work, we considered it of interest to compare it with the caseinolytic method for determining plasminogen recommended by Johnson, Kline, and Alkjaersig (1969). The immunochemical method we used was a slight modification of the method of Ganrot and Niléhn (1968) by Ekelund, Hedner, and Nilsson (1970). To avoid activation of plasminogen *in vitro* the blood was collected in tubes containing EACA. The determinations were made according to Laurell (1966) with electrophoresis in agarose gel containing a specific rabbit antiserum to human plasminogen. The serum samples diluted 1:5 migrates into the gel and precipitation peaks are formed. The height of these peaks obtained is a measure of the amount of antigen in the samples. Mixed serum from 20 apparently healthy volunteers was used as standard. The normal range was found to be 75-137% (44 normal persons examined).

In the caseinolytic method for determining plas-

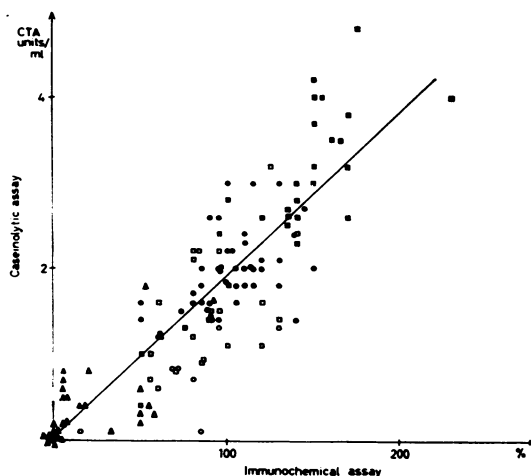


Fig. Comparison of plasma plasminogen levels assayed by caseinolytic and immunochemical methods.

minogen (Johnson *et al.* 1969) the plasminogen in plasma was precipitated by 30% ammonium sulphate. After removal of the supernatant containing the plasmin inhibitors the precipitate was resuspended and its plasminogen content was determined with a caseinolytic method. Removal of the plasmin inhibitors in this way is claimed to be more effective than by acidification. The results were expressed in CTA units. Among 44 normal individuals the mean value was found to be 1.9 CTA units/ml (SD  $\pm$  0.46) and the normal range 1.0-2.8 CTA units/ml.

Plasminogen determinations were performed with both methods in 44 normal subjects (aged 18-45 years), 25 pregnant women in the third trimester, seven patients with septicaemia, nine foetuses obtained by legal induced abortion, 24 patients with liver diseases, and 20 patients during thrombolytic therapy.

In all 129 subjects the plasminogen values varied as measured with the caseinolytic method between 0 and 4.8 CTA units/ml and as measured with the immunochemical method between 0 and 230%. From the figure it is apparent that there was a close correlation ( $r = 0.9$ ;  $P < 0.001$ ) between the plasminogen values determined immunochemically and as caseinolytic activity both for high and low plasminogen levels.

The immunochemical plasminogen assay has proved reproducible and as sensitive as the caseinolytic method. Inhibitors of plasminogen activation do not influence the results.

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