there was no purification over the initial whole homogenate.

Cells grown anaerobically in the presence of 10% (w/v) glucose contained no detectable cytochrome oxidase or catalase. Cells grown anaerobically with 5% (w/v) glucose (and maintained as anaerobic as possible during sphaeroplast production and lysis) did, however, show low activities of both these enzymes. The percentage sedimentability of enzymes in homogenates of these cells was as follows: protein, 7%; acid phosphatase and esterase, not sedimentable; NADH-cytochrome *c* oxidoreductase, 19%; NADPH-cytochrome *c* oxidoreductase, 4%; NADPH-ferri-cyanide reductase, 33%.

Cells grown anaerobically with galactose in the presence of Tween 80 and ergosterol (Wallace, Huang & Linnane, 1968) do possess detectable activities of cytochrome oxidase and catalase and the percentage sedimentability of these enzymes was 87% and 55% respectively. The median density of sedimentable protein (1.19) corresponded with that of NADH- and NADPH-cytochrome *c* oxidoreductases and NADH- and NADPH-ferri-cyanide oxidoreductases; acid phosphatase was 47% sedimentable.

R.B. was I.C.I. Fellow during the course of this work.

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The Subcellular Acquisition of Carbohydrates by Glycoproteins

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Several reports have indicated that the microsomal membranes are of primary importance in the addition of carbohydrate to the protein moiety of glycoproteins (Lawford & Schachter, 1966; Molnar, Robinson & Winzler, 1965). We have investigated the subcellular sites of acquisition of carbohydrate by protein in the immunoglobulin A-secreting plasmacytoma, MPC-1, by using a technique of incubation *in vitro*.

Homogenization and fractionation of incubated myeloma tissue on a continuous sucrose gradient gives a number of distinct bands. The specific radioactivity of protein from these bands after incubation of the tissue with [¹⁴C]lysine is largely uniform, whereas there are marked differences between the specific radioactivities of bands obtained after incubation with [¹⁴C]glucosamine. Uptake of both [¹⁴C]lysine and [¹⁴C]glucosamine is linear with time up to 140 min. The radioactive myeloma protein synthesized in these subcellular

fractions was identified by immunoelectrophoresis of sonicated extracts of the fractions, followed by radioautography of the resulting precipitin lines.

In the presence of puromycin at a concentration that inhibits protein synthesis by 98%, [¹⁴C]-glucosamine incorporation into trichloroacetic acid-insoluble glycoprotein is decreased by only 50–60%. Further, there is a decrease of only 20–30% in radioactive glycoprotein appearing in the incubation medium. Thus there must exist in the cell a large pool of partially glycosylated protein that continues to be glycosylated and secreted after protein synthesis has virtually ceased.

Whether ribosomes are involved in the initial attachment of hexosamine to glycoprotein is a matter of contention. Preparations of ribosomes from tissue incubated with [¹⁴C]glucosamine showed a linear increase in specific radioactivity up to 140 min. Tissue preincubated with [¹⁴C]glucosamine for 30 min. and 70 min., and subsequently incubated for a further 10 min. with added puromycin, showed an exactly similar decrease in the specific radioactivity of the ribosomes after the puromycin addition. However, this decrease constituted only 29% and 16% respectively of the total specific radioactivity of the ribosomes. Thus it is suggested that two factors contribute to ribosomal labelling: glucosamine attached to nascent polypeptides, and radioactivity from adsorbed labelled material.

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Studies on Sialoglycoprotein Complexes of Human Erythrocyte Membranes

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Proteins and glycoproteins have been prepared by the disruption of erythrocyte membranes in several ways (Rega, Weed, Reed, Berg & Rothstein, 1967; Blumenfeld, 1968; Harris, 1968; Marchesi & Steers, 1968; Rosenberg & Guidotti, 1968; Schneiderman & Junga, 1968). We have modified the techniques of Marchesi & Steers (1968) and Blumenfeld (1968) to enable us to prepare two glycoprotein complexes from human erythrocyte 'ghosts'.

Erythrocyte 'ghosts' (water-washed, 2%, w/w) were treated with mercaptoethanol (50 mM) and