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cell-retained fraction includes an unusually small heparan sulphate proteoglycan, less than M_r 30 000. This is less than half the size of the cell-surface heparan sulphate proteoglycan from liver (Oldberg *et al.*, 1979), previously the smallest known molecule of this type.

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- Levitt, D. & Ho, P. L. (1983) *J. Cell Biol.* **97**, 351–358
 Hart, G. W. (1982) *Biochemistry* **24**, 6088–6096
 Oldberg, A., Kjellen, L. & Hook, M. (1979) *J. Biol. Chem.* **254**, 8505–8670
 Rider, C. C. & Hart, G. W. (1987) *Mol. Immunol.* in the press
 Wasteson, A. (1971) *J. Chromatogr.* **59**, 87–97

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Further evidence that galactosyltransferase activity is not localized to the brush border membrane of human duodenum

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Galactosyltransferase (EC. 2.4.1.22) catalyses the transfer of galactose from UDP-galactose to glycoproteins with a terminal *N*-acetylglucosamine residue. It is considered as a suitable marker enzyme for Golgi [1]. It has been claimed, using immunocytochemical techniques with an antibody raised against galactosyltransferase partially purified from milk, that galactosyltransferase is located at the liminal

surface of absorptive enterocytes in human duodenal biopsies [2].

Using analytical subcellular fractionation of human jejunum biopsy specimens by a single-step sucrose density gradient centrifugation, we demonstrated that galactosyltransferase activity is located in the Golgi fraction and not in the brush border membrane fraction [3]. In this paper we present the results of three separate fractionation experiments, together with fraction mixing and time-course experiments further substantiating our previous report.

Human jejunal biopsies were homogenized and fractionated in a single-step sucrose density gradient centri-

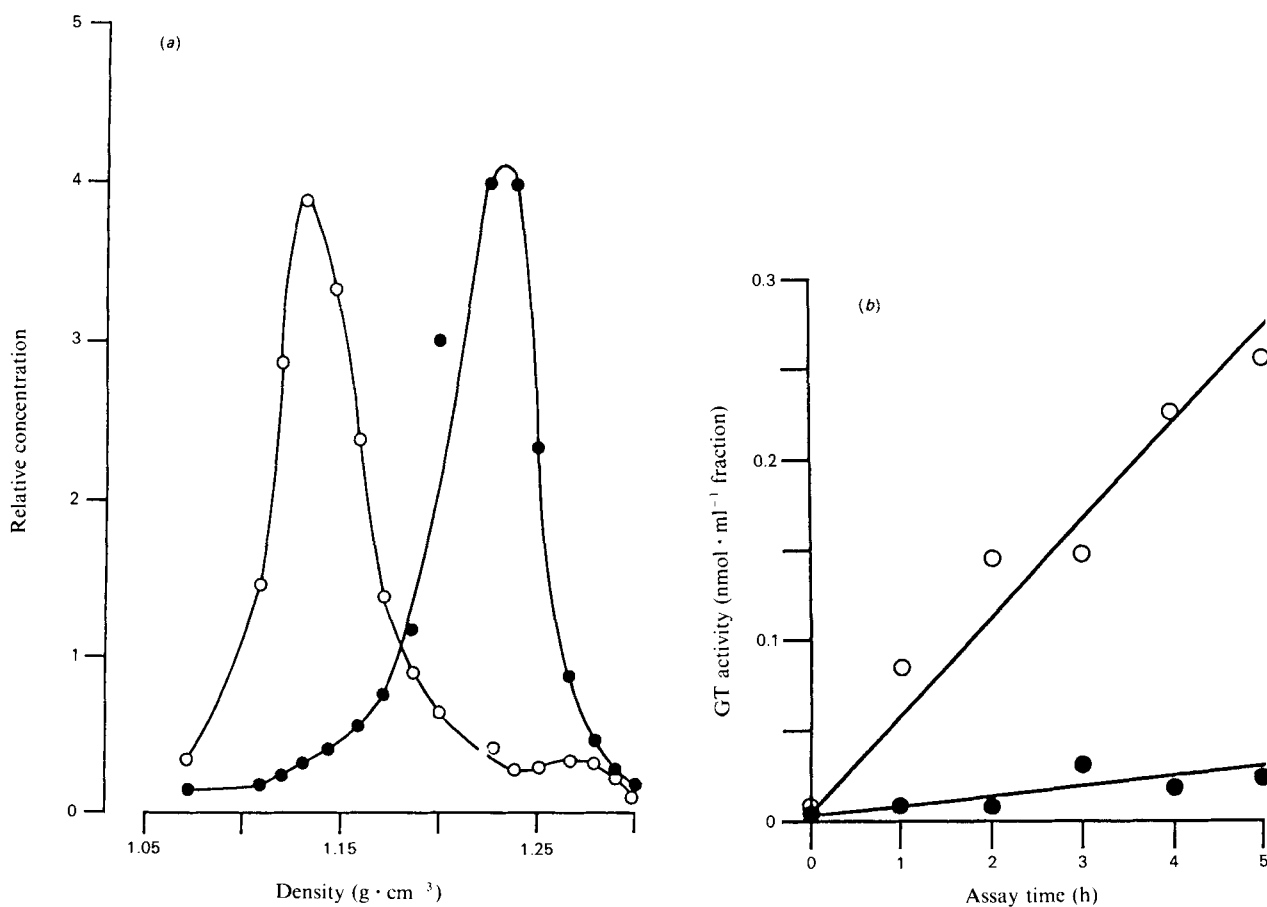


Fig. 1. Fractionation of human jejunum biopsy (a) and time courses of galactosyltransferase activity (b)

(a) Relative concentration — density distributions [7] of galactosyltransferase (○) and α -glucosidase (●). Averaged data of duplicate assays for three experiments. Mean recovered enzyme activities are 77 and 71% for galactosyltransferase and α -glucosidase, respectively. (b) Galactosyltransferase activity assayed in the peak fraction (see a) densities 1.14 g · cm⁻³ (○) and 1.23 g · cm⁻³ (●).

fugation procedure as before [3]. Galactosyltransferase activity, using ovalbumin as an acceptor in a 4 h assay [4], and brush border α -glucosidase activity [5] were assayed in each gradient fraction.

Fig. 1(a) shows the averaged distribution of galactosyltransferase and α -glucosidase activities from three separate experiments. Galactosyltransferase shows a peak relative concentration at a density of $1.14 \text{ g} \cdot \text{cm}^{-3}$, typical of Golgi distribution [6]. α -Glucosidase activity was well separated from galactosyltransferase, with a peak activity at $1.22 \text{ g} \cdot \text{cm}^{-3}$ typical of brush border membrane [5]. Fig. 1(b) shows a time course of galactosyltransferase activities in each of these two peak fractions, which were linear over 5 h. Galactosyltransferase activity in the peak α -glucosidase fraction was <10% of that in the peak galactosyltransferase fraction. Mixing these two peak fractions together caused no inhibition of galactosyltransferase activity compared to an equivalent dilution of the peak galactosyltransferase fraction with buffer alone. Thus, the absence of galactosyltransferase activity in the brush border membrane fraction cannot be attributed to the presence of an inhibitor, or inactivation of the enzyme substrates.

These studies provide further evidence confirming that enzymically active galactosyltransferase is localized to the Golgi and is not found significantly in the brush border membrane.

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1. Ram, B. P. & Munjal, D. D. (1985) *CRC Crit. Rev. Biochem.* **17**, 257-311
2. Roth, J., Lentze, M. J. & Berger, E. G. (1985) *J. Cell. Biol.* **100**, 118-125
3. Boyle, F., Snape, S., Duane, P., Cook, N. & Peters, T. (1986) *Biochem. J.* **234**, 171-175
4. Leake, D., Lieberman, G. E. & T. J. (1983) *Biochim. Biophys. Acta* **762**, 52-57
5. Peters, T. J. (1976) *Clin. Sci. Mol. Med.* **51**, 557-574
6. Smith, G. & Peters, T. J. (1980) *Eur. J. Biochem.* **104**, 305-311
7. Smith, G. D., Osterloh, K. R. S. & Peters, T. J. (1987) *Anal. Biochem.* **160**, 17-23

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Binding and endocytosis of exogenous glycosaminoglycans in cultures of rat rhabdomyosarcoma cells

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Recent studies suggest that interactions of proteoglycans or glycosaminoglycans secreted by malignant cells or by the adjacent host cells may be involved in the control of tumour growth and metastasis formation (Iozzo, 1985; Redini *et al.*, 1986). Exogenous heparin, and to a lesser extent heparan sulphate, inhibits specifically the growth of strongly metastatic rat rhabdomyosarcoma cells (F. Redini, E. Moczár & M.-F. Poupon, unpublished results). In this paper we examine the binding and mode of internalization of glycosaminoglycans by strongly (RMS0) and weakly (RMS8) metastatic rat rhabdomyosarcoma cells in culture.

The cell lines were obtained by cloning from a nickel-induced rat rhabdomyosarcoma (Sweeney *et al.*, 1982). Heparin (Choay, Paris); heparan sulphate (from ovine placenta, Bioetica, Lyon and from bovine kidney, Miles); dermatan sulphate (pig skin, Sigma); chondroitin 4- and 6-sulphates (Sigma) were *N*-acetylated with [^3H]acetic anhydride to yield [^3H]glycosaminoglycans (Höök *et al.*, 1982). The time course of the binding and internalization of the glycosaminoglycans were studied in confluent cultures of the rhabdomyosarcoma cells (3×10^6 cells/dish of 6 cm diameter). The cells were incubated for 1, 2, 6 and 24 h with the labelled glycosaminoglycans ($4 \mu\text{g}/\text{ml}$) in Dulbecco's minimum essential medium with 7.5% (v/v) fetal calf serum at 37°C . The radioactivities were determined in the extracellular (culture medium), pericellular (trypsinate) and intracellular (cell residue) compartments.

Heparin, heparan sulphate and dermatan sulphate were taken up and internalized in a time-dependent way by both cell types. The internalization of the glycosaminoglycans reached an equilibrium after 6 h for the RMS0 and after 24 h for the RMS8 cells. At 24 h, the amount of the cell-associated heparin, heparan and dermatan sulphates was respectively: 2-, 1.6- and 2.6-fold higher in the pericellular and 1.5-, 1.6- and 5-fold higher in the intracellular compart-

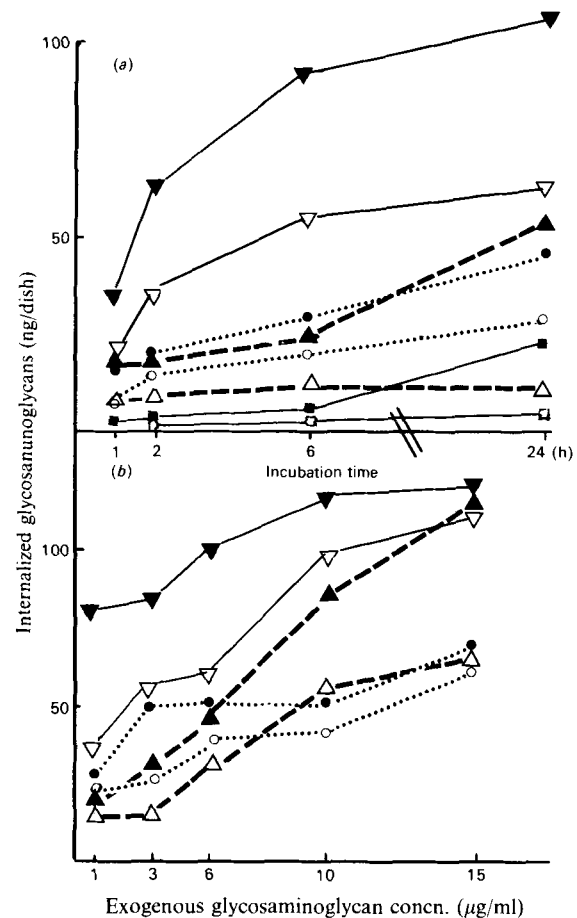


Fig. 1. Internalization of exogenous [^3H]glycosaminoglycans by rat rhabdomyosarcoma cells in culture

(a) Time course of the internalization ($4 \mu\text{g}/\text{ml}$) and (b) dose dependency of the internalization of the glycosaminoglycans in 6 h: ∇ — ∇ , heparin; \circ — \bullet , heparan sulphate (ovine placenta); \triangle — \blacktriangle , dermatan sulphate; \square — \blacksquare , chondroitin 4- and 6-sulphates; for RMS0 cells (open symbols) and RMS8 cells (closed symbols). Results are means \pm S.D. > 15% ($n = 3$).