or methanolysed, reacylated and examined by g.l.c. as their trimethylsilyl derivatives (Clamp *et al.*, 1967).

The compounds fell into two classes, namely those that were neutral at pH 5.3 but separated on electrophoresis at pH 2.0, and acidic compounds, with very low electrophoretic mobilities at pH 2.0, but separable at pH 5.3. Three major neutral components were seen, namely components N₁, N₂ and N₃ with respectively one, two and three additional neutral carbohydrate residues attached to the aspartylglycosamine residue. Traces of higher-molecular-weight neutral compounds were also present. Component N₁ had an additional galactose residue, and component N₂ had galactose and fucose. Component N₃ could be resolved into two components on prolonged electrophoresis at pH 2.0; one had two mannose residues and one galactose residue, and the other had two mannose residues and one additional *N*-acetylglucosamine residue.

At least nine acidic compounds were present, with some members containing more than one acidic group/molecule. Most of these compounds contained sialic acid and were converted into neutral compounds on treatment with 0.1 M-HCl at 80°C; however, at least one of the more acidic compounds was stable to this treatment, and it possibly contained a sulphated residue as it moved slowly to the anode even at pH 2.0.

K. M. P. is in receipt of a Medical Research Council Scholarship.

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The Iodination of Mammalian Cells Catalysed by Lactoperoxidase as a Probe for Externally Located Proteins of the Plasma Membrane

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The use of lactoperoxidase to catalyse the iodination of tyrosine residues exposed on the outside of human erythrocyte membranes has been described by Phillips & Morrison (1971). We have been able to confirm their finding that only two peptide-chain components (as revealed by polyacrylamide gel

electrophoresis in sodium dodecyl sulphate) are labelled. One of these components is the glycoprotein of the membrane. In addition, we have obtained evidence that the sites on both components can saturate, and exhibit similar kinetic behaviour in this iodination reaction. Since the macromolecule lactoperoxidase must participate in this specific iodination, this reaction should provide an excellent means of identifying protein components of cells that could possibly participate in specific intercellular interactions. We are therefore applying this label to the case of the cultured BHK 21 cell, and preliminary results will be reported.

We wish to acknowledge the financial assistance of the Tenovus Organization in this work.

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Isolation of Plasma-Membrane-Associated Tumour-Specific Antigen from Rat Hepatoma Cells

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Carcinogen-induced rat hepatomas and fibrosarcomas possess tumour-specific antigens, comparable with weak histocompatibility antigens, against which the host may evoke an immune response (Baldwin *et al.*, 1971*a,b*). These antigens are specific products of individual tumours and are viewed to be new discrete components of the transformed cell surface. Limited papain digestion of cell membranes has been used extensively to release H-2 and HL-A transplantation antigens as water-soluble glycoproteins (Reisfeld & Kahan, 1970). Similar techniques have now been applied to an amino-azodye-induced rat hepatoma (hepatoma D23) to characterize tumour-specific antigens.

Antigenic activity present in individual or pooled preparations of solubilized hepatoma D23 cell membranes was determined by their capacity to neutralize the reaction of antibody in hepatoma D23 immune serum with tumour-specific antigens expressed on viable hepatoma D23 cells. This inhibition of antibody reactivity was measured by using the indirect membrane immunofluorescence test (Baldwin & Barker, 1967).

An 'extranuclear' membrane preparation of hepatoma D23 tissue was subjected to limited papain digestion (1 mg of enzyme/30 mg of membrane protein) for 60 min at 37°C. The soluble extract was fractionated by DEAE-cellulose column chromatography by the technique of Baldwin & Glaves (1972). Velocity sedimentation of eluted fractions exhibiting

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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