Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 4736–4739, July 1985
Cell Biology

Localization of galactosyl- and sialyltransferase by immunofluorescence: Evidence for different sites

(bovine kidney cells/fibroblasts/Golgi apparatus/GERL)

ERIC G. BERGER AND FRANCIS J. HESFORD*

Medizinisch-Chemisches Institut, University of Berne, Postfach CH-3000, Bern 9, Switzerland

Communicated by A. Frey-Wyssling, April 1, 1985

ABSTRACT **Polyclonal rabbit antisera against soluble human milk galactosyltransferase and bovine colostrum sialyltransferase were used to localize by indirect immunofluorescence the respective intracellular enzymes in primary cultures from bovine fetal kidneys and established cell lines of human and bovine fibroblasts. Staining for galactosyltransferase was juxtanuclear and crescent shaped in epitheloid cells; a similar staining, occasionally perinuclear and sparsely distributed in the cytoplasm, was found in fibroblasts. In contrast, staining for sialyltransferase in epitheloid kidney cells derived from the same primary culture was observed predominantly in cytoplasmic vesicles that were spread over the whole cytoplasm. Sialyltransferase-positive vesicles had a similar distribution in fibroblasts and often appeared concentrated around an unstained Golgi area. Thus, in both cell types galactosyl- and sialyltransferase were localized in different subcellular compartments. Since both galactosyl- and sialyltransferase participate in formation of the terminal glycan NeuAc(α2→6)Gal(β1→4)GlcNAc (Neu, neuraminic acid) present in many *N*-glycosidic complex types of glycans, different subcellular compartments for these enzymes support a model of functional compartmentalization of the Golgi apparatus that is compatible with an assembly-line model for glycan chain elongation and termination.**

N-glycosylation of secretory and membrane proteins proceeds in a series of posttranslational modifications known as core glycosylation, processing, chain elongation, and terminal glycosylation (for review, see ref. 1). Some of these modifications take place in the Golgi apparatus, which is organized in subcompartments the function of which is as yet poorly defined. On the basis of cytochemical evidence the Golgi stack appears to be divided in *cis, median, trans*, and *transmost* cisternae (for reviews, see refs. 2 and 3); however, until enzymes with defined activities can be localized to distinct subcompartments or individual cisternae, the biological significance of these subcompartments will remain unclear. Such localization has recently been rendered possible by the immunocytochemical labeling of galactosyltransferase (EC 2.4.1.22). Evidence obtained by immunofluorescence (4, 5) and immunoelectron microscopy (6) demonstrated the presence of this enzyme exclusively in two to three *trans* Golgi cisternae in HeLa cells. This finding was in accord with the already known *trans* localization of thiamine pyrophosphatase and it lent support to a model of functional compartmentalization of the Golgi cisternae as recently discussed by Tartakoff (7).

The exact delimitation of galactosyltransferase in the *trans* cisternae has led to the assumption that glycosyltransferases involved in terminal glycosylation in general might be restricted to specific subcompartments. This assumption, however, does not appear to be compatible with the concept of multiglycosyltransferase systems proposed by Roseman (8). The latter concept implied a close topological association of the sequentially acting glycosyltransferases and was supported mainly by the apparent co-distribution of several chain-elongating glycosyltransferases after subfractionation of the Golgi complex (9). To test for this alternative—i.e., a multiglycosyltransferase system versus a "confined function model" (7)—we selected two glycosyltransferases known to participate in the formation of a commonly found outer glycan chain—e.g. NeuAc(α2→6)Gal(β1→4)GlcNAc-R; since these enzymes act stepwise on the same acceptor substrate, they appear ideally suited to function as part of a multiglycosyltransferase complex and, when localized, would produce an identical picture, namely, the visualization of a juxtanuclear structure typical of the Golgi apparatus (4–6).

This work represents a further step in a project aimed at defining by immunocytochemistry the topology of glycosyltransferases along the secretory pathway and provides evidence for a typical juxtanuclear Golgi-localization for galactosyltransferase and a more distant Golgi-associated localization for sialyltransferase.

MATERIALS AND METHODS

Biological Material. Galactosyltransferase was isolated from pooled human milk (10); sialyltransferase was prepared from bovine colostrum (11) and stored at −20°C for several months. Antisera were raised in rabbits. Primary cultures of bovine fetal kidneys were prepared essentially as described (12) and grown for 5 days in minimal essential medium supplemented with 5% fetal calf serum. Bovine nasal cartilage fibroblasts were obtained from W. Bommeli (Diagnostische Laboratorien, Berne, Switzerland) and human fibroblasts were obtained as described (4). Both cell lines were grown to confluency in minimal essential medium supplemented with 5% fetal calf serum.

Chemicals. All reagents were of the highest purity available and were purchased either from Merck (Germany) or Fluka.

Glycosyltransferase Assays. Galactosyltransferase was assayed as described (10). Sialyltransferase was assayed using sialic acid-free fetuin prepared by mild acid hydrolysis (13). The standard assay mixture contained, in a final volume of 40 μl, 10 nmol of CMP-[^{3}H]NeuAc (specific activity, 10 Ci·mol^{-1}; 1 Ci = 37 GBq), 4.4 mg of asialo-fetuin, and 15 μl of enzyme in 0.25 M Na cacodylate at pH 6.8. Incubation was at 37°C for 1 hr and stopped by addition of 0.5 ml of an ice-cold solution containing 5% (vol/wt) phosphotungstic acid and 5% (wt/vol) trichloroacetic acid. Precipitated protein was collected by suction filtration on Whatman GF/A glass fiber filters. The filter containing the precipitated [^{3}H]NeuAc-fetuin was washed with ethanol, dried, and assessed for radioactivity by liquid scintillation counting with a counting efficiency of 29%.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*Present address: Institut für Tierzucht Tierspital, Universität Bern, CH-3012 Bern, Switzerland.

Dot-ELISA. To test the specificity of the antisera against sialyltransferase, the procedure described by Hawkes *et al.* (14) was used because the antigen was available in limited amounts only. Briefly, 1 μl of a homogeneous preparation of sialyltransferase [as determined by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (11)] was spotted onto a nylon sheet (GeneScreen, New England Nuclear) on which an array of squares of 4 × 4 mm was drawn in pencil. The sheet was saturated with a solution of bovine serum albumin [3% (wt/vol)] in Tris-buffered saline (Tris·HCl, 25 mM; NaCl, 150 mM; pH 7.4) during 30 min at room temperature. Individual squares were cut out and placed into the wells of a flat-bottomed microtiter plate. One hundred microliters of serially diluted antibody or control serum was added to each well and left overnight at 4°C. The wells containing the squares were washed three times with Tris-buffered saline and 100 μl of a suitably diluted solution of protein A-peroxidase (Diagnostische Laboratorien, Berne, Switzerland) was added. After three washes with Tris-buffered saline, 100 μl containing 0.05% diaminobenzidine and 0.01% H_2O_2 was added to each well.

ELISA. Antisera to galactosyltransferase were tested by ELISA as described (4).

Immunofluorescent Staining. Primary cultures from fetal calf kidney were grown on coverslips in 3-cm Petri dishes for 5 days in minimal essential medium supplemented with 5% heat-inactivated fetal calf serum (GIBCO). Two protocols for fixation and making cells permeable yielded positive results. *Fixation 1*: The coverslips were placed in acetone (4°C, 15 min) then dipped in phosphate-buffered saline (P_i/NaCl) and distilled water and air dried. Acetone treatment also made cells permeable. *Fixation 2*: All steps were at room temperature: The coverslips were treated with *p*-formaldehyde, 1.5% (wt/vol), in P_i/NaCl for 15 min at room temperature, washed with P_i/NaCl, blocked with NH_4Cl, 50 mM in P_i/NaCl for 10 min, washed, and treated with Triton X-100, 0.1% in P_i/NaCl for 4 min followed by three washes with P_i/NaCl. The coverslips were rinsed with a solution containing 0.2% gelatin (wt/vol) in P_i/NaCl then placed upside down on a 25-μl drop of antibody or control serum spotted onto Parafilm and incubated for 20 min at room temperature. After two washes in P_i/NaCl/gelatin and P_i/NaCl, a second antibody, fluorescein isothiocyanate-labeled swine IgG against rabbit Ig (Sevac), was applied. Incubation and washes were as above.

Fixed and stained coverslips were mounted in a medium described by Heimer and Taylor (15) and observed in a Leitz Diavert microscope equipped for epifluorescence. Photographs were taken on an Ilford HP5 film (400 ASA, 27 DIN).

RESULTS

A globulin fraction prepared by 33% ammonium sulfate precipitation of a rabbit anti-human milk galactosyltransferase antiserum was tested for crossreactivity against bovine milk galactosyltransferase (Fig. 1, dotted line); similarly, a globulin fraction of a rabbit antiserum against purified bovine colostrum sialyltransferase was incubated with defatted bovine colostrum (Fig. 1, solid line). Both antisera inhibited the activity of the corresponding glycosyltransferase without precipitating it. In order to ascertain further the specificity of the anti-sialyltransferase antibody and because the antigen was available in only limited amounts, the dot-ELISA method of Hawkes *et al.* (14) was used. Purified sialyltransferase (11) was spotted on a nylon sheet and incubated with serially diluted native antiserum (Fig. 2*A*) or fractions thereof (Fig. 2 *C* and *D*) or preimmune serum (Fig. 2*B*). Retained antibody was visualized by a protein A-peroxidase conjugate developed by diaminobenzidine and H_2O_2. It is easily seen (dark squares on the bottom of the wells) that the antiserum reacted

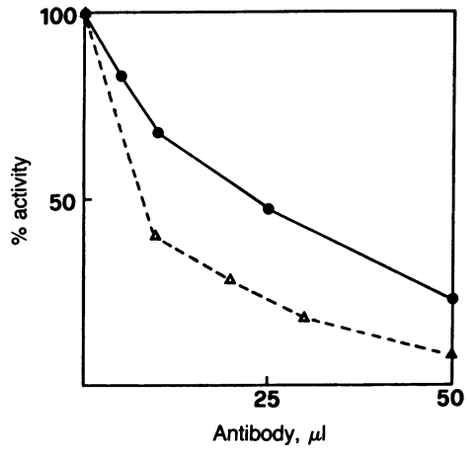

FIG. 1. Inhibition of enzyme activity by antibody. ———, Inhibition of sialyltransferase activity in defatted bovine colostrum by a 33% globulin fraction obtained by ammonium sulfate precipitation of anti-sialyltransferase antiserum (16 mg/ml); ——— inhibition of galactosyltransferase activity in defatted bovine colostrum by a 33% globulin fraction of anti-galactosyltransferase antiserum. Volumes of added antibody were equalized with a corresponding globulin fraction obtained from a nonimmune rabbit. These globulin fractions were shown to be devoid of endogenous glycosyltransferase activity.

positively with the purified preparation of sialyltransferase whereas the preimmune serum was negative. The specificity of the anti-galactosyltransferase antiserum has been documented (4, 6). Possible crossreactivity of anti-sialyltransferase with galactosyltransferase has been excluded by ELISA and of anti-galactosyltransferase with sialyltransferase by dot-ELISA (results not shown). We conclude from these results that the antiserum used to identify sialyltransferase is highly specific for this enzyme, however, attempts to demonstrate the enzyme in colostrum or in homogenates of the cell cultures used in this study by immunoblotting (16) were not successful. This may be ascribed to the denaturing effect of sodium dodecyl sulfate.

Immunofluorescent staining of both enzymes was carried out in subconfluent primary cultures of bovine kidney cells, which are known to consist of both epitheloid and fibroblastic cells (12). Staining for galactosyltransferase in epitheloid cells is shown on Fig. 3*A*. In these cells, a compact or punctate, crescent-shaped juxtanuclear structure oriented on the apical side of the cell can be observed. In some instances, galactosyltransferase antigenic sites were almost perinuclear (see ref. 4, figure 4b). However, in no instances did we observe peripheral cytoplasmic staining. Control staining with preimmune serum produced a high background but no specific fluorescent staining (Fig. 3*C*).

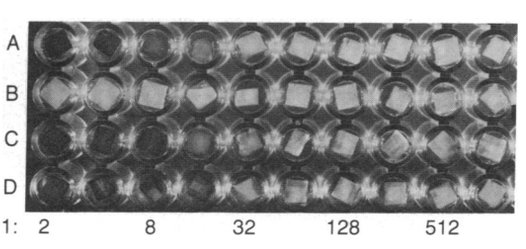

FIG. 2. Specificity testing of anti-sialyltransferase by the dot-ELISA method. Serially diluted antiserum was incubated with purified sialyltransferase spotted on squares of GeneScreen, which were then cut out and placed in wells of a microtiter plate. Rows: A, native antiserum; B, preimmune serum; C, 33% globulin fraction of the antiserum to sialyltransferase (16 mg/ml); D, IgG fraction of the same antiserum prepared by DEAE-cellulose chromatography.

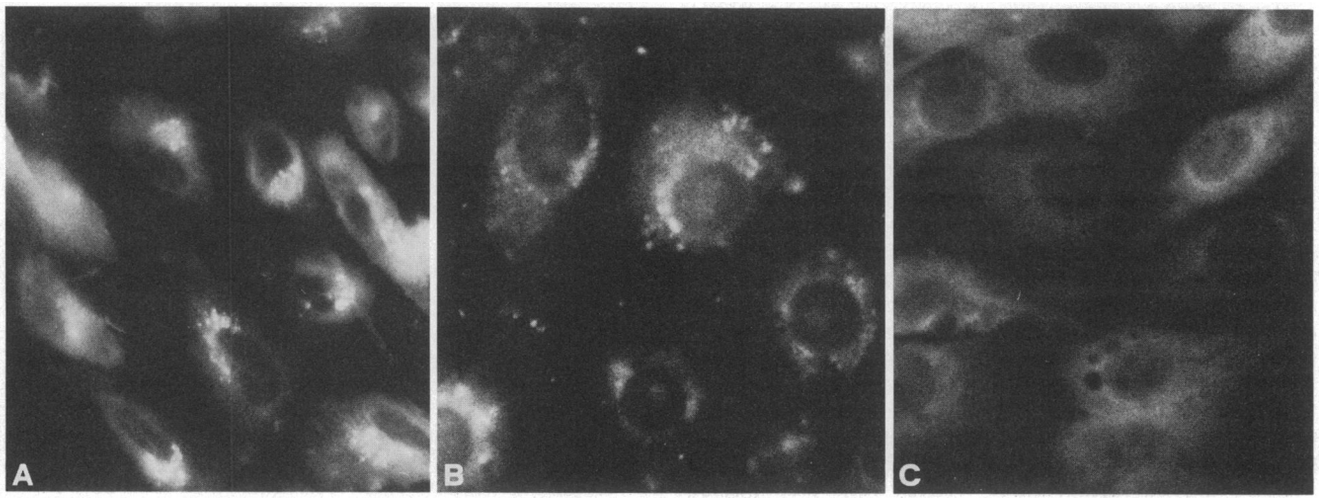

FIG. 3. Localization of galactosyl- and sialyltransferase by immunofluorescence in epitheloid cells of a primary fetal calf kidney culture. Cells were exposed to the following serums: (*A*) Undiluted anti-galactosyltransferase antiserum. (*B*) Globulin fraction of anti-sialyltransferase antiserum (1.6 mg/ml). (*C*) Undiluted preimmune serum as control. (×585.)

In contrast to galactosyltransferase, sialyltransferase antigenic sites in epitheloid cells were located more distant from the nucleus, usually in cytoplasmic vesicles (Fig. 3*B*); some labeling also appeared to be associated with the Golgi apparatus. The staining for sialyltransferase was also more heterogeneous than that of galactosyltransferase. In the center of areas of confluency, weak labeling was observed. The most intense staining was always found at the periphery of a confluent area (results not shown). In order further to substantiate the differences in localization of galactosyl- and sialyltransferase, human skin fibroblasts and bovine nasal cartilage fibroblasts were stained with anti-galactosyltransferase and anti-sialyltransferase fluorescent antibodies. Galactosyltransferase was again localized in a compact, juxta- or perinuclear structure (Fig. 4*A*, and figures 5a and 5b in ref. 4) whereas sialyltransferase appeared to be dispersed in vesicles over the whole cytoplasm (Fig. 4*B*). Characteristically in many cells a juxtanuclear area remained unstained while positive vesicles appeared to be clustered around this "empty zone" (Fig. 4 *C* and *D*, arrowheads). These results indicate different compartments for galactosyl- and sialyltransferase but do not exclude a partial co-distribution of both enzymes at the ultrastructural level.

DISCUSSION

In this report, we provide a comparative immunocytochemical study of the intracellular localization of sialyl- and galactosyltransferase. The results demonstrate that the predominant subcellular localization of these enzymes is different and the conclusion is supported by the difference in the staining patterns of the enzymes in epitheloid cells and in fibroblasts. Although the exact definition of the sialyltransferase-containing subcompartments will necessitate an immunoelectronmicroscopic study, our results obtained by light microscopy show that sialyltransferase antigenic sites are found primarily in peripheral cytoplasmic vesicles (Figs. 3*B* and 4*B*). A comparison with galactosyltransferase, an

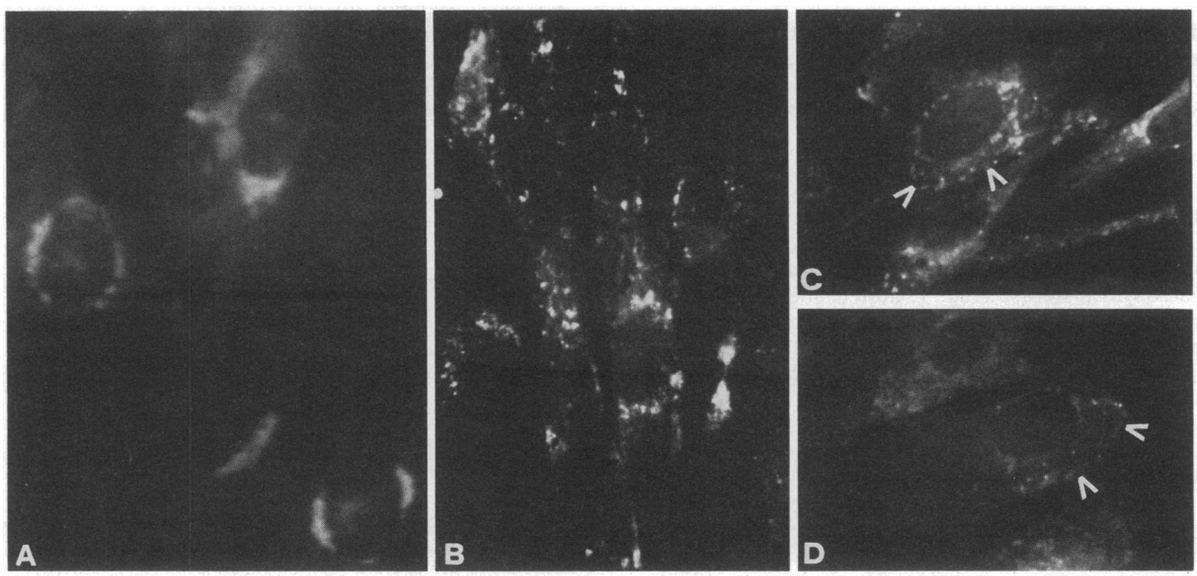

FIG. 4. Localization of galactosyl- and sialyltransferase by immunofluorescence in fibroblasts. Cells were exposed to the following serums: (*A*) Anti-galactosyltransferase antiserum diluted 1:10. (*B*) Globulin fraction of anti-sialyltransferase antiserum (16 mg/ml). (*C* and *D*) As in *B*; a vesicular peri-Golgi staining is indicated by arrowheads. (×585.)

established *trans* Golgi marker (6) which usually appears as a compact juxtanuclear structure (Figs. 3*A* and 4*A*; refs. 4–6), shows that sialyltransferase was distributed over the whole cytoplasm and, in fibroblasts especially, was accumulated around an unstained Golgi area (Fig. 4, *C* and *D*, arrow heads). Thus, sialyltransferase appears to be present in a compartment known as the transtubular network (3). It can also be shown that galactosyltransferase and sialyltransferase antigenic sites are not (or are only partially) congruent, as one might have anticipated on the basis of fractionation studies (17).

A different localization of both enzymes favors the "confined function model" (7) of the Golgi apparatus in which sequentially acting glycosyltransferases are organized in a way analogous to an assembly line. This model predicts that during transport of a secretory or a membrane glycoprotein across the Golgi apparatus, incorporation of sugar residues occurs stepwise in different cisternae; thus, *N*-acetylglucosaminyltransferases act in *median* cisternae as recently shown by immunocytochemistry (18), whereas galactose transfer occurs on the *trans* side as already established (6, 18). Addition of sialic acid appears to take place on the *trans* side as suggested by autoradiographic evidence (19). According to the generally accepted notion of the secretory pathway (20), the compartment adjacent to the *trans* cisternae consists of a complicated network of tubules which may extend through the whole cytoplasm and in some cases may appear as peripheral cytoplasmic vesicles (2, 3). This Golgi subcompartment, called GERL on the basis of acid phosphatase cytochemistry (21), may also include elements known as rigid lamellae. In HeLa cells, galactosyltransferase is completely absent from this compartment (6). In the work presented here, Golgi elements as visualized by immunofluorescent staining for galactosyltransferase closely imitate the site and shape of this organelle as described earlier (4–6). Therefore it is reasonable to assume that the compartment that stains for sialyltransferase represents more peripheral Golgi vesicles or the transtubular network as mentioned above.

Two implications of this assumption need to be discussed: First, it allows extension of the concerted action model proposed for galactosyltransferase and UDPase [thiamine pyrophosphatase (3, 6, 22)] to sialyltransferase and CMPase (acid phosphatase, ref. 23). Co-distribution of glycosyltransferases with those phosphatases that cleave the nucleotide products of the respective transferase reaction prevents accumulation of UDP in the *trans* and CMP in the "*transmost*"cisternae. Interestingly, these nucleotides not only inhibit the transferase reaction kinetically (24, 25), but they also lead to dead-end complexes by immobilizing soluble acceptor substrates to the membrane-bound enzyme or vice-versa (unpublished results). This phenomenon has been amply exploited for the efficient affinity chromatography of glycosyltransferases (26). Second: the likely presence of sialyltransferase in the transtubular network may be of importance in the functional definition of this compartment. Regoeczi *et al.* (27) has found partial resialylation of asialotransferrin after endocytosis, a finding which has found recent support by an analysis of the intracellular pathway taken by transferrin after endocytosis. Yamashiro *et al.* (28) reported that transferrin is transported to a para-Golgi compartment whose shape and cytoplasmic location could be compatible with sialyl- and perhaps galactosyltransferase containing subcompartments. Moreover, work by Reutter and co-workers (29) indicated a higher turnover of peripheral carbohydrate residues in glycoproteins as compared with the core sugar or amino acid residues. This may suggest that the *trans* side of the Golgi apparatus which consists of the *trans* (galactosyltransferase positive) and *transmost* (probably sialyltransferase positive) compartments may be involved in the endocytotic pathway.

In summary, our comparative localization studies of sialyl- and galactosyltransferase carried out in two different cell types show a more distal localization of sialyltransferase and thus may contribute to a redefinition of the function of those compartments which arise from the trans Golgi cisternae. These results also render the model of multiglycosyltransferase systems for chain elongation and termination unlikely and support a model in which sequentially acting glycosyltransferases are compartmentalized and arranged in an assembly line.

This work is dedicated to the memory of the late Prof. H. E. Aebi, former chairman of this Department. We are indebted to Dr. W. Bommeli and Ms. Dorothy Etter for providing the primary cell cultures and to Mrs. Therese Mandel and Mr. Roland Buehler for technical assistance. This work was supported by Grants 3.093.081 from the Swiss National Science Foundation and 205 from the Roche Research Foundation.

1. Berger, E. G., Buddecke, E., Kamerling, J. P., Kobata, A., Paulson, J. C. & Vliegenthart, J. F. G. (1982) *Experientia* **38,** 1129–1258.
2. Farquhar, M. G. & Palade, G. (1981) *J. Cell Biol.* **91,** 77s–103s.
3. Goldfischer, S. (1982) *J. Histochem. Cytochem.* **30,** 717–733.
4. Berger, E. G., Mandel, Th. & Schilt, U. (1981) *J. Histochem. Cytochem.* **29,** 364–370.
5. Bouchilloux, S., Gabrion, J. & Delori, P. (1981) *Biol. Cell.* **40,** 143–146.
6. Roth, J. & Berger, E. G. (1982) *J. Cell Biol.* **93,** 223–229.
7. Tartakoff, A. M. (1983) *Int. Rev. Cytol.* **85,** 221–252.
8. Roseman, S. (1970) *Chem. Phys. Lipids* **5,** 270–297.
9. Schachter, H., Jabbal, I., Hudgin, R. L., Pinteric, L., McGuire, E. J. & Roseman, S. (1970) *J. Biol. Chem.* **245,** 1090–1100.
10. Gerber, A. Ch., Kozdrowski, I., Wyss, S. R. & Berger, E. G. (1979) *Eur. J. Biochem.* **93,** 453–460.
11. Hesford, F. J., Berger, E. G. & Van Halbeek, H. (1984) *Glycoconjugate J.*, 141–153.
12. Adams, R. L. P. (1980) in *Laboratory Techniques in Biochemistry and Molecular Biology*, eds. Work, T. S. & Burdon, R. H. (Elsevier/North-Holland, Amsterdam), pp. 75–79.
13. Schmid, K., Polis, A., Hunziker, K., Fricke, R. & Yayoshi, M. (1967) *Biochem. J.* **104,** 361–368.
14. Hawkes, R., Niday, E. & Gordon, J. (1982) *Anal. Biochem.* **119,** 142–147.
15. Heimer, G. V. & Taylor, C. E. (1974) *J. Clin. Pathol.* **27,** 254–256.
16. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
17. Bretz, R., Bretz, H. & Palade, G. E. (1980) *J. Cell Biol.* **84,** 87–101.
18. Dunphy, W. G., Brands, R. & Rothmann, J. E. (1985) *Cell* **40,** 463–472.
19. Bennett, G. & Oshaughnessy, D. (1981) *J. Cell Biol.* **88,** 1–15.
20. Palade, G. (1975) *Science* **189,** 347–358.
21. Novikoff, A. B. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2781–2787.
22. Kuhn, N. J. & White, A. (1977) *Biochem. J.* **168,** 423–433.
23. Novikoff, P. M., Novikoff, A. B., Quintana, N. & Hauw, J. J. (1971) *J. Cell Biol.* **50,** 859–886.
24. Khatra, B. S., Herries, D. G. & Brew, K. (1974) *Eur. J. Biochem.* **44,** 537–560.
25. Klohs, W. D., Bernacki, R. J. & Korytnyk, W. (1979) *Cancer Res.* **39,** 1231–1238.
26. Sadler, J. E., Beyer, T. A., Oppenheimer, C. C., Paulson, J. C., Prieels, J. P., Rearick, J. I. & Hill, R. L. (1982) *Methods Enzymol.* **83,** 458–514.
27. Regoeczi, E., Chindomi, P. A., Debanne, M. T. & Charlwood, P. A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2226–2230.
28. Yamashiro, D. J., Tycko, B., Pluss, S. R. & Maxfield, F. R. (1984) *Cell* **37,** 787–800.
29. Kreisel, W., Volk, B. A., Büchsel, R. & Reutter, W. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1828–1831.