The Degradation and Turnover of Fucosylated Glycoproteins in the Plasma Membrane of a Neuroblastoma-Cell Line

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When monolayer cultures of neuroblastoma N2a cells were prelabelled with [^3H]fucose to steady state, and then reincubated in complete medium in the presence of unlabelled 40 mM-L-fucose, there was a rapid metabolism of fucosylated cellular macromolecules and the specific radioactivity of the acid-insoluble material decreased by 22% within 2 h. After this period of time the remaining radioactive glycoproteins appeared to be more stable and the rate of loss of specific radioactivity markedly decreased. Since fucose is known to be associated predominantly with plasma-membrane components, the analysis of fucosylated glycoproteins was characterized in plasma-membrane fractions by polyacrylamide-gel electrophoresis. Two experimental approaches were used to measure glycoprotein degradation and turnover in the cell-surface membranes. In one set of experiments, with a similar incubation procedure to that used with intact cells, three membrane components were rapidly degraded (150 000, 130 000 and 48 000 daltons), but another surface glycoprotein (68 000 daltons) appeared to be more slowly metabolized than the mean rate of glycoprotein degradation. The relationship of the degradation of membrane glycoproteins to their turnover was analysed by dual-label experiments that used both [^{14}C]fucose and [^3H]fucose. Glycoproteins of the surface membrane of neuroblastoma cells were found to turn over at heterogeneous rates. The components mentioned above that exhibited significantly rapid rates of degradation, were also shown to turn over more rapidly than the average surface component. In addition to the membrane components detected by the use of only [^3H]fucose, dual-label experiments illustrated that numerous surface glycoproteins were metabolized more rapidly or slowly than most of the cell-surface constituents.

The literature encompassing eukaryotic membrane metabolism primarily involves work carried out with liver cells (Simon *et al.*, 1970; Kawasaki & Yamashina, 1971; Dehlinger & Schimke, 1971; Gurd & Evans, 1973; Landry & Marceau, 1975; Tweto & Doyle, 1976). Most of these studies have suggested that membranes are metabolically dynamic structures, with their macromolecular components continually being degraded and replaced. Perhaps this has been illustrated best in the endoplasmic reticulum of liver cells, where different rates of turnover can be ascribed to individual enzyme constituents (Omura *et al.*, 1967; Arias *et al.*, 1969; Bock *et al.*, 1971). Heterogeneous rates of turnover have been demonstrated in the plasma-membrane proteins (Dehlinger & Schimke, 1971; Landry & Marceau, 1975; Kaplan & Moskowitz, 1975; Billington & Nayudu, 1976), glycoproteins (Gurd & Evans, 1973; Kaplan & Moskowitz, 1975; Landry & Marceau, 1975; Tweto & Doyle, 1976) and phospholipids (Pasternak &

Friedrichs, 1970) of some eukaryotic cells, although reports of a few exceptions to this generality exist (Hubbard & Cohn, 1975; Tweto & Doyle, 1976).

This study of the metabolism and turnover of membrane glycoproteins was carried out with membranes of neuroblastoma cells grown in cell culture. These cells can be grown selectively in suspension as a population of 'blast' cells with essentially spherical morphology, or in monolayer culture where the cells attach to the culture-vessel surface and undergo a morphological change that leads to a considerable amount of neurite extension. The flexibility of neuroblastoma-cell-membrane properties was illustrated in a serology study where Akeson & Herschman (1974) reported that the monolayer form, which possesses axon- or dendrite-like extensions, had an additional antigen exposed on the plasma-membrane surface. Both Brown (1971) and Glick *et al.* (1973) have demonstrated the presence of surface glycoproteins on neuroblastoma cells, and Truding *et al.* (1974) have shown that a protein of 78 000 mol.wt. is associated with the morphological 'differentiation' of neuroblastoma cells in culture. More recently we have extended the study of neuroblastoma-cell

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membranes to include the metabolism of surface proteins and have reported that they exhibit heterogeneous rates of turnover (Mathews *et al.*, 1976). Studies with intact neuroblastoma cells suggested that glycoproteins also show heterogeneous rates of metabolism (Hudson & Johnson, 1977). In the present study, the rate of glycoprotein degradation and turnover in neuroblastoma-plasma-membrane preparations were characterized by using radioactively labelled L-fucose, a monosaccharide of known specificity for the plasma membrane of eukaryotic cells (Bennett & Leblond, 1970; Atkinson & Summers, 1971; Bennett *et al.*, 1974; Atkinson, 1975).

Materials and Methods

Materials

Radioactive chemicals were purchased from New England Nuclear (Boston, MA, U.S.A.), cell-culture-medium components and antibiotics from Grand Island Biological Co. (Grand Island, NY, U.S.A.) and non-radioactive fucose was from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The [^3H]fucose (12.1 Ci/mmol) was diluted with unlabelled L-fucose and used at 1–10 $\mu\text{Ci/ml}$ (2.6–3.3 nmol/ml), and [^{14}C]fucose (53.2 mCi/mmol) was used undiluted at 0.2 $\mu\text{Ci/ml}$ (3.7 nmol/ml) of complete media.

Neuroblastoma cell culture

The N2a line of neuroblastoma cells was obtained from Dr. A. Telsar, Northwestern University Medical School. The cells were maintained over the course of the experiments as monolayers in Falcon tissue-culture flasks (75 cm²) in humidified incubators kept at 37°C and supplied with CO₂/air (1:19). The cultures were transferred by Viokase (Mathews *et al.*, 1976) treatment and reincubated in fresh medium which was composed of Dulbecco's Modified Eagle's Medium (Grand Island Biological Co.) supplemented with dextrose (3.5 g/l), NaHCO₃ (3.7 g/l), penicillin (1000 units), streptomycin (1 mg/l), fungizone (2.5 mg/l) and 10% γ -globulin-free newborn-calf serum (Hudson & Johnson, 1977).

Incubation of neuroblastoma cells with radioactive precursors

Subconfluent monolayer cultures were maintained as a routine for 2–3 days after being transferred to allow time for recovery from enzyme removal before they were incubated in the presence of [^3H]fucose. During this incubation period the cells continued to grow at exponential rates, with a generation time of 18–22 h. Neuroblastoma cells were radioactively labelled with [^3H]fucose in complete medium supplemented with unlabelled 2.5 μM -L-fucose to avoid the possibility that fucose was rate-limiting (Hudson & Johnson, 1977).

Plasma-membrane isolation

Plasma membranes from neuroblastoma cells were isolated by a modification of the Zn²⁺ method of Warren *et al.* (1966) as described by Mathews *et al.* (1976). Briefly, monolayer cultures were scraped into, and washed twice with, ice-cold 0.15 M-NaCl. The cells were pelleted by centrifugation and the cell pellet was resuspended in 0.15 M-NaCl and 1 mM-ZnCl₂ (1:3, v/v), the mixture was incubated at 22°C for 10 min and the cells were then disrupted by homogenization. The resulting homogenate was made to 20% (w/v) sucrose, layered over a gradient containing 13 ml of 65% and 10 ml of 40% sucrose and centrifuged at 8000g for 10 min in a Beckman SW-27 rotor. The material at the 65%/40% sucrose interface was removed, diluted with water, pelleted by centrifugation at 3400g for 10 min and resuspended in 35% sucrose. The membrane suspension was layered over a gradient containing 6 ml each of 65, 55, 50, 45 and 40% sucrose and centrifuged at 9000g for 90 min. The membrane fraction at the 65%/55% sucrose interface was removed, diluted with water, concentrated by centrifugation at 9000g for 180 min, and the final membrane pellet was resuspended in 0.15 M-NaCl and frozen at –80°C.

Polyacrylamide-gel electrophoresis

Membrane glycoproteins from purified plasma membranes were separated on SDS*/urea/polyacrylamide gels by the method of Fairbanks *et al.* (1971), except that 0.5 M-urea was included in the gels and the sample buffer. Samples (<120 μl) were layered over 12 cm 5.6% polyacrylamide gels containing 1% SDS and 0.5 M-urea, and electrophoresed at 5 mA per gel for 18–20 h (Mathews *et al.*, 1976).

Evaluation of gels

Electrophoretic profiles of the gels containing radioactive glycoproteins were obtained by freezing the gels in solid CO₂/ethanol and slicing them into 1.1 mm fractions with a Bio-Rad Gel Divider (model 190). Each fraction was heated with 0.5 ml of Nuclear Chicago Solubilizer (Nuclear-Chicago, Chicago, IL, U.S.A.) at 55°C for 2 h. Toluene-based scintillation fluid (White, 1968) was added to each vial and the radioactivity was measured in a Packard Tri-Carb scintillation counter (model 3310) with an efficiency of 35% for ^3H and 90% for ^{14}C . The lower 5 cm of the gels was cut off and discarded as a routine, since radioactive material was never found in this region.

The data obtained with polyacrylamide gels were processed on a Wang 2200 system programmable calculator. A program was devised that would assemble the data from two independent gels, calculate the ratio of counts between gels at each slice, and statistically analyse the ratio values. The pro-

* Abbreviation: SDS, sodium dodecyl sulphate.

gram was also capable of shifting one gel pattern relative to the other to align major peaks. For dual-label experiments, the program was modified to carry out the above as well as compensate for cross-over of ^{14}C into the ^3H channel.

Molecular-weight calibration of polyacrylamide gels

Approximate molecular weights of membrane glycoproteins were estimated by comparing their electrophoretic mobilities with those of proteins of known molecular weight. Bovine serum albumin (68000), γ -globulin heavy chain (50000), egg albumin (45000), chymotrypsinogen (25000), γ -globulin light chain (25000) and cytochrome *c* (13500) were used as standards. Staining, destaining and scanning of polyacrylamide gels were done as reported by Mathews *et al.* (1976).

Results

In a previous paper the metabolism of fucosylated glycoproteins was studied in monolayer cultures of neuroblastoma cells (Hudson & Johnson, 1977). In

Fig. 1. Degradation of fucosylated macromolecules in intact neuroblastoma cells

Cells were incubated for 24h in complete medium containing L- ^3H fucose, washed twice with fresh medium, overlaid with complete medium supplemented with unlabelled 40mM-L-fucose and then reincubated. At the times indicated the monolayers were removed from incubation, washed twice with ice-cold 0.15M-NaCl, solubilized in 0.5ml of 0.15M-NaCl containing 0.2M-NaOH, and the acid-insoluble macromolecules were precipitated by the addition of an equal volume of 10% (w/v) trichloroacetic acid. Precipitates were pelleted by centrifugation at 2000g for 10min, resolubilized in 0.5ml of 0.15M-NaCl containing 0.2M-NaOH and both the radioactivity and protein content were measured (Hudson & Johnson, 1977). The bars represent the range in specific radioactivity measured in triplicate cultures,

those experiments cells were preincubated for 24h with L- ^3H fucose in complete medium, washed with unlabelled medium and then reincubated in complete medium supplemented with non-radioactive 40mM-fucose. The experimentally observed decrease in cellular specific radioactivity (Fig. 1, solid line) was compared with a calculated rate for the dilution of specific radioactivity solely due to cell growth in unlabelled medium when degradation or turnover is assumed to be absent (broken line). Over short periods of reincubation, neuroblastoma glycoproteins were rapidly metabolized (22% by 2h), but after longer periods of incubation (10h) the rate of degradation decreased and the measured ranges of specific radioactivity increased because of radioisotope reutilization (Hudson & Johnson, 1977). Since fucose has been shown to be incorporated primarily into the plasma membrane of other cell types (Bennett & Leblond, 1970; Atkinson & Summers, 1971; Bennett *et al.*, 1974; Atkinson, 1975), and because it is principally located in the glycoprotein fraction of neuroblastoma cells (Hudson & Johnson, 1977), the kinetics of fucose metabolism in intact cells probably reflected glycoprotein metabolism in the surface membrane. To establish whether the biphasic kinetics of degradation depicted in Fig. 1 represented a general loss of all glycoproteins, or were indicative of the existence of at least two classes of glycoproteins with markedly different rates of metabolism, membrane-bound glycoproteins were separated by polyacrylamide-gel electrophoresis. Cell monolayer cultures were incubated with ^3H fucose for 24h, then half of the cultures were harvested by scraping and plasma membranes were isolated as described in the Materials and Methods section. The remainder of the monolayer cultures were washed twice with fresh medium and reincubated in complete medium containing non-radioactive 40mM-L-fucose. After 2.5h of reincubation, these cultures were harvested and a plasma-membrane fraction was prepared. A sample of each membrane preparation was separately electrophoresed, sliced and the radioactivity per slice was measured (Fig. 2a). The ratio of radioactivity was calculated for the plasma-membrane profiles at each gel slice (Fig. 2b) in order to discern those glycoproteins that are rapidly degraded and responsible for the kinetics of fucose metabolism obtained with intact neuroblastoma cells (Fig. 1). Ratios above the broken line ($P=0.05$) identify fucosylated glycoproteins that are more rapidly degraded, and ratios below the line are degraded more slowly than most membrane components. Three glycoproteins that migrate on polyacrylamide gels as proteins of mol. wts. 150000, 130000 and 48000 were more rapidly degraded, and an additional constituent (mol.wt. 68000) was more slowly degraded than most of the fucosylated components. The molecular-weight scale was constructed to compare distinct fucosylated

Fig. 2. Degradation of neuroblastoma-cell plasma-membrane glycoproteins as measured by polyacrylamide-gel electrophoresis

Neuroblastoma-cell monolayers were incubated for 24 h with [³H]fucose (2.0 μ Ci/ml) in complete medium. After incubation, half of the cultures were removed and their plasma membranes were isolated (○). The remaining half of the cultures were washed twice with fresh medium and reincubated in complete medium supplemented with unlabelled 40 mM-L-fucose. After 2.5 h of reincubation the plasma membranes of these cells were also isolated (●). The purified plasma-membrane preparations were electrophoresed as described in the text, after which the amount of ³H in each slice was determined (a) and the ratio between the two preparations ($A/B = \text{○}/\text{●}$) was calculated (b). The broken lines represent the tolerance limits of variability where $P = 0.05$. The molecular-weight scale was constructed from protein standards as described in the Materials and Methods section.

glycoproteins, although it cannot be utilized directly to assign molecular-weight values, since glycoproteins have been shown to migrate anomalously on polyacrylamide gels (Segrest *et al.*, 1971).

We observed some variability in the amount of metabolism found in distinct membrane peaks, as well as in the number of components that showed rapid or slow metabolic behaviour from one experiment to another. Although the components shown in Fig. 3 were quite consistent in their heterogeneous rates of metabolism, sometimes other glycoproteins appeared to be metabolized at a faster or slower rate in individual experiments. To identify those com-

ponents whose metabolism was significantly different from that of most membrane glycoproteins, a two-tailed Student's *t* test was performed on the data from three separate sets of gels (Table 1). The four fucosylated glycoproteins (Fig. 2) were shown by this method to deviate significantly in their rate of metabolism from most of the components (Table 1). Since a precise analysis of glycoprotein metabolism required an accurate comparison of two separate gels, at least part of the variability encountered between experiments may have been attributable to small differences in slicing or electrophoretic migration in the gels. In addition, these experiments were limited to measurements of glycoprotein degradation and may not have been applicable to turnover. The following experiments were designed to determine whether the loss of glycoproteins as illustrated in Table 1 was related to turnover.

Control experiments were first carried out by the method of Arias *et al.* (1969) to test the possible differential incorporation of the two labelled forms of fucose as well as to measure the reliability of the gel-fractionation procedure. Monolayer cultures were incubated simultaneously in [³H]- and [¹⁴C]-fucose until steady-state conditions were reached, plasma membrane was isolated, electrophoresed and sliced, and the ³H/¹⁴C ratios were statistically considered at a confidence level of $P = 0.01$. In Fig. 3 the ratios are bounded by broken lines that represent the 99% tolerance limits of variability. A preliminary experiment designed to assess turnover was performed under the experimental conditions used in the measurement of glycoprotein degradation. Cells were incubated for 24 h in medium containing [¹⁴C]fucose, washed and then immediately reincubated for 2.5 h in medium containing [³H]fucose. After reincubation the plasma membranes were isolated, electrophoresed, and the radioactivity per slice was determined for

Table 1. Statistical analysis of glycoprotein metabolism in neuroblastoma-cell plasma membranes as measured on polyacrylamide gels

The data from three separate experiments as shown in Fig. 3 were combined and a two-tailed Student's *t* test was performed to determine whether glycoprotein components were more or less rapidly metabolized, at a statistically significant level, than most of the membrane components.

Electrophoretic mobility (mol.wt.)	<i>P</i> value
(Rapidly metabolized)	
150 000	<0.01
130 000	<0.001
48 000	<0.01
(Slowly metabolized)	
68 000	<0.02

Fig. 3. *Relative incorporation of [^3H]fucose and [^{14}C]fucose into neuroblastoma-cell plasma-membrane glycoproteins* Neuroblastoma cells were incubated in complete medium containing both [^3H]fucose ($2.0\mu\text{Ci/ml}$) and [^{14}C]fucose ($0.2\mu\text{Ci/ml}$) for 15h. At that time the cultures were removed and plasma membranes were prepared as described in the Materials and Methods section. The ratio of $^3\text{H}/^{14}\text{C}$ was calculated at each gel slice and the 99%-tolerance limits of variability from the mean ratio (1.9) are depicted by the broken lines. The molecular-weight scale was constructed from protein standards as described in the Materials and Methods section.

both radioactive labels. When cells were reincubated under these experimental conditions, there did not appear to be a significant amount of measurable turnover (results not shown). We reasoned that large intracellular pools of low-specific-radioactivity [^{14}C]fucose, in conjunction with reutilization of the ^{14}C -labelled precursor (Hudson & Johnson, 1977), may have been responsible for the lack of apparent turnover of glycoproteins in the plasma membrane. To circumvent this problem, a procedure was developed that would deplete the cellular [^{14}C]fucose before [^3H]fucose was added. To test this possibility, neuroblastoma cells were incubated as monolayer cultures for 24h with [^{14}C]fucose in complete medium, washed, reincubated for 6h in fresh non-radioactive medium, then washed and incubated for an additional 2.5h in medium containing [^3H]fucose. Plasma-membrane fractions from these cells were electrophoresed, sliced and the radioactivity per slice was determined for both radioactive markers. The electrophoretic profile of neuroblastoma plasma membrane for both radioactive labels is illustrated in Fig. 4(a) and the $^3\text{H}/^{14}\text{C}$ ratios were calculated for each gel slice (Fig. 4b). As has been demonstrated in other cell types (Kaplan & Moskowitz, 1975; Tweto & Doyle, 1976) the metabolism of neuroblastoma plasma-membrane glycoproteins was quite heterogeneous. Three high-mol.wt. glycoproteins (180000, 150000 and 130000), and a relatively low-mol.wt. region (48000–35000), turn over rapidly. A broad area of the gel containing slowly metabolized constituents (100000–56000 mol.wt.) appeared to be associated with the more highly radioactive membrane components. In general, there appeared to be a correlation between the degradation (Table 1) and the turnover (Fig. 4) of fucosylated glycoproteins. In both cases, glycoproteins that migrate on gels as

Fig. 4. *Turnover of fucosylated glycoproteins in the neuroblastoma cell surface membrane*

Neuroblastoma cells were incubated for 24 h with complete medium containing $0.2\mu\text{Ci}$ of [^{14}C]fucose/ml, then washed twice with fresh medium, reincubated for 6h in complete medium without fucose, and reincubated for 2.5h in complete medium containing $10\mu\text{Ci}$ of [^3H]fucose/ml. After incubation plasma membranes were prepared and electrophoresed on polyacrylamide gels, the gels were sliced and the ^3H ($\circ$) and ^{14}C ($\bullet$) in each slice were measured (a). The ratio of $^3\text{H}/^{14}\text{C}$ in each slice was determined (b) and the broken lines represent the tolerance limits of variability where $P=0.01$. The molecular-weight scale was constructed as described in the Materials and Methods section.

proteins of mol.wts. 150000, 130000, 48000 and 68000 were similarly metabolized. Although the use of only [^3H]fucose (Fig. 3) certainly gave results similar to those obtained in the initial studies with intact neuroblastoma cells (Fig. 1), the use of dual-labelled membranes (Fig. 4) probably gave a truer picture of the heterogeneous metabolism of glycoproteins at the cell surface.

Discussion

A number of important cellular functions, including structural and receptor properties, have been attributed to glycoproteins of the surface membrane (Hughes, 1975; Nicolson, 1976). The ability of cells to alter their surface glycoprotein composition and maintain functionally active components may be the result of membrane metabolism. At least some glycoproteins, including transplantation antigens (Turner

et al., 1972), virus receptors (Philipson *et al.*, 1968) and insulin receptors (El-Allawy & Gliemann, 1972), turn over at a rapid rate, since all of these membrane components are regenerated rapidly after the cells were treated with proteolytic enzymes. It has been suggested that the ability of a cell to replace surface receptors rapidly might provide a selective advantage by allowing the cell to respond continually to external stimuli (Gurd & Evans, 1973).

Although most of the fucosylated macromolecules of neuroblastoma cells appeared to be relatively stable, approx. 22% of the acid-insoluble material was lost within 2h of reincubation (Fig. 1). Since fucose is primarily incorporated into plasma membranes and because neuroblastoma cells preferentially incorporate this monosaccharide into glycoproteins (Hudson & Johnson, 1977), it appeared that these rapidly metabolized macromolecules were glycoproteins associated with the surface membrane. A similar loss of surface-membrane components has been observed with plasma-membrane proteins iodinated by the lactoperoxidase method in intact L cells (Hubbard & Cohn, 1975) and hepatoma cells (Tweto & Doyle, 1976). However, when these investigators evaluated the metabolism of membrane constituents separated by polyacrylamide-gel electrophoresis, all labelled components appeared to be metabolized at equivalent rates. In contrast with these previous studies, polyacrylamide-gel electrophoresis of isolated plasma membranes of neuroblastoma cells labelled with [³H]fucose did show a heterogeneous rate of glycoprotein metabolism (Fig. 2). Three populations of glycoproteins were rapidly degraded compared with most of the plasma-membrane glycoproteins. These three populations constituted 16% of the total [³H]fucose that was incorporated into cell-surface glycoproteins, and therefore correlated well with experiments carried out with intact cells. Although the use of [³H]fucose with both intact cells and isolated plasma membranes allowed direct correlations of glycoprotein degradation, this procedure did not permit estimates of metabolic turnover.

When membrane glycoprotein turnover was measured by dual-label techniques with [¹⁴C]fucose and [³H]fucose, all three populations of molecules were found to turn over rapidly (Fig. 4). Under these experimental conditions the metabolism of surface-membrane components appeared to be even more heterogeneous. As stated in the Results section, the dual-label method may reflect a more accurate representation of neuroblastoma-membrane metabolism, since there was no selective incorporation of the two labelled forms of the precursor (Fig. 3), high concentrations of unlabelled fucose were unnecessary during the reincubation period and a comparison of separate gels was not necessary.

Monolayer cultures of neuroblastoma N2aE cells

have been shown to release fucosylated glycoproteins (87000, 66000 and 55000 daltons) into the cell-culture medium (Truding *et al.*, 1975). These authors considered the release of these glycoproteins to be either the result of rapid plasma-membrane metabolism or a product of selective secretion. If these components were the by-product of membrane metabolism they might have been shed either as intact macromolecules or, since transformed cells may possess relatively high proteinase activity in the surface membrane (Schnebli & Burger, 1972), as glycopeptides resulting from the hydrolysis of surface glycoproteins. The shedding of antigenic glycopeptides is a characteristic of many transformed cell lines (Nicolson, 1976). On the basis of the results presented here, it seems unlikely that plasma-membrane glycoproteins were released into the culture fluid as complete molecules, since the fucosylated components that Truding *et al.* (1975) described correspond to glycoproteins that turn over relatively slowly (Fig. 5). This is consistent with a previous observation that the rapid loss of glycoproteins from the cell surface of neuroblastoma cells was not directly related to their transport into the culture medium (Hudson & Johnson, 1977).

Previously, using radioactive amino acids, we identified two external proteins (180000 and 130000 daltons) on the surface membranes of neuroblastoma cells grown in monolayer form that turned over more rapidly than did most of the surface components (Mathews *et al.*, 1976). Since both of these proteins co-migrated on polyacrylamide gels with membrane constituents labelled with radioactive glucosamine and fucose, they appeared to be glycoproteins. Consistent with this earlier report, the present study shows that two fucosylated glycoproteins (180000 and 130000 daltons) turned over significantly faster than the mean rate of fucosylated membrane components (Fig. 4). However, in contrast with the results obtained with radioactive amino acids, a large number of fucosylated macromolecules were metabolized significantly faster than the mean ($P=0.01$). These observations may have resulted from a more rapid rate of turnover of the carbohydrate region of some glycoproteins than of their respective polypeptide backbones. Glycosyltransferase enzymes have been shown to be localized in the plasma membrane of some cells (Roth *et al.*, 1971) and may be responsible for this form of selective metabolism. This is not without precedent, since some membrane-bound lipids are apparently metabolized in a manner that allows the polar head group and the fatty acyl chain to turn over independently (Omura *et al.*, 1967). This may be particularly evident with fucose, since it is a terminal sugar (Hughes, 1975), and such selective metabolism of membrane carbohydrates and polypeptides has been observed before (Tweto & Doyle, 1976).

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