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# SURFACE ALTERATIONS OF P388 LEUKEMIA CELLS BY 2-DEOXY-D-GLUCOSE*

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**Abstract**—Treatment of P388 murine leukemia cells *in vitro* with relatively low concentrations of 2-deoxy-D-glucose results in altered plasma membrane architecture, as detected by an increase in the binding of and in the subsequent rate of cellular agglutination by concanavalin A. In contrast, binding of and agglutination by wheat germ agglutinin in 2-deoxy-D-glucose-treated cells are reduced, while these parameters are not changed when soybean agglutinin is employed. These effects of 2-deoxy-D-glucose on surface architecture are not due to partial synchrony of the cell population by the carbohydrate analog since the DNA content distribution profiles are not altered markedly. The changes detected in the surface membrane appear to be partially due to 2-deoxy-D-glucose incorporation into membrane glycoproteins and glycolipids; in support of this concept, 2-deoxy-D-glucose was found to compete with P388 cells for concanavalin A binding, but did not compete for the binding of either wheat germ or soybean agglutinins. CMP-sialic acid:glycoprotein sialyl transferase and UDP-galactose:glycoprotein galactosyl transferase activities, and the availability of acceptor glycoproteins were measured in 2-deoxy-D-glucose-treated cells. Exposure of cells to this agent decreased sialyl transferase activity and the availability of endogenous acceptor glycoprotein molecules for this enzyme, suggesting that the observed decrease in wheat germ agglutinin binding might be due to decreased sialylation of the cell surface.

Structural and functional changes in plasma membranes have been implicated in many facets of neoplastic cell behavior, such as invasiveness, adhesiveness, metastatic capacity, antigenicity and growth rate [1, 2]. Thus, it is conceivable that therapeutic gain might be obtained by the exploitation of differences between normal and malignant cell membranes. To explore this possibility, we have studied the effects of 2-deoxy-D-glucose (dGlc) on the surface properties of P388 leukemia cells. dGlc, like other hexoses, has been reported to be accumulated by transformed cells to a greater extent than by normal cells [3]. Furthermore, dGlc produces a variety of metabolic effects including inhibition of the synthesis of viral glycoproteins [4, 5]; this agent is also incorporated into viral glycoproteins [4] and into mammalian cell glycoproteins [4, 6, 7] and glycolipids [8]. In the P388 leukemia, we have demonstrated that inhibition of cellular growth by dGlc is associated with the presence of this carbohydrate in cellular glycoprotein and with alterations in cell surface architecture, as detected by the degree of responsiveness to agglutination by plant lectins. In the present paper, evidence is presented which suggests that the surface alterations induced by dGlc in P388 cells are due both to the presence of exposed dGlc residues on the surface and to altered patterns of glycosyl transferase activity.

## MATERIALS AND METHODS

Fischer's medium and horse serum were obtained from the Grand Island Biological Co., Grand Island, NY. Fetuin, β-galactosidase (EC 3.2.1.23) from *Escherichia coli*, grade IV, dGlc, and 2-deoxy-D-glucose-6-phosphate were purchased from the Sigma Chemical Co., St. Louis, MO. Concanavalin A (Con A) and wheat germ agglutinin (WGA) were obtained from Miles-Yeda, Inc., Rehovot, Israel. Radiochemicals were supplied by the New England Nuclear Corp., Boston, MA; these included [$^{14}$C]dGlc, 50 μCi/μmole; CMP-[$^{14}$C]sialic acid, 150 μCi/μmole; UDP-[$^{14}$C]galactose, 230 μCi/μmole; [$^{3}$H]Con A, 0.6 μCi/μg; [$^{3}$H]WGA, 0.73 μCi/μg; and [$^{3}$H]soybean agglutinin (SBA), 1.37 μCi/μg.

P388 murine leukemia cells were grown in suspension culture in Fischer's medium containing 5.6 mM glucose and supplemented with 10% horse serum, as described previously [6]. Cultures were routinely seeded at $10^4$ cells/ml and treated with 5–500 μM dGlc in 0.9% NaCl or an equivalent volume of 0.9% NaCl for control cells, and cultured for 68–72 hr. Cells were collected by centrifugation at 750 *g* at room temperature for 6 min prior to experimentation, and their numbers and mean volumes were determined with a Coulter Counter, model B. Protein was determined by

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the method of Lowry *et al.* [9], using bovine serum albumin as a standard.

Glycosyl transferase activities were measured in cells grown in the presence or absence of 125 μM dGlc. The cells were collected by centrifugation, washed three times in cold 0.9% NaCl, and homogenized in 0.1% Triton X-100 using a glass/glass Potter–Elvehjem homogenizer at a concentration of $10^8$ cells/ml to solubilize the transferase enzymes. After removal of insoluble material by centrifugation at 10,000 *g* at 4°, the supernatant solution was stored at −20° and used as the enzyme source for glycosyl transferase assays. Exogenous acceptors for sialyl transferase and galactosyl transferase were prepared as described previously [10]. Native fetuin was subjected to acid hydrolysis for removal of sialic acid and then to β-galactosidase treatment for 72 hr for removal of galactose. The final stock solution of fetuin minus sialic acid consisting of 10 mg/ml contained 3 per cent of its original sialic acid content, as determined by the method of Warren [11]. Stock solutions of fetuin minus both sialic acid and galactose, employed at concentrations of 20 mg/ml, contained 88 per cent of the anthrone detectable carbohydrate of the precursor fetuin minus sialic acid, as determined by the method of Spiro [12].

Sialyl transferase assay mixtures contained 17 mM $MgCl_2$, 8.3 mM Tris–HCl (pH 7.0), 0.4 mg fetuin minus sialic acid, 20 μl enzyme, and 0.04 μCi CMP-[$^{14}$C]sialic acid in a total volume of 120 μl. Galactosyl transferase mixtures contained 8.3 mM $MnCl_2$, 8.3 mM Tris–HCl (pH 7.0), 1 mg fetuin minus sialic acid minus galactose, 20 μl enzyme, and 0.04 μCi UDP-[$^{14}$C]galactose. Reactions were incubated for 60 min at 37°; under these conditions, incorporation was linear with time. Incorporation of labeled monosaccharide was terminated by the addition of cold 0.5% phosphotungstic acid in 0.1 N HCl. Precipitated protein was washed twice with 10% trichloroacetic acid, once with ethanol–ether (2:1, v/v), and dissolved in 0.025 N NaOH; radioactivity was measured with Aquasol (New England Nuclear Corp.) by scintillation spectrometry.

Agglutination of P388 cells by plant lectins was performed as described previously [6, 13]. Cells washed in $Ca^{2+}$, $Mg^{2+}$-free phosphate buffered 0.9% NaCl (pH 7.4) were mixed with 1000 μg/ml of Con A, 200 μg/ml of WGA, or 400 μg/ml of SBA, and agglutination was measured by both the rate of sedimentation of cell clumps and the delay in onset of sedimentation (lag time). Agglutinin binding assays were conducted using cells washed twice in $Ca^{2+}$, $Mg^{2+}$-free phosphate buffered 0.9% NaCl (pH 7.4) at room temperature. Cells were resuspended in the same buffer at a concentration of $8 \times 10^6$ cells/ml. Binding assays consisted of 200 μl of cell suspension to which was added 50 μl of assay buffer containing various concentrations of unlabeled lectin and 10 μl of labeled lectin, which consisted of either 0.084 μCi [$^3$H]Con A, 0.38 μCi [$^3$H]WGA, or 0.63 μCi [$^3$H]SBA, in round bottom tubes. Following incubation for 30 min with gentle shaking at room temperature, the reaction was stopped by the addition of 5 ml of cold assay buffer. Cells were centrifuged at 4° and the supernatant solution was removed. The pelleted cells were washed three times with cold buffer, dissolved in 0.5 ml of 0.5 N NaOH, and radioactivity was determined by scintillation spectrometry using Aquasol. In some cases, competing monosaccharides at a concentration of 0.1 M were added to the assays and washing buffers.

The DNA distribution content of dGlc-treated cell populations was ascertained using cells grown in 0, 6.4, 91.4 and 914 μM dGlc for 70 hr. Samples containing $5 \times 10^6$ cells were collected by centrifugation and resuspended in 5 ml of cold hypotonic citrate/propidium iodide solution, as described by Krishan [14]. Samples were analyzed for DNA content distribution as described previously [15].

## RESULTS

Growth of P388 leukemia cells in medium containing dGlc results in altered rates of agglutination by the plant lectins Con A and WGA [6]. These lectins bind to mannosyl- (Con A) and *N*-acetylglucosaminyl- or *N*-acetyl-neuraminic acyl(WGA)-residues. The altered agglutination of cells exposed to dGlc suggests that this agent, which is incorporated into cellular glycoprotein, contributes to changes in cell surface architecture. Agglutination by certain other plant lectins, such as SBA, which binds to *N*-acetylgalactosaminyl- and galactosyl-residues, is unchanged in dGlc-treated cells (M. W. Myers-Robfogel and A. C. Sartorelli, unpublished results). These findings suggest that there is specificity to the action of dGlc on the cell surface which leads to effects detected by certain lectins.

To determine whether the observed changes in the rate of lectin-induced agglutination of dGlc-treated cells were the result of alterations in the number of lectin binding sites, the effects of this sugar on the binding of $^3$H-labeled lectins to P388 cells were measured. Binding of [$^3$H]Con A to dGlc-treated cells was enhanced significantly over that of untreated control cells at each concentration of lectin tested (Fig. 1). Under the conditions employed for binding, a two-phase saturation curve was obtained as described previously [13]; high affinity binding of Con A to P388 cells was saturable at about 50 μg Con A/$1.6 \times 10^6$ cells, but at high levels of Con A low affinity binding was not saturable up to 200 μg lectin/$1.6 \times 10^6$ cells. A Scatchard plot of the data suggests the existence of multiple types of binding sites for Con A in both untreated and dGlc-treated P388 cells. The specificity of Con A binding to either untreated or dGlc-treated P388 cells was investigated by measuring the degree of binding of Con A in the presence of 0.1 M mannose, α-methylglucose, or dGlc. With each of these three monosaccharides, Con A binding was reduced to 24–28 per cent in both control and dGlc-treated cells. These findings indicate that about 75 per cent of the Con A binding to P388 cells is prevented by the monosaccharides, mannose and α-methylglucose, which are known to bind specifically to Con A, and also by dGlc, which appears to compete equally well for Con A binding sites (Table 1).

Exposure of P388 leukemia cells to dGlc resulted in a decrease in the binding of [$^3$H]WGA when compared to untreated control cells (Fig. 2). The amount of WGA bound to dGlc-treated P388 cells reached a saturation plateau at a concentration of 50 μg WGA/$1.6 \times 10^6$ cells; under these conditions, the binding of WGA to untreated cells remained linear. The specificity of the interaction between the lectin and cells was investigated

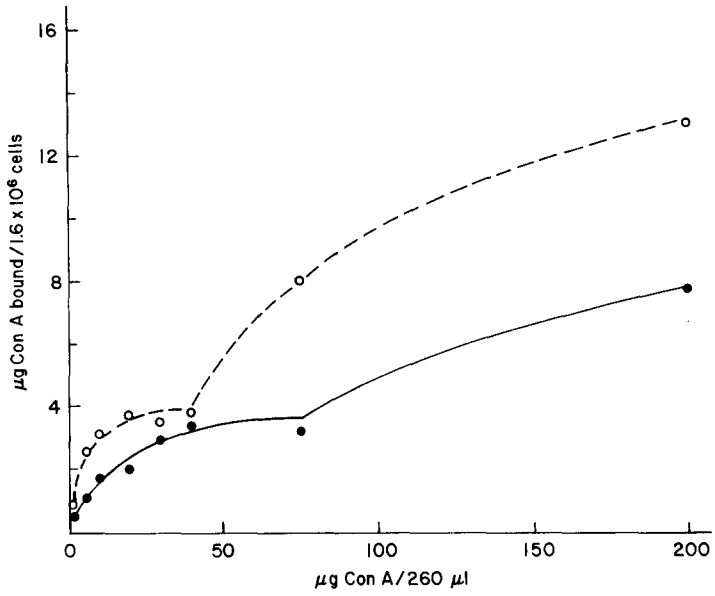


Fig. 1. Binding of [$^3$H]Con A to dGlc-treated P388 cells. Key: (●) control cells; and (○) cells grown in 120 μM dGlc for 72 hr. Data points represent the means of triplicate determinations. The results are representative findings of four separate experiments.

by measurement of the binding of [$^3$H]WGA in the presence of monosaccharides (i.e. 0.1 M *N*-acetylglucosamine, *N*-acetylgalactosamine, dGlc). None of these monosaccharides competed for WGA binding in this assay system by more than 10 per cent. Similar findings showing lack of reversibility of WGA binding have been reported by others [16].

Measurement of the degree of [$^3$H]SBA binding to P388 leukemia cells revealed no difference between control and dGlc-treated cells in the amount of lectin bound over a range of 5 to 62 μg SBA/1.6 × $10^6$ cells (Fig. 3). The specificity of binding was tested by competitive monosaccharide inhibition; of the monosaccharides used, only *N*-acetylgalactosamine decreased SBA binding (by 30 per cent).

Since lectin binding to and agglutination of cells are different in various stages of the cell cycle [17], we have examined the possibility that dGlc alters the surface of P388 cells by inducing partial synchronization of the cell population. To accomplish this, DNA content distribution profiles for the population of P388 cells grown in the absence or the presence of 6.4, 91.4 and 914 μM dGlc for 70 hr were measured, and the results obtained are shown in Fig. 4. At a level of 914 μM dGlc, cell growth was inhibited severely, with cells reaching only 25 per cent of the number present in parallel untreated control cultures. However, the alterations in the DNA distribution curves, as compared to the untreated control, were not pronounced. At 6.4 μM, there is a suggestion of a shift of G1 cells into early S

Table 1. Effects of 2-deoxy-D-glucose on lectin binding to P388 leukemia cells

| | % Lectin bound* | | |
|---|---|---|---|
| Monosaccharide | [$^3$H]Con A | [$^3$H]WGA | [$^3$H]SBA |
| α-Methylglucose | 27.3 | | |
| Mannose | 23.9 | | |
| 2-Deoxyglucose | 28.1 | 108.0 | 82.2 |
| *N*-Acetylglucosamine | | 89.4 | |
| *N*-Acetylgalactosamine | | 92.9 | 70.4 |
| Galactose | | | 82.6 |

* Per cent radioactive lectin bound to P388 leukemia cells in the presence of 0.1 M monosaccharide. One hundred per cent = the amount of lectin bound in the absence of competing monosaccharide. The concentrations of lectins employed were: [$^3$H]Con A, 420 μg/ml; [$^3$H]WGA, 203 μg/ml; and [$^3$H]SBA, 211 μg/ml.

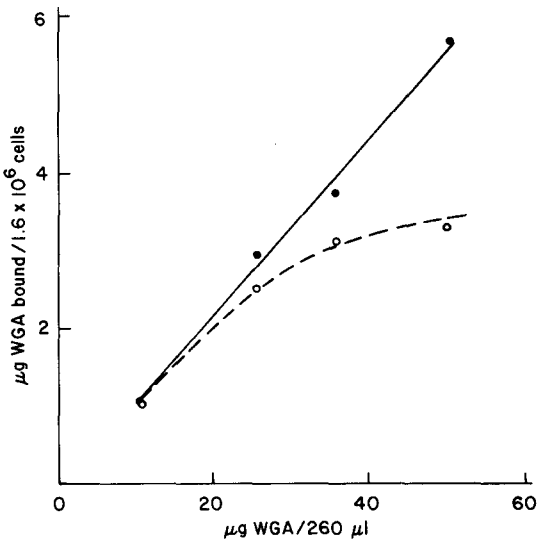


Fig. 2. Binding of [$^3$H]WGA to dGlc-treated P388 cells. Key: (●) control cells; and (○) cells grown in 120 μM dGlc for 72 hr. Data points represent means of triplicate determinations. The results are representative findings of four separate experiments.

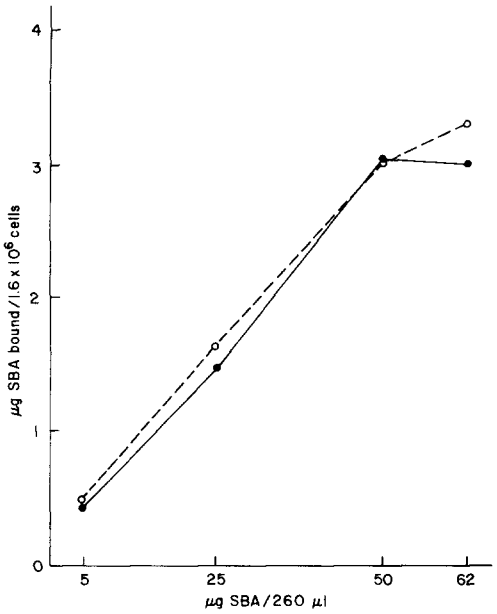


Fig. 3. Binding of [$^3$H]SBA to dGlc-treated P388 cells. Key: (●) control cells; and (○) cells grown in 120 μM dGlc for 72 hr. Data points represent means of triplicate determinations. The results are representative findings of two separate experiments.

phase, which appears to be of slightly greater magnitude for the 91.4 μM dGlc concentration. At 914 μM dGlc, there is a depletion of S and G2 cells with an increase in the pre-G1 area. These findings would seem to indicate that dGlc has relatively little, if any, phase specificity.

Further support for the absence of cell synchrony by dGlc was obtained by demonstrating that the mean cell volume was identical for logarithmically growing P388 leukemia cells and for those grown in 120 μM dGlc (M. W. Myers-Robfogel and A. C. Sartorelli, unpublished results).

Since previous studies [6] have shown changes in the content of fucose and sialic acid in glycoproteins and glycolipids of dGlc-treated P388 cells, the effects of dGlc treatment on glycosyl transferase activity were examined in Triton X-100 homogenates of P388 cells grown in the absence or presence of 120 μM dGlc. Assays were performed which determine both the level of transfer of labeled monosaccharide onto endogenous cellular glycoprotein acceptors present in the enzyme preparations and onto added exogenous acceptors prepared from purified glycoproteins. Table 2 summarizes the results of measurements of the activities of CMP-sialic acid:glycoprotein sialyl transferase and UDP-galactose:glycoprotein galactosyl transferase. Enzyme activity is expressed as radioactivity incorporated per $2 \times 10^6$ cells; however, since dGlc-treated cells have

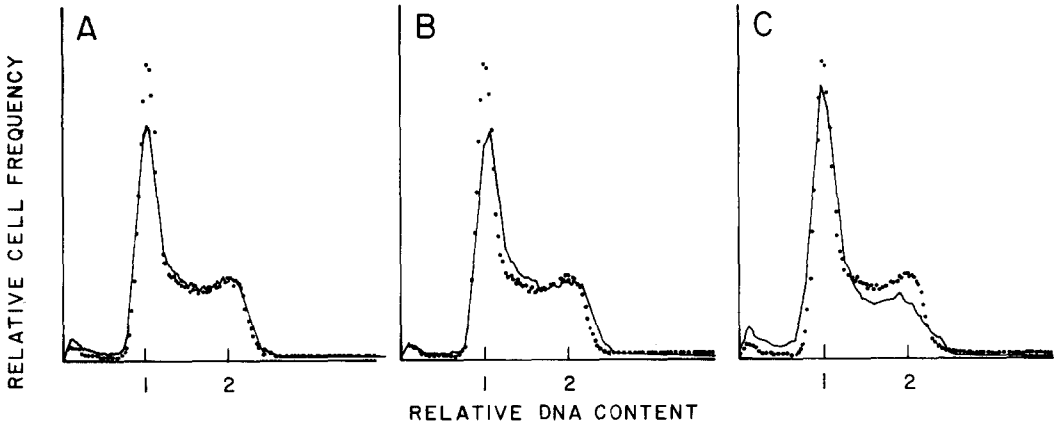


Fig. 4. DNA content distribution profiles of cells grown for 70 hr in the presence of various concentrations of dGlc. The solid line in all panels represents results from cells exposed to dGlc and the dots represent those obtained from untreated control cells. Panel A: 6.4 μM dGlc; panel B: 91.4 μM dGlc; and panel C: 914 μM dGlc.

Table 2. Glycosyl transferase activity of 2-deoxy-D-glucose-treated P388 leukemia cells*

| | Control | | | dGlc-treated | | |
|---|---|---|---|---|---|---|
| Substrate | A (endogenous) | B (+acceptor) | C (B − A) | A (endogenous) | B (+ acceptor) | C (B − A) |
| CMP-sialic acid | 190 | 3310 | 3220 | 290† | 2360† | 2070† |
| UDP-galactose | 1110 | 2950 | 1850 | 1000 | 2910 | 1910 |

* Data are expressed as cpm/2 × $10^6$ cells.
† Significantly different from control, $P < 0.05$, using five different enzyme preparations.

the same amount of protein per cell as the control cells, these data also represent relative levels of enzyme activity per mg of protein [6]. Control P388 cells had higher CMP-sialic acid:fetuin minus sialic acid:sialyl transferase activity than did dGlc-treated neoplastic cells; however, treated cells appeared to contain more available sites for sialylation, as demonstrated by higher endogenous activity than was present in untreated control samples. The activity of UDP-galactose:fetuin minus sialic acid minus galactose: galactosyl transferase was identical in homogenates of untreated and dGlc-treated cells; furthermore, the amount of available acceptor glycoprotein for galactosyl transferase appeared to be the same, as demonstrated by the equal activity obtained in endogenous assays.

To ascertain whether the presence of dGlc or a metabolite thereof in the enzyme assay might directly alter activity, assays of sialyl and galactosyl transferase activities in cell homogenates were performed in the presence of added dGlc and 2-deoxy-D-glucose-6-phosphate. dGlc did not inhibit either sialyl or galactosyl transferase activities at concentrations up to 0.01 M; however, at this level, 2-deoxy-D-glucose-6-phosphate inhibited the enzyme activities by 32 and 23 per cent, respectively.

## DISCUSSION

Previous studies have shown that dGlc alters surface membrane architecture, as detected by plant lectin agglutination in P388 [6] and hamster [18] cells grown in culture. These effects are accompanied by the incorporation of radioactive dGlc into membrane glycoproteins and glycolipids [6–8]. Agglutination of P388 cells by Con A, a plant lectin which binds to glucosyl- and mannosyl-residues, is increased in dGlc-treated cells [6, 18]. The present study supports the concept that this enhanced agglutination is due to an increased binding of Con A to surface receptors of dGlc-treated P388 cells. The presence of 0.1 M dGlc in the assay system inhibited Con A binding to P388 cells, indicating that dGlc is capable of interacting with this lectin. These findings suggest that the increased binding of Con A and the resulting increase in cellular agglutination in dGlc-treated cells are reflections of the interaction of this plant lectin with dGlc residues present in glycoproteins and glycolipids on the surface of cells exposed to this mannosyl analog.

Studies on the interactions of P388 cells with two other plant lectins (i.e. WGA, which binds to *N*-acetyl-D-glucosaminyl- and *N*-acetyl-neuraminic acyl-residues, and SBA, which binds to *N*-acetyl-D-galactosaminyl-residues) have provided data which are indicative of the specificity of the changes in the binding and subsequent agglutination produced by Con A in dGlc-treated cells. Thus, both cellular agglutination caused by WGA [6] and the binding of this plant lectin to dGlc-treated P388 leukemia cells were decreased; in contrast, the action of SBA, with respect to both cellular agglutination and lectin binding, was unaltered by exposure of cells to dGlc. These findings also imply that the quantity of lectin bound was the major determinant in the process of agglutination.

Cell growth parameters which are known to affect surface binding of agglutinins, such as cell cycle stage and mean cell volume, were essentially unaltered by treatment of P388 cells with dGlc. Since dGlc inhibits the proliferation of P388 cells [6], the finding that this agent does not cause a major accumulation of cells in any particular phase of the cell cycle indicates that dGlc creates a metabolic lesion(s) which interferes with cellular replication in a manner independent of the phases of the cycle.

Measurement of the synthesis of glycoprotein in cells exposed to low levels of dGlc indicates that the transfer of several monosaccharides onto glycoprotein is altered [3, 19]. Furthermore, inhibition of the synthesis of viral coat glycoprotein [4, 5] and immunoglobins [20] by this carbohydrate or its nucleotide derivatives has been demonstrated [21]. Data presented in this report show further that, in dGlc-treated cells, alterations occur in both enzymatic activity for transfer of sialic acid onto glycoprotein (i.e. CMP-sialic acid:glycoprotein sialyl transferase) and in the availability of suitable acceptor glycoproteins. In contrast, however, neither acceptors for nor activity of a galactosyl transferase were altered. These findings are supportive of earlier results [6] which demonstrated a slight but significant decrease in the concentration of glycoprotein-bound sialic acid of P388 cells treated with dGlc. This latter type of action may be responsible at least in part for changes at the cell surface resulting in decreased binding of WGA.

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