toxin (1 µg/ml) or dibutyryl cyclic AMP (0.2mM) to the culture medium in which the cells are propagated.

The altered distribution of glycosylation in cells exposed to cholera toxin and dibutyryl cyclic AMP, which is less evident in cells exposed to conditions that restrict the expression of transformation, is also manifested in immune-precipitation reactions, in which cellular glycoproteins are allowed to react with antisera versus murine leukaemia virus components. In such experiments, immune serum detects a differential effect of cholera toxin on glycosylation, which appears to depend on conditions that allow or restrict the expression of malignant transformation in vivo.

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Liver Sinusoid Surface Membranes and Glycoprotein Secretion

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The liver is the source of most serum proteins (Miller & John, 1970). These are synthesized in the rough endoplasmic reticulum, transported to the Golgi apparatus and there packaged into secretion granules. The same route is apparently followed by cell-coat proteins (Keenen & Morré, 1975). In both cases, the secretion granules fuse with the plasma membrane and discharge their contents, although serum proteins appear to be temporarily associated with the plasma membrane and may be detected immunologically (Riordan et al., 1974). Export proteins will presumably be transported to the sinusoidal surface of the cell, but this will not necessarily be the case with cell-coat proteins.

The sheets of plasma membrane, which sediment with the crude nuclear fraction, are derived principally from the bile-canaliculcar face of the cell (Wisher & Evans, 1975) so one might expect that sinusoid membrane fragments would be found principally in the microsomal fraction. This is supported by the concentration in microsomal plasma membrane of glucagon-activated adenylate cyclase (Wisher & Evans, 1975). However, the plasma-membrane fragments found in the microsomal fraction are themselves heterogeneous (Norris et al., 1974), and it is not clear whether all the vesicles are derived from the sinusoid membranes. One approach to this problem is to label the sinusoid membrane in situ. The dye SITS (4-acetamido-4'-isothiocyanato stilbene-2,2'-disulphonic acid), which binds covalently to surface membranes without penetrating into the cells (Knauf & Rothstein, 1971), appears to be suitable.

In the present experiments, sinusoid membranes were labelled by perfusion of the livers with 50 µM-SITS (BDH Chemicals, Poole, Dorset, U.K.). In separate experiments newly made glycoproteins were labelled by the intravenous injection of L-[1-14C]glucose (The Radiochemical Centre, Amersham, Bucks., U.K.) 15 min before the death of the animal (Riordan et al., 1974). Plasma-membrane sheets were separated from the crude nuclear fraction of the liver homogenates as described by Hinton et al. (1970); the microsomal preparations were subfractionated as described by Norris et al. (1974). Marker enzymes were assayed as described by Prospero et al. (1973). The distribution of newly made glycoprotein was determined by precipitation with 5% (w/v) trichloroacetic acid, collection on glass-fibre filters, and dispersing and counting for radioactivity as described by Scherrer (1969). SITS fluorescence was measured at 483 nm with excitation at 350 nm by using a Perkin–Elmer (Beaconsfield, Bucks., U.K.) spectrofluorimeter. KOH was added to a concentration of 15% (w/v) immediately before taking the reading.

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The rats were anaesthetized with diethyl ether and perfused through the portal vein with 0.25 M sucrose/5 mM Tris, pH 8.0, until the livers were completely blanched. Each liver was then perfused with 30 ml of SITS dye (50 μM in 0.25 M sucrose/5 mM Tris, pH 8.0) and then extensively perfused with 0.25 M sucrose/5 mM Tris, pH 8.0, to remove unbound SITS dye. Homogenization and centrifugation were done as described by Hinton et al. (1970). The results were corrected for endogenous fluorescence by comparison with a control experiment in which the SITS dye perfusion was omitted. Fluorescence, enzyme activities and protein concentrations are in arbitrary units. ●, SITS fluorescence; ..., protein; ○, 5’-nucleotidase activity; ——, density (20°C).

After homogenization of the livers, large particulates were removed by centrifugation for 15 min at 11500 g. The microsomal fraction was collected by centrifugation for 1 h at 122000 g resuspended in 2 M sucrose and layered under a linear sucrose gradient in a B-XIV zonal rotor. Enzyme activities are given in arbitrary units. ●, 14C radioactivity; ○, 5’-nucleotidase activity; ▲, alkaline phosphodiesterase activity; ..., protein; ——, density.

Fluorescence microscopy of livers perfused with SITS showed that labelling was restricted to blood vessels and sinusoids. Difficulties were, however, encountered in localizing the SITS label in the subcellular fractions owing to the high endogenous fluorescence. After correction, it was clear that the distribution of fluorescence among subfractions...
separated from the nuclear fraction was generally similar to the distribution of 5'-nucleotidase (Fig. 1), but that there was proportionately more label in plasma-membrane fragments of a microsomal size (fractions 4–6) than in plasma-membrane sheets (fractions 21–23). The fluorescence/5'-nucleotidase ratio in the microsomal fraction was similar to that of the crude nuclear microsomal fraction. The high endogenous fluorescence, however, prevented clear localization of the SITS dye in submicrosomal fractions, although there were some indications that the label was concentrated in the lower-density material.

The distribution of rapidly labelled glycoproteins among particles in the crude nuclear fraction was extremely similar to the distribution of SITS fluorescence. Subfractionation of microsomal preparation (Fig. 2) showed that newly made glycoproteins were concentrated in very-low-density vesicles presumably derived from the Golgi apparatus (fractions 1–5) and in the lower-density plasma-membrane vesicles (fractions 11–14). As it was thought possible that this apparent localization could be due to secretion granules, the vesicles were treated with 0.15M-NaCl, to remove loosely bound protein, and 0.2M-NaHCO₃, pH 9.0, to remove vesicle contents (Issa & Hinton, 1974). The results showed that about 45% of the labelled glycoprotein could be solubilized from the plasma-membrane vesicles as against 30% of the label in plasma-membrane sheets from the crude nuclear fraction and over 60% of the material from the low-density Golgi vesicles. Solubilization of the extracted membranes by 1% sodium deoxycholate and 0.5% Lubrol W (Blomberg & Perlman, 1971) and analysis by immunodiffusion against anti-liver plasma membrane confirmed that newly made membrane glycoproteins were concentrated in plasma-membrane vesicles banding at a density of 1.14g/cm³ (fractions 11–14, Fig. 2).

The results presented above show that newly made serum and cell-coat glycoproteins appear first in plasma-membrane fragments, which appear to be derived from the sinusoidal surface of the cell. This suggests that there is no difference in packaging in the Golgi apparatus between proteins destined for release into the serum and those that will be incorporated into the cell coat. Proteins destined for eventual release into the serum are probably associated with the plasma membrane, so explaining the immunological cross-reaction between well-washed plasma-membrane preparations and anti-rat serum (Issa & Hinton, 1974). The subsequent release of the proteins into the serum would then be explained by their low affinity for the membrane as compared with the proteins that form a permanent part of the cell coat.

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