hepatoma D23-specific activity was performed in a titanium BXIV zonal rotor with a sucrose density gradient [5–20% (w/w), linear with volume]. After centrifugation for 5.5 h at 115000g (r_{av} , 4.5 cm) at 5°C, a range of large-molecular-weight components (up to approx. 22S), which retained specific antigenic determinants, were recovered. These gave predominantly single bands on analytical polyacrylamide electrophoretic gels and were revealed in the electron microscope by negative-contrast staining as discrete molecular entities. The bulk of the antigenic activity was associated with a much-smaller-molecular-weight fraction that did not sediment far into the gradients. Preparative polyacrylamide-gel electrophoresis of this fraction further resolved the antigenic component as a defined zone. This material gave a single diffuse migratory band on analytical polyacrylamide-gel electrophoresis, and by using the velocity sedimentation method of Martin & Ames (1961) an approximate molecular weight of 55000 was estimated.

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Studies on Fibrinogen Degradation in the Rat

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The precise mechanism by which any plasma protein is degraded remains uncertain, although evidence is accumulating in favour of engulfment by pinocytosis and subsequent degradation within secondary lysosomes. Such a sequence has been demonstrated for denatured albumin (Mego & McQueen, 1965, 1967), and lysosomes have also been implicated in the breakdown of undenatured plasma proteins (Gordon & Jacques, 1966). The present report describes attempts to investigate the mechanism of fibrinogen degradation in the rat.

In preliminary experiments isolated rat livers were perfused with homologous rat blood (Curtis *et al.*, 1971) containing screened rat ^{125}I -labelled fibrinogen. Samples of the perfusate were removed every hour for periods up to 7 h, and the plasma was assayed for

total and trichloroacetic acid-soluble ^{125}I . On the assumption that the screened ^{125}I -labelled fibrinogen was indistinguishable from the native fibrinogen in the perfusate, the average rate of fibrinogen degradation was calculated to be 0.91 mg/h per 12.5 g liver. At the end of each perfusion the plasma was subjected to column chromatography on Sephadex G-25. The elution profiles showed radioactive peaks that corresponded in mobility to [^{125}I]iodinated tyrosine, free [^{125}I]iodide, large-molecular-weight ^{125}I -labelled material and possibly some ^{125}I -labelled peptides.

In subsequent experiments rats were injected with screened ^{125}I -labelled fibrinogen and after 30 min the livers were removed and subfractionated (Davies *et al.*, 1971). The distribution of ^{125}I showed that 20% of the total radioactivity in the liver was associated with the lysosome-rich fraction. Incubation (at 22°C) of this lysosome-rich fraction over 2 h showed a progressive decrease in the trichloroacetic acid-insoluble ^{125}I -labelled material within the particles. This was accompanied by a progressive increase in the trichloroacetic acid-soluble ^{125}I outside the particles, demonstrating that appreciable degradation had taken place. The other crude subcellular fractions showed little or no such activity.

In a further series of experiments partially purified lysosomal fractions were prepared from rat liver (Ragab *et al.*, 1967). The lysosomes were ruptured by freezing and thawing and centrifuged at 52000g for 30 min. Samples (0.5 ml) of the supernatant (containing 1.5 mg of protein) were incubated (up to 24 h at 37°C) with screened ^{125}I -labelled fibrinogen (1.5 mg dissolved in 1 ml of 0.1 M-sodium acetate-acetic acid buffer, pH 5.5). Under these conditions the amount of trichloroacetic acid-soluble ^{125}I increased to over 96% of the total ^{125}I , showing that the labelled fibrinogen had been completely degraded by the lysosomal enzymes.

These findings support the view that *in vivo* the liver makes a significant contribution to the overall degradation of fibrinogen.

We are grateful to The Wellcome Trust for financial support. T. R. I. is grateful to the Trust for a fellowship.

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