Isolation of a Golgi-Apparatus-Enriched Fraction from Leukaemic Cells

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1. A Golgi-apparatus-enriched fraction was isolated from acute leukaemic lymphoblasts of AKR mice by using an homogenate stabilized with 1 mM-glutaraldehyde. 2. The isolated fraction, which was shown morphologically to be enriched in dictyosomes, possessed between 44- and 76-fold increase in specific activity, compared with the tumour homogenate, of UDP-galactose-glycoprotein galactosyltransferase and between 3- and 10.5-fold increase in relative specific activity of UDP-*N*-acetylgalactosamine-polypeptide *N*-acetylgalactosaminyltransferase. 3. Plasma membranes isolated from the leukaemic lymphoblasts also possessed glycoprotein galactosyltransferase activity, though in contrast with Golgi-apparatus-enriched material had no detectable polypeptide *N*-acetylgalactosaminyltransferase. 4. The difficulties associated with maintaining the morphological integrity of the Golgi apparatus in subcellular fractionation are discussed.

There is a considerable body of evidence that complex carbohydrates, in particular glycoproteins, are important constituents of cell surfaces. Much interest has centred on the activity of these molecules during malignant transformation of the cell, as structural variations in surface carbohydrates may be responsible for the altered social behaviour of malignant cells. Cell-electrophoretic studies of the acute lymphoblastic leukaemic cells of AKR mice (Cook & Jacobson, 1968) had demonstrated that the electrokinetic properties of malignant cells differed from those of normal lymphoid cells. This was interpreted as resulting from structural differences in the membrane glycoproteins. Lectin-binding studies by Presant & Kornfeld (1972) on leukaemic lymphoblasts and normal lymphocytes of AKR mice indicate a quantitative difference in the surface carbohydrate residues. Zatz *et al.* (1972) demonstrated that the leukaemic cells of AKR mice are defective in the surface components necessary for normal recirculation. Their studies and previous investigations (Woodruff & Gesner, 1968) indicate that these components are glycoprotein in nature.

A major site within the cell for the glycosylation of membrane proteins and lipids is the Golgi apparatus (Bennett *et al.*, 1974; Fishman, 1974). Any differences in the structure of membrane glycoproteins in normal and leukaemic cells are likely to be reflected in this organelle by an altered spectrum of glycosyltransferases. In order to investigate this hypothesis further it will be necessary to isolate fractions enriched in the Golgi apparatus from normal lymphoid and leukaemic cells, and in this paper a method for isolating such material from

lymphoblastic leukaemic cells of AKR mice is reported.

Materials and Methods

Chemicals

All solutions were prepared in water that had been distilled from an electrically heated metal still (Manesty Machines Ltd., Liverpool, U.K.) and immediately redistilled twice in Pyrex glass stills fitted with suitable spray and soda-lime traps. Chemicals and materials for enzyme assays were of analytical grade unless otherwise stated and were from BDH Chemicals Ltd., Poole, Dorset, U.K., or Fisons Scientific Apparatus Ltd., Loughborough, Leics., U.K. Bovine serum albumin, prepared as a protein standard, was from Armour Pharmaceutical Co., Eastbourne, Sussex, U.K. Dextran T.250 was from Pharmacia (G.B.) Ltd., London W5 5SS, CsCl (optical grade) was from Gallard Schlesinger, New York, NY, U.S.A.

Fetuin (Spiro Method; 99% pure; lot number AG 156D) was from Gibco Bio-Cult Ltd., Paisley, Renfrewshire, Scotland, U.K., and a sample of α_1 acid glycoprotein was kindly made available by Dr. Yu-Lee Hao, American National Red Cross Blood Research Laboratory, Bethesda, MD, U.S.A., under the scheme run by the National Fractionation Center [with partial support of NIH grant HE 13881 (HEM)]. Sheep submaxillary mucin from which the sialic acid and *N*-acetylgalactosamine residues had been removed enzymically (Hagopian & Eylar, 1968), to produce a suitable acceptor (SNF-OSM; sialic acid- and *N*-acetylgalactosamine-free sheep submaxillary mucin) for assaying polypeptide *N*-acetylgalactosaminyltransferase activity (EC 2.4.1.41), was a generous gift from Dr. E. H. Eylar, Medical

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UDP-D-[U-¹⁴C]galactose (210 mCi/mmol), UDP-N-acetyl-D-[1-¹⁴C]galactosamine (61 mCi/mmol) and *n*-[1-¹⁴C]hexadecane (0.11 mCi/mmol; reference standard for liquid-scintillation counting) were from The Radiochemical Centre, Amersham, Bucks., U.K.

Preparation of Golgi-apparatus-enriched fractions

Tumour tissue was harvested as a routine from AKR mice 14 days after the subcutaneous implantation of acute lymphoblastic leukaemic cells (described in detail by Cook & Jacobson, 1968). A modification of the procedure of Morré *et al.* (1970) was used to fractionate these cells.

Tumours were homogenized in 0.5 M-sucrose (3:1, w/v) containing 37.5 mM-Tris/maleate, pH 6.4, 1% dextran and 5 mM-MgCl₂ by using a Polytron PT 20 homogenizer (Northern Media Company Ltd., Hull, U.K.) operated at setting no. 2 for 90 s. In a number of experiments a range of concentrations (100–1 mM) of glutaraldehyde was incorporated into the homogenization medium to stabilize the Golgi apparatus. Homogenate (2.5 ml) was layered directly on a discontinuous gradient consisting of 1.1 M-sucrose (6 ml) layered over 1.25 M-sucrose (6 ml), both sucrose solutions containing 37.5 mM-Tris/maleate, pH 6.4, 1% dextran and 5 mM-MgCl₂. The gradient was placed in a 3 × 23 ml swing-out rotor and centrifuged in a MSE Superspeed 50 centrifuge for 5 h at 4°C at 99000g (*r*_{av}, 9.7 cm). Golgi-apparatus-enriched material collected at the homogenate/1.1 M-sucrose interface and was removed by aspiration with a Pasteur pipette, added to water (15 ml) and pelleted by centrifugation for 30 min at 99000g (*r*_{av}, 9.7 cm) as described above.

Homogenates (1 ml) stabilized with glutaraldehyde were also fractionated on a discontinuous gradient consisting of 0.7 M-sucrose (4 ml), 1.3 M-sucrose (4 ml) and 1.7 M-sucrose (7 ml), all containing 0.1 M-Tris/HCl, pH 7.6, 0.01 M-MgCl₂ and 1% dextran, by the procedure of Schachter *et al.* (1970). The centrifugation conditions and collection procedure were identical with that described above.

Preparation of plasma membranes

The method described in detail by Warley & Cook (1973) for the isolation of plasma membranes from acute lymphoblastic leukaemic cells of AKR mice was used.

Preparation of rough and smooth microsomal fractions

Leukaemic tissue was homogenized as detailed above in 0.25 M-sucrose as the homogenization medium (ratio of tumour to homogenization medium, 1:4, w/v). After centrifugation at 10000g_{av} for 20 min, the supernatant fluid was aspirated and fractionated into rough and smooth microsomal fractions by

using the CsCl-gradient method of Dallner *et al.* (1966).

Electron microscopy

Pelleted material was fixed in 2.5% (w/v) glutaraldehyde dissolved in 0.09 M-sodium cacodylate buffer, pH 7.2, containing 3 mM-CaCl₂ for 1 h and then washed in the cacodylate buffer. The pellets were separated into a number of fragments, post-fixed in 1% (w/v) OsO₄ dissolved in cacodylate buffer, pH 7.2, for 1 h, stained with 0.5% uranyl acetate for 1 h, dehydrated in ethanol and embedded in Araldite. Thin sections were stained with 0.3% lead citrate and examined in a GEC AEI EM6B electron microscope. Care was taken to examine all regions of the pellet thoroughly.

Assay of enzymic activities

UDP-galactose-glycoprotein galactosyltransferase activity (glycoprotein galactosyltransferase; EC 2.4.1.38) was assayed by the method of Caccam & Eylar (1970) and UDP-N-acetylgalactosamine-polypeptide *N*-acetylgalactosaminyltransferase activity (polypeptide *N*-acetylgalactosaminyltransferase) was assayed by the method of Hagopian *et al.* (1968). The radioactive products were counted on glass-fibre squares in toluene-based scintillant (Stoddart & Northcote, 1967). 5'-Nucleotidase (EC 3.1.3.5) activity was determined by using the procedure described by Persijn *et al.* (1968). All determinations were performed in duplicate, and where sufficient material was available, in triplicate. Where appropriate in the Results section values are quoted as the mean ± s.d.

Chemical determinations

Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. Galactose was measured by using Galactostat reagent (Cambrian Chemicals, Croydon, Surrey, U.K.).

Preparation of exogenous acceptors

Sialic acid-free agalactofetuin was prepared from desialylated fetuin by the method of Spiro (1966). This method has been used by others (Molnar *et al.*, 1969; Kim *et al.*, 1971) to prepare a suitable exogenous acceptor for assaying glycoprotein galactosyltransferase activity. Sialic acid was removed from fetuin (268 mg) by suspending the glycoprotein in 0.05 M-H₂SO₄ (25 ml) and incubating at 80°C for 1 h. The hydrolysate was neutralized by the addition of 1 M-NaOH and then dialysed exhaustively against water at 4°C; the non-diffusible material was isolated by freeze-drying. The sialic acid-free agalactofetuin contained no demonstrable galactose.

Sialic acid and galactose were also removed from α₁ acid glycoprotein in a similar manner to that described for fetuin, except that the molar ratio of periodate

EXPLANATION OF PLATE I

Electron micrographs of thin sections of a Golgi-apparatus-enriched fraction isolated from leukaemic cells

The fractions were isolated, after stabilization of the homogenate by the presence of 1 mM- or 100 mM-glutaraldehyde (Plates 1b and 1c), by following the procedure described in detail in the text. (a) Section through a pellet of the fraction obtained at the homogenate/sucrose interface when homogenate was layered over a discontinuous 1.1 M-/1.25 M-sucrose density gradient. The fraction is devoid of mitochondria (cf. Plate 1b). Magnification $\times 31\,000$; the bar represents $1\,\mu\text{m}$. (b) Section through a pellet of the fraction obtained at the homogenate/sucrose interface when homogenate was layered directly over a 1.25 M-sucrose barrier. A number of dictyosomes (arrows) are present together with some mitochondria (double arrows) (cf. Plate 1a). Magnification $\times 48\,000$; the bar represents $1\,\mu\text{m}$. (c) Section through same material as in (b). This enlarged area shows more clearly the stacks of Golgi cisternae that form a dictyosome. Magnification $\times 75\,000$; the bar represents $0.5\,\mu\text{m}$.

to protein was 300:1 and the reaction was allowed to proceed for 2h, conditions that Eylar & Jeanloz (1962) demonstrated destroyed in excess of 90% of the galactose present. The sialic acid-free α_1 acid glycoprotein contained 4.7% galactose and the sialic acid-free agalactoprotein 0.1% galactose, expressed as free sugar.

Results

Morphology of the isolated fractions

Thin sections of material pelleted from the fraction collecting at the top of the discontinuous gradient were examined by transmission electron microscopy and were found to contain numerous stacks of Golgi cisternae (dictyosomes), together with smooth vesicles (Plate 1a). Where an homogenate was layered directly over 1.25M-sucrose, the highest concentration used by Morré *et al.* (1970) for isolating Golgi-apparatus-enriched material, the pelleted fraction was found to consist of dictyosomes, smooth-membrane vesicles and a significant number of mitochondria (see Plates 1b and 1c). The concentration of glutaraldehyde present in the material illustrated in Plate 1(a) was 1 mM and in Plates 1(b) and 1(c) was 100 mM. Variation

of the concentration of glutaraldehyde from 100 to 1 mM with the same batch of tumour had no effect on the morphology of the isolated Golgi-apparatus-enriched fraction.

When the alternative procedure of Schachter *et al.* (1970) was used to fractionate glutaraldehyde-stabilized homogenates, material collecting at the homogenate/0.7M-sucrose interface consisted of smooth-membrane vesicles, some collagen fibres and a few free ribosomes. Smooth-membrane vesicles were also recovered at the 0.7M-/1.3M-sucrose interface, and the fraction collecting at the 1.3M-/1.7M-sucrose interface contained numerous free ribosomes in addition to vesicles formed from the rough endoplasmic reticulum and some smooth-membrane fragments. Nuclear debris and mitochondria were recovered in the pellet from the base of the gradient. Dictyosomes were not identifiable in any fraction.

Glycoprotein glycosyltransferases in the Golgi-apparatus-enriched fraction

The distribution of exogenous glycoprotein galactosyltransferase and polypeptide *N*-acetylgalactosaminyltransferase activities in the Golgi-apparatus-

Table 1. *Glycoprotein glycosyltransferases in Golgi-apparatus-enriched and accompanying fractions obtained from leukaemic cells*

Four separate batches of tumour were fractionated as described in the Materials and Methods section. Two batches were used to examine the distribution of UDP-galactose-glycoprotein galactosyltransferase within the subcellular fractions by using sialic acid-free agalactofetuin (82 μ g) as acceptor, and the other two batches were examined for the distribution of UDP-*N*-acetylgalactosamine-polypeptide *N*-acetylgalactosaminyltransferase. In the latter assays SNF-OSM (80 μ g) was used as acceptor. Both glycosyltransferase activities were assayed by using the procedures described in the Materials and Methods section. 'Purification' refers to the relative specific activity of the fraction compared with that of the homogenate.

Glycoprotein galactosyltransferase	Preparation I			Preparation II		
	$10^{-3} \times$ Specific activity (d.p.m./h per mg of protein)	$10^{-3} \times$ Total activity (d.p.m./h)	Purification	$10^{-3} \times$ Specific activity (d.p.m./h per mg of protein)	$10^{-3} \times$ Total activity (d.p.m./h)	Purification
Homogenate	8.3	1238		2.4	498	
Material at 0.5M-/1.1M-sucrose interface	630	63	76	105	32	44
Material at 1.1M-/1.25M-sucrose interface	87.6	23	11	*	*	
Pellet of material at bottom of gradient	17.5	895	2	2.2	196	0.9

Polypeptide <i>N</i> -acetylgalactosaminyl transferase	Preparation III			Preparation IV		
	$10^{-3} \times$ Specific activity (d.p.m./h per mg of protein)	$10^{-3} \times$ Total activity (d.p.m./h)	Purification	$10^{-3} \times$ Specific activity (d.p.m./h per mg of protein)	$10^{-3} \times$ Total activity (d.p.m./h)	Purification
Homogenate	5.7	400		1.09	217	
Material at 0.5M-/1.1M-sucrose interface	18.3	9	3.2	11.5	6	10.5
Material at 1.1M-/1.25M-sucrose interface	4.6	44	0.8	*	*	
Pellet of material at bottom of gradient	2.0	111	0.4	2.2	136	2.0

* Insufficient material recoverable for assay purposes.

enriched fractions obtained by subcellular fractionation of the tumour are given in Table 1. Four different batches of tumour were fractionated, and two batches examined for each enzyme activity. Material collecting at the homogenate/1.1 M-sucrose interface and shown morphologically to be enriched with dictyosomes had the highest specific exogenous activity relative to the homogenate for both enzyme activities (see Table 1). In all batches this fraction contained low amounts of endogenous activity. The result for the total assay after incubation for 1 h for glycoprotein galactosyltransferase was 5148 ± 2467 (3 determinations) d.p.m. and 2606 ± 158 (3) d.p.m., as opposed to 141 ± 107 (3) d.p.m. and 32 ± 6 (3) d.p.m. respectively in the absence of appropriate acceptor. Polypeptide *N*-acetylgalactosaminyltransferase activity values of 839 ± 285 (3) d.p.m. and 667 ± 84 (3) d.p.m. were found after incubation for 1 h with added acceptor; the corresponding endogenous activity was zero and 185 ± 39 (3) d.p.m. The recovery of glycoprotein galactosyltransferase activity in the two separate batches of tumour fractionated was 5 and 6.4%, corresponding to 0.06 and 0.15% of protein recovered. For polypeptide *N*-acetylgalactosaminyltransferase, recoveries of 2.3 and 2.8% of the enzyme activity in the fraction collecting at the homogenate/1.1 M-sucrose interface were associated with protein recoveries of 1.3 and 0.25% respectively.

Although material collecting at the base of the gradient showed high enzyme activity (see Table 1), this was associated with sizeable recoveries of the total cell protein, of which 32–46% was recovered as a routine in this fraction. In contrast with the dictyosome-enriched fraction, this material also possessed appreciable endogenous galactosyltransferase activity. In the total assay, values of 7899 ± 1224 (3) d.p.m. and 4336 ± 283 (3) d.p.m. were obtained after 1 h incubation, whereas the corresponding values in the absence of appropriate acceptor were 1929 ± 1128 (3) and 2096 ± 41 (3) d.p.m. respectively. Similarly, when UDP-*N*-acetylgalactosamine was present as donor, appreciable quantities of endogenous activity were demonstrable; values of 1002 ± 851 (3) d.p.m./h and 7961 ± 199 (3) d.p.m./h as opposed to 1323 ± 1120 (3) and 9705 ± 901 (3) d.p.m.h.

Contrasting results were obtained when the cell homogenates were fractionated on the gradient described by Schachter *et al.* (1970). No single fraction showed enrichment in both glycosyltransferases (see Table 2). The highest relative specific activity of the glycoprotein galactosyltransferase was found in the fractions collecting at the 0.5 M-/0.7 M- and 0.7 M-/1.3 M-sucrose interfaces. The only appreciable purification of polypeptide *N*-acetylgalactosaminyltransferase was in the pellet from the base of the gradient.

The properties of the glycosyltransferases examined were similar to those described by Caccam & Eylar (1970) and Hagopian *et al.* (1968). The modified

Table 2. Glycoprotein glycosyltransferases in subcellular fractions obtained from leukaemic cells

	Glycoprotein galactosyltransferase						Polypeptide <i>N</i> -acetylgalactosaminyltransferase					
	Preparation V			Preparation VI			Preparation VII					
	$10^{-3} \times$ Specific activity (d.p.m./h per mg of protein)	$10^{-3} \times$ Total activity (d.p.m./h)	Purification	$10^{-3} \times$ Specific activity (d.p.m./h per mg of protein)	$10^{-3} \times$ Total activity (d.p.m./h)	Purification	$10^{-3} \times$ Specific activity (d.p.m./h per mg of protein)	$10^{-3} \times$ Total activity (d.p.m./h)	Purification	$10^{-3} \times$ Specific activity (d.p.m./h per mg of protein)	$10^{-3} \times$ Total activity (d.p.m./h)	Purification
Homogenate	5.5	752		2.3	303		10	580				
Material at 0.5 M-/0.7 M-sucrose interface	87.8	31	16	42	12	18	6.9	1.9	0.7			
Material at 0.7 M-/1.3 M-sucrose interface	60.0	237	11	37.8	94	16	9.5	11	0.95			
Material at 1.3 M-/1.7 M-sucrose interface	11.2	110	2	11.2	68	5	19.4	305	1.9			
Pellet of material at bottom of gradient	5.2	144	0.9	5.9	170	2.6	38.2	12	3.8			

Three separate batches of tumour were fractionated by the method of Schachter *et al.* (1970) as described in the Materials and Methods section. Two batches were examined for glycoprotein galactosyltransferase activity within the subcellular fractions and the other batch was assayed for polypeptide *N*-acetylgalactosaminyltransferase activity. Sialic acid-free agalactofetuin (82 µg) and SNF-OSM (80 µg) were used as acceptors in the respective assay procedures, which are described in the Materials and Methods section. 'Purification' refers to the relative specific activity of the fraction compared with that of the homogenate.

glycoprotein, sialic acid-free α_1 acid glycoprotein, also acted as an acceptor in the glycoprotein galactosyltransferase assay; values of 86% of the activity measured with an equal amount of sialic acid-free agalactofetuin were obtained.

When the material collecting at the 0.7M-/1.3M-sucrose interface was examined for glycoprotein galactosyltransferase activity from the same batch of tumour fractionated in the absence and presence of glutaraldehyde (1mM), the former had a specific activity of 58×10^3 d.p.m./h per mg of protein and the aldehyde-treated preparation had a value of 62×10^3 d.p.m./h per mg of protein, indicating that under the conditions used this aldehyde did not destroy glycoprotein galactosyltransferase activity in the isolated fractions.

Glycosyltransferases in isolated plasma-membrane fractions

The same reaction mixtures were used to investigate the presence of glycoprotein galactosyltransferase and polypeptide *N*-acetylgalactosaminyltransferase activity in plasma membranes. Plasma membranes equal to 15 μ g of protein for each assay were isolated by the method of Warley & Cook (1973).

Isolated plasma membranes showed virtually no endogenous glycoprotein galactosyltransferase activity after 1 h, 4.3 ± 3.0 (3) d.p.m. as opposed to an appreciable amount of exogenous activity, 1119 ± 87 (3) d.p.m., in the same material. The specific activity of the isolated plasma membranes was 74.3×10^3 d.p.m./h per mg of protein and compares with 630.3×10^3 and 105.1×10^3 d.p.m./h per mg of protein for the Golgi-apparatus-enriched fractions. In the absence of Triton X-100, 64% of the exogenous activity was retained, as opposed to 29% for Golgi-apparatus-enriched material.

Isolated plasma membranes possessing glycoprotein galactosyltransferase activity showed no detectable polypeptide *N*-acetylgalactosaminyltransferase activity, in contrast with Golgi-apparatus-enriched fractions.

Polypeptide N-acetylgalactosaminyltransferase in rough and smooth microsomal fractions

Rough and smooth microsomal fractions prepared by the procedure of Dallner *et al.* (1966) showed no polypeptide *N*-acetylgalactosaminyltransferase activity.

5'-Nucleotidase activity in the Golgi-apparatus-enriched fraction

A sample of the Golgi-apparatus-enriched fraction was examined for 5'-nucleotidase activity by the method of Persijn *et al.* (1968) and found to possess a specific activity of 2.3 nmol/min per mg of protein. This value represents 2.4% of the lower value and 1.2% of the higher value found previously for isolated

plasma membranes from this cell (Warley & Cook, 1973).

To establish whether glutaraldehyde had an effect on 5'-nucleotidase activity associated with this tumour, the same batch of leukaemic cells was divided into two portions, one of which was homogenized in medium with no aldehyde and the other in the presence of 1mM-glutaraldehyde. The latter material had a specific activity of 187 nmol/min per mg of protein, whereas in the absence of glutaraldehyde a value of 163 nmol/min per mg of protein was obtained. As glutaraldehyde stabilization does not lower 5'-nucleotidase activity, the low activity of this enzyme in the Golgi-apparatus-enriched fraction, as opposed to isolated plasma membranes, indicates that the latter membranes are not concentrated to any extent in the fraction.

Discussion

The isolation of Golgi-apparatus-enriched fractions has relied heavily on morphological criteria as a means of identification. Indeed, as Morré *et al.* (1970) have stated, 'morphology is so characteristic that it serves as a reliable marker'. Initially morphological details were used to identify Golgi-apparatus-enriched fractions obtained from the leukaemic cells.

As with other studies on the isolation of Golgi-apparatus-enriched fractions, homogenization and fractionation conditions are critical if morphologically identifiable dictyosomes are to be obtained. Cook (1973) reported that when using this cell type it was necessary to include glutaraldehyde in the homogenization medium as a stabilizing agent, a situation reported previously for plant tissues by Morré *et al.* (1965). Low ratios of homogenization medium to tissue were also found to be necessary for maintaining dictyosomal integrity, as reported by Hudgin *et al.* (1971), who found that a lower ratio of homogenization fluid to hepatoma was required than that used for the isolation of Golgi-apparatus-enriched fractions from normal rat liver. Initially the procedure described by Cook (1973) was used for fractionating the acute lymphoblastic leukaemic cells; however, electron-microscopic examination showed that although numerous dictyosomes were present the fractions also contained varying amounts of other organelles, principally mitochondria and ribosomes. Leelavathi *et al.* (1970) were able to purify a Golgi-apparatus-enriched fraction from rat liver by flotation of a 'crude membrane felt' on a 1.1M-sucrose barrier. When material derived from leukaemic cells from the homogenate/1.25M-sucrose interface, was purified further on a 1.1M-sucrose barrier, although a large amount of ribosomal material was removed, no dictyosomal profiles were identified among the smooth membranes collecting

above the 1.1M-sucrose barrier. By combining the two barriers not only was the time taken to isolate a Golgi-apparatus-enriched fraction from the leukaemic cells greatly decreased, but the problem of the extreme lability of this organelle to fractionation procedures was largely overcome; material collecting at the homogenate/1.1M-sucrose interface in the discontinuous gradient was found to be enriched in dictyosomes.

The extreme lability of the Golgi apparatus of these cells during isolation is also illustrated in those cases where the fractionation procedure devised by Schachter *et al.* (1970) was used. Though this method was used successfully by Hudgin *et al.* (1971) to isolate Golgi apparatus from Morris hepatoma, in the present work no fraction was obtained that could be identified by morphological means as being derived from this organelle. Material collecting at the 0.7M-/1.3M-sucrose interface, where Golgi-apparatus-enriched material would be expected (Schachter *et al.*, 1970) to collect, though containing principally smooth membranes, was devoid of any dictyosomes.

Ovtracht *et al.* (1973), who examined the sub-fractionation of the Golgi apparatus, point out that the difficulty of isolating this organelle from tumour lines may be due to extremely rapid unstacking of the dictyosomes in tumour homogenates, perhaps owing to increased activities of lysosomal enzymes.

With the successful isolation of the Golgi apparatus from a number of non-malignant mammalian cells (Morré *et al.*, 1970; Leelavathi *et al.*, 1970), increased interest has been focused on this organelle as a major site of glycosylation within the cell, and a number of authors have reported enrichments of glycosyltransferases in fractions derived from the Golgi apparatus that complement the results of radioautography (Bennett *et al.*, 1974). In leukaemic cells the greatest enrichment of glycoprotein glycosyltransferases was found in the dictyosomal-rich material. The enrichment of glycoprotein galactosyltransferase in this fraction is in good agreement with the 100- and 40-fold purifications reported for rat liver (Morré *et al.*, 1969) and bovine liver (Fleischer *et al.*, 1969) respectively. Recovery of 5% of the activity in a Golgi-apparatus-enriched fraction from the leukaemic cells compares well with 2% recovery reported (Fleischer *et al.*, 1969) for a comparable fraction from bovine liver and 0.9–4% for rat testis (Letts *et al.*, 1974). The glycoprotein galactosyltransferase activity, which we have established is not destroyed by our method of glutaraldehyde stabilization, is associated with less than 0.15% of the total cell protein, a finding similar to that reported by Fleischer *et al.* (1969) and Letts *et al.* (1974). Dictyosomal-enriched fractions also showed increased polypeptide *N*-acetylgalactosaminyltransfer-

ase, an enzyme that initiates glycosylation of the polypeptide chain.

As Molnar *et al.* (1965) suggest that initial glycosylation occurs simultaneously with, or immediately after, synthesis of the polypeptide in the rough endoplasmic reticulum it would be interesting to know if the activity found in the Golgi-apparatus-enriched fraction represents contamination with rough endoplasmic reticulum.

Attempts to demonstrate the presence of polypeptide *N*-acetylgalactosaminyltransferase in rough and smooth microsomal preparations of leukaemic cells prepared by the method of Dallner *et al.* (1966) were unsuccessful. This result contrasts with that of Wagner *et al.* (1973), who were able to demonstrate glycosyltransferases in such fractions of rat liver, but may be due to the lability of the enzyme under the conditions of a low ratio of tumour to homogenization fluid that are used in the method of Dallner *et al.* (1966).

A consideration of the question of contamination of Golgi-apparatus-enriched material with rough endoplasmic reticulum may, however, be misplaced, as it is probable that functionally there is no clear distinction between the two regions of the cell (see Schachter & Roden, 1973). Indeed Morré *et al.* (1970) point out that in a microscopic examination of the Golgi-apparatus-enriched fraction, fragments of rough endoplasmic reticulum are often closely associated with the tubular peripheries of the Golgi apparatus and they suggest that this close topographical relationship in the pellets of the isolated organelle may indicate sites of continuity between the Golgi-apparatus tubules and the rough endoplasmic reticulum in the intact cell.

If glycosyltransferases are associated with different regions of the Golgi complex it might be expected that in those cases where the dictyosomes become unstacked the different activities might be recovered in more than one fraction. This would appear to be the case when the gradient procedure of Schachter *et al.* (1970) is used to fractionate the leukaemic cells. The recovery of 30% of the glycoprotein galactosyltransferase with 2.7–3.8% of the cell protein at the 0.7M-/1.3M-sucrose interface compares well with the findings of Schachter *et al.* (1970); however, where these authors reported considerable galactosyltransferase activity (40%) in the fraction collecting at the 1.3M-/1.7M-sucrose interface much less (15–22%) was found in the corresponding fraction from AKR mice. Neither of these fractions showed any enrichment in polypeptide *N*-acetylgalactosaminyltransferase; some slight increase in specific activity was found in the denser fraction, where the majority of the activity is recovered.

These results, in addition to amplifying the difficulties associated with isolating Golgi-apparatus-enriched fractions from leukaemic cells, would

be in agreement with the membrane-flow mechanism for the biosynthesis of glycoprotein, where it is considered that initial glycosylation takes place on the rough endoplasmic reticulum, or at the forming face of the dictyosomes, followed by polymerization of the carbohydrate groups as the polypeptide transverse the Golgi apparatus. The finding of polypeptide *N*-acetylgalactosaminyltransferase activity in the fractions consisting of rough and smooth membranes, but absent from the plasma membrane would be in accord with this mechanism. Whether the glycoprotein galactosyltransferase activity found in the plasma membrane serves a special function in adhesive recognition (Roseman, 1970), accounting for behavioural differences in normal and transformed cell lines (Roth, 1973), is beyond the scope of this paper. However, the failure to demonstrate activity of this enzyme in plasma membranes from normal lymphocytes (A. Warley & G. M. W. Cook, unpublished work) would be in agreement with the suggestion (Webb & Roth, 1974) that the expression of this activity on the cell surface may be a growth-related phenomenon. The production of a Golgi-apparatus-enriched fraction from leukaemic cells may be useful for the further elucidation of the role of this organelle in membrane biogenesis and control of the expression of enzymes in the plasma membrane.

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