Phosphodiesterase and Ribonuclease Activities of the Plasma Membrane of Rat Liver and Hepatoma

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Plasma-membrane fractions isolated from the crude nuclear fraction of liver homogenates possess considerable alkaline phosphodiesterase and ribonuclease activities (Emmelot, Bos, Benedetti & Rümke, 1964; Lansing, Belkhome, Lynch & Lieberman, 1967; Erecinska, Sierakowska & Shugar, 1969). Until recently, however, methods were not available to isolate the 40% of the plasma-membrane fragments that sediment as microsomal fraction, or to separate plasma-membrane sheets simultaneously with other cell organelles. We have used zonal rotors to investigate the subcellular distribution of phosphodiesterase and ribonuclease activities, employing techniques that separated plasma-membrane sheets from the crude nuclear fraction (Hinton, Fitzsimons, Dobrota & Reid, 1970) and plasma-membrane vesicles from the microsomal fraction (El-Aaser, Norris & Reid, 1970; Hinton, Norris & Reid, 1971), while simultaneously giving other elements in good condition. Methods developed for liver microsomal fraction were equally applicable to hepatoma, but attempts to separate large sheets of membrane from hepatoma failed.

The results indicate that about one-half of the particulate alkaline ribonuclease and a considerably higher proportion of alkaline phosphodiesterase (assayed with bis-*p*-nitrophenyl hydrogen phosphate) are associated with the plasma-membrane. The distribution of enzymes in microsomal sub-fractions indicates that a major part both of microsomal ribonuclease (de Lamirande, Boileau & Morais, 1966) and microsomal phosphodiesterase (Razzell, 1961; de Lamirande *et al.* 1966) is due to plasma-membrane fragments.

Comparison of 'nuclear' and 'microsomal' plasma-membrane phosphodiesterase and ribonuclease activities revealed no significant differences in amounts or properties. The pH optimum of the phosphodiesterase was between 7.4 and 7.8, but with considerable residual activity in the acidic region. These determinations were, however, complicated by the inhibitory action of some buffers, notably phosphate and 3,3-dimethylglutarate. The phosphodiesterase was inhibited by EDTA and activated by Mg^{2+} , the activity in the presence of 5mM-magnesium chloride being four times that in the presence of 5mM-EDTA. The pH optimum of the ribonuclease was very similar, but this enzyme was less activated by Mg^{2+} than was the phospho-

diesterase. Preliminary experiments indicate that the properties of tumour and liver plasma-membrane ribonuclease activities are similar. Liver plasma-membrane ribonuclease had little activity towards poly(A), activity towards this substrate being largely confined to mitochondria (cf. Morais, Blackstein & de Lamirande, 1967).

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Amino-Azo-Dye-Binding Protein in the Soluble Cytoplasm of the Rat Liver

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It has been the belief for some time that amino-azo-dye carcinogens bind covalently to proteins of the soluble cytoplasm of the rat liver and that the major part is bound to a basic protein fraction (Sorof, Young, McCue & Fetterman, 1963). Further, a basic amino-azo-dye-binding protein, thought to be the major dye-binding component, has been isolated as a pure substance and some of its properties have been described (Ketterer, Ross-Mansell & Whitehead, 1967).

A claim that the principal amino-azo-dye-binding protein is not basic has been made (Sugimoto & Terayama, 1970), and this is based on experiments in which liver extracts are subjected to mild heat treatment followed by several chromatographic separations. However, we have found that if the heat treatment is followed by electrophoresis in an