Glycosyltransferase and Glycosidase Activities in Ovarian Cancer Patients

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ABSTRACT

In order to elucidate the mechanism of appearance of abnormal glycoproteins in cancer, activities of glycoprotein glycosyltransferases and glycosidases were determined in the homogenates prepared from normal ovaries and ovarian epithelial adenocarcinomas. Significantly high activities (more than normal mean + 2 S.D.) of these enzymes were found as follows: galactosyltransferase and sialyltransferase in 100%; fucosyltransferase 1 (exogenous acceptor, fetuin minus sialic acid and galactose) in 86%; fucosyltransferase 2 (fetuin minus sialic acid, acceptor) in 45%; N-acetylglucosaminyltransferase 1 (ovalbumin acceptor) in 53%; N-acetylglucosaminyltransferase 2 (ribonuclease A as acceptor) in 10% of the samples analyzed. Among the glycosidases, substantially elevated activities above normal controls were found as follows: N-acetyl-\(\beta\)-D-galactosaminidase in 85%; N-acetyl-\(\beta\)-D-galactosaminidase in 63%; N-acetyl-\(\beta\)-D-galactosidase in 50%, and those of \(\alpha\)-L-fucosidase in 35% of the tumors. In serum of these cancer patients, only levels of galactosyltransferase were consistently elevated compared to controls. Increases in serum levels for other transferases were as follows: fucosyl-1, 10%; fucosyl-2, 60%; sialyl-, 20%; N-acetylglucosaminyl-1, 90%; N-acetylglucosaminyl-2, in 80% of the serum samples from ovarian carcinoma patients. Galactosyltransferase thus appears to be an excellent marker for ovarian carcinoma.

INTRODUCTION

In malignant ovaries, antigenically distinct glycoproteins are found that are either absent or occur in trace amounts in normal ovaries (3). Comparative studies of the synthesis and breakdown of glycoproteins in these tumors and normal ovarian tissue may elucidate the mechanism by which such tumor-associated glycoproteins are formed in a malignant disease. With this objective, we initiated studies on a number of glycosyltransferases and glycosidases present in normal and malignant ovarian tissues and sera. Glycosyltransferases sequentially add individual monosaccharides to the incomplete oligosaccharide part of the glycoconjugates and are specific for the donor nucleotide sugar and for the acceptor molecule (32). Glycosidases, on the other hand, are degrading enzymes that specifically hydrolyze monosaccharide residues from such glycoconjugates (1). In preliminary studies, we have reported that the specific activities of galactosyltransferase in ovarian tumor homogenate are significantly higher than those from normal ovaries (6). In patients with this type of cancer, the levels of galactosyltransferase in the sera are also elevated and appear to correlate with tumor volume as well as the clinical status of the patients (6, 12). Six glycoprotein glycosyltransferases and 4 glycosidases have been assayed in about 20 samples of normal ovaries and compared with a similar number of malignant ovarian tumors. Levels of the glycosyltransferases were also determined in the sera from 30 patients with ovarian epithelial carcinoma and compared with a control group of 30 healthy women. The results of these studies are presented in this report.

MATERIALS AND METHODS

Materials. UDP-[U-14C]galactose (301 Ci/mol), CMP-[4, 5, 6, 7, 8, 9-14C]NANA (235 Ci/mol), CMP-[4-14C]NANA (1 Ci/mol), UDP-[1-14C]N-acetyl-D-glucosamine (43 Ci/mol), and GDP-[U-14C]fucose (174 Ci/mol) were obtained from New England Nuclear, Boston, Mass. ATP, NAD+, CTP, dithiothreitol, unlabeled UDP-galactose, unlabeled UDP-N-acetylgalactosamine, p-nitrophenyl derivatives of \(\beta\)-D-galactopyranoside, \(\alpha\)-L-fucose, \(\beta\)-D-N-acetylglucosaminidase, \(\beta\)-D-N-acetylgalactosaminidase, \(\beta\)-D-N-acetylglucosaminidase, and RNase A were obtained from Sigma Chemical Co., St. Louis, Mo. Ovalbumin (5 times crystallized) was obtained from Miles Laboratories, Inc., Elkhart, Ind. Other chemicals were the purest available from commercial sources.

Tumor and Normal Tissues. Ovarian tissues were obtained at surgery, whereas normal ovaries were from patients who underwent oophorectomies for nonovarian diseases. All tissues were examined histologically to determine the type and grade of malignancy.

Preparation of Tissue Homogenate. The tissue was washed with cold 0.85% NaCl solution to remove adherent mucus and blood, and then it was dissected free of necrotic tissue and fat. After the tissue was minced with surgical scissors and blades, the fragments were placed in 0.85% NaCl solution (1 g wet tissue/3 ml of solution) for homogenization. Three 60-sec periods of homogenization, each followed by 60 sec cooling, were done with a Polytron homogenizer, (Brinkman Instruments Inc., Westbury, N. Y.). The solution was maintained at 0° while homogenizing at maximum speed by ethanol-ice. After a brief sonication (three 30-sec treatments, with 60 sec cooling), the material was filtered through 4 layers of surgical gauge and stored at −20° before use.

Assay of Glycosyltransferases. These enzymes were assayed essentially as described earlier (6) for galactosyltransferase. Composition and total volume of reaction mixtures as well as the time of incubation for each enzyme are summarized in Table 1. The specific activities of galactosyltransferase in ovarian tumor homogenate are significantly higher than those from normal ovaries (6). In patients with this type of cancer, the levels of

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2 To whom requests for reprints should be addressed.

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Table 1
Composition of the incubation media for the assay of glycosyltransferases in the ovarian tissues and serum samples

<table>
<thead>
<tr>
<th>Transferases</th>
<th>Nucleotide sugar</th>
<th>Exogenous acceptor</th>
<th>Homogenate protein (mg/ml)</th>
<th>Reaction volume (µl)</th>
<th>Additional divalent cation</th>
<th>Incubation time (hr)</th>
<th>Optimum pH</th>
<th>Pyrophosphatase inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fucosyl-1-</td>
<td>GDP-fucose (12 µM)</td>
<td>Fetuin (-NANA, galactose) (8.0 mg/ml)</td>
<td>0.2</td>
<td>25</td>
<td>None^d</td>
<td>1</td>
<td>6.9</td>
<td>NAD^+</td>
</tr>
<tr>
<td>Fucosyl-2-</td>
<td>GDP-fucose (12 µM)</td>
<td>Fetuin (-NANA) (18.6 mg/ml)</td>
<td>0.2</td>
<td>25</td>
<td>None^d</td>
<td>1</td>
<td>6.9</td>
<td>NAD^+</td>
</tr>
<tr>
<td>Sialyl-</td>
<td>CMP-NANA (1 µM)</td>
<td>Fetuin (-NANA) (4.7 mg/ml)</td>
<td>1.0</td>
<td>100</td>
<td>None</td>
<td>2</td>
<td>7.0</td>
<td>None</td>
</tr>
<tr>
<td>N-Acetylglucosaminyl-1-</td>
<td>UDP-N-acetylgalactosamime (1 µM)</td>
<td>Ovalbumin (20 mg/ml)</td>
<td>0.4</td>
<td>50</td>
<td>Mn^2+ (50 mM)</td>
<td>3</td>
<td>7.1</td>
<td>NAD^+</td>
</tr>
<tr>
<td>N-acetylglucosaminyl-2-</td>
<td>UDP-N-acetylgalactosamime (1 µM)</td>
<td>RNase A (20 mg/ml)</td>
<td>0.4</td>
<td>50</td>
<td>Mn^2+ (50 mM)</td>
<td>3</td>
<td>7.1</td>
<td>NAD^+</td>
</tr>
<tr>
<td>Galactosyl-</td>
<td>UDP-galactose (100 µM)</td>
<td>Fetuin (-NANA, galactose) (5 mg/ml)</td>
<td>0.2</td>
<td>100</td>
<td>Mn^2+ (20 mM)</td>
<td>1</td>
<td>6.2</td>
<td>ATP</td>
</tr>
</tbody>
</table>

* Five to 10 µl of serum were added.
* Tris-maleate buffer (50 mM) was used.
* Concentrations of these inhibitors were 1 mM when present.
* EDTA (2.5 mM) inhibited the reaction.

Continuous stirring in a Dubnoff metabolic shaker (Fisher Scientific Co., Pittsburgh, Pa.). Each tube contained 5 µl of the radioactive nucleotide sugar (100,000 to 200,000 dpm) together with the unlabeled compound to achieve the required substrate concentration. For sialyltransferase CMP-[4,14C]sialic acid (1 Ci/mol) was used. For the assay of these enzymes in serum, similar incubation conditions were used, and 5 to 10 µl of serum were added per tube. The dpm were calculated by using external standard method of quench correction.

Assay of Glycosidases. Activities of glycosidases were determined by the modification of the method of Aronson and DeDuve (2). Solutions of the p-nitrophenylglycosides were prepared in 0.1 M sodium acetate buffer, pH 4.6, to obtain a substrate concentration of 1.6 mM in the final incubation mixture. Triton X-100 was added to the homogenate to obtain a 5% solution. The reaction was terminated after 6 hr with the addition of an equal volume of cold 95% ethanol. The resulting precipitate was removed by centrifugation at 4°, and clear supernatant was applied as a band on S & S orange ribbon papers. The chromatograms were developed with Solvent A for 17 to 24 hr when the solvent front reached 1 cm above the paper tip. The chromatograms were scanned in a Packard Model 7201 radiocromatogram scanner (Packard Instrument Co., Inc., Downers Grove, Ill.). Appropriate spots were cut from the paper and eluted with water, and the radioactivity was determined in the liquid scintillation counter using 10 ml of ACS (Amersham Corp., Arlington Heights, Ill.).

Identification of the Incorporated Monosaccharide. To determine the identity of incorporated radioactivity into the high-molecular-weight acceptors, the standard incubation mixture was scaled up 4-fold, and all unlabeled nucleotide sugars were replaced by labeled ones. For sialyltransferase, substrate CMP-[4, 5, 6, 7, 8, 9,14C]sialic acid (235 Ci/mol) was used. All incubations, except N-acetylglucosaminyltransferases, were terminated after 6 hr with the addition of an equal volume of 2% phosphotungstic acid in 1 N HCl. The pellet was collected by centrifugation and washed (2 ml each time) with 1% phosphotungstic acid in 0.5 N HCl (2 times), 10% trichloroacetic acid (once), 1:1 (v/v) ethanol:diethyl ether (2 times), and finally once with diethyl ether. The dried powder obtained from the reaction mixture of fucosyltransferase and galactosyltransferases was taken in Pyrex ampuls, and hydrolysis at 100° with double-distilled HCl was performed in sealed tubes in the presence of nitrogen. The concentration of protein in each sample was maintained at 2 mg/ml, and concentration of HCl and time of hydrolysis were: 1 N HCl, 1 hr for fucosyltransferase; 2 N HCl, 8 hr for galactosyltransferase product. Product of sialyltransferase was hydrolyzed with 0.1 N H2SO4 at 80° for
1 hr. In each case, unlabeled monosaccharide (0.1 mM) was added prior to hydrolysis. The hydrolysate was neutralized with solid NaHCO₃ and deionized by passage through a mixed bed of AG-501-X8 resin (Bio-Rad Laboratories, Richmond, Calif.). The eluents from the columns were lyophilized, reconstituted in 50 μl of 50% ethanol, and applied as a band on S & S orange ribbon papers. Reactions for N-acetylglucosaminyltransferases were stopped after 6 hr by boiling at 100°C for 5 min, and the product was dialyzed against 0.1 mM sodium acetate buffer, pH 4.0, for 16 hr. The hydrolysis of the product was accomplished by 1 unit of β-N-acetylglucosaminidase in 0.1 mM sodium acetate buffer, pH 4.0, for 200 hr at room temperature by continuous rotation in a Fisher Model 343 Roto-Rack (Fisher Scientific Co.). The residual protein was precipitated by the addition of an equal volume of cold ethanol containing 0.2 mM N-acetylglucosamine. The supernatant was separated by centrifugation and dried by the passage of nitrogen, the residue obtained was reconstituted and banded on papers as above. All paper chromatograms were developed with Solvent B, and radioactivity on the paper was determined as above for pyrophosphatase assay. Reference-unlabeled sugars were run in parallel, and neutral sugars on papers were detected by alkaline AgNO₃ reagent (37), while sialic acid and N-acetylglucosamine were by periodate resorcinol (20) and Elson-Morgan (29) reagents, respectively.

**SDS-Gel Electrophoresis of the Products of Glycosyltransferases.** The incubation conditions were changed from those described in Table 1 in order to obtain sufficient radioactivity for counting. Incubations were run in duplicate, and total volume in each case was 50 μl. Unlabeled nucleotide sugars were replaced by 10 to 25 μl of radioactive compounds. For sialyltransferase CMP-[4, 5, 6, 7, 8, 9-14C]sialic acid was used. Concentrations of acceptor proteins were also cut down to 100 μg/tube. After 16 hr of incubation at 37°C, the reaction of one set of tubes was terminated by phosphotungstic acid-HCl, and the radioactivity of the precipitate was determined as described earlier (6). The second set of tubes were chilled, and the products were dialyzed at 0–2°C for 16 hr first against 0.1 mM NaCl, followed by distilled water. The dialyzed products were lyophilized, and 100 μl of a solution containing 4 mM urea, 0.01 sodium phosphate buffer (pH 7.0), 0.1% SDS, and 1% β-mercaptoethanol were added to the contents of each tube. Incubation at 37°C was then done for 3 hr for the denaturation of the proteins. One drop of glycerol and 2 μl of bromophenol blue (5%) were added to the tubes, and 50-μl aliquots were placed on each gel (50 μg protein per gel). The lengths of the gels were 7.2 cm, and they were made up of 10% polyacrylamide containing 6 mM urea, 0.01 mM sodium phosphate buffer (pH 7.0), and 0.1% SDS. Electrophoresis buffer was 0.01 mM sodium phosphate (pH 7.0) containing 0.1% SDS. The time of run was 7 hr at 20 V with a current of 1 mA/gel. Ovalbumin, fetuin (–NANA), fetuin (–NANA, galactose), and RNase (100 μg each) were denatured as described above for radioactive products and run in parallel tubes as standards. The gels containing radioactive products were fractionated by a liquid column Aliquogel fractionator (Gibson Electronics, Inc., Middleton, Wis.), and 2-mm slices were collected in liquid scintillation vials. Radioactivity was determined after the addition of 1 ml of H₂O and 10 ml of Aquasol 2 (New England Nuclear, Boston, Mass.). The standard proteins were fixed for 1 hr with 12.5% trichloroacetic acid and stained by Coomassie brilliant blue. Destaining was done by diffusion against 50% methanol:7.5% acetic acid for 2 hr with 4 changes at 30-min intervals and finally against 5% methanol:7.5% acetic acid overnight.

**RESULTS**

**Effect of Pyrophosphatase on Glycosyltransferase.** Homogenates from normal as well as malignant ovaries had varying levels of pyrophosphatases. These enzymes interfered with the assay of glycosyltransferases in these crude extracts since they share the same nucleotide sugar substrates. In general, the normal ovaries had higher activities of pyrophosphatases than did the malignant ones. These enzymes differed in their affinities for the nucleotide sugars, the UDP-N-acetylgalactosamine being the most preferred substrate while CMP-NANA was the least preferred. In an initial experiment when N-acetylglucosaminyltransferase was determined in the homogenates from each of a normal and malignant ovary using a substrate concentration of 20 μM, the activity in the malignant tissue was 3- to 4-fold higher. Increasing the substrate concentration to 1 mM closed up this difference, and by the inclusion of 1 mM NAD⁺ in the incubation media, the enzyme levels became comparable in the 2 homogenate preparations. Further increase of the concentration of NAD⁺ was, however, inhibitory in both preparations. Effect of a number of nucleotides on the hydrolysis of the donor substrate was examined for all the glycosyltransferases, and the results for representative assays are presented in Table 2. Hydrolysis of CMP-NANA was less than 20% under the assay conditions. Better protection of the substrate could not be achieved by addition of nucleotides such as CTP. Action of pyrophosphatases on other nucleotide sugar could be minimized by the inclusion of low concentrations of nucleotides and by using minimum amounts of enzyme protein. With galactosyltransferase at 15 μM UDP-galactose concentration, the ATP requirement was marginal (6), but at 7 μM, ATP significantly stimulated the activity of this enzyme. Using 100 μM UDP-galactose together with 1 mM ATP, sub-

### Table 2

**Hydrolysis of nucleotide sugars by normal and malignant ovarian homogenates and its inhibition by various nucleotides**

<table>
<thead>
<tr>
<th>Nucleotide sugar</th>
<th>% of residual substrate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Pyrophosphatase inhibitor&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Normal</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDP-fucose (3 μM)</td>
<td>None</td>
<td>None</td>
<td>41</td>
<td>64</td>
</tr>
<tr>
<td>GDP-fucose (3 μM)</td>
<td>NAD⁺</td>
<td>NAD⁺</td>
<td>50</td>
<td>69</td>
</tr>
<tr>
<td>GDP-fucose (12 μM)</td>
<td>None</td>
<td>NAD⁺</td>
<td>67</td>
<td>73</td>
</tr>
<tr>
<td>GDP-fucose (12 μM)</td>
<td>NAD⁺</td>
<td>NAD⁺</td>
<td>94</td>
<td>88</td>
</tr>
<tr>
<td>GDP-fucose (12 μM)</td>
<td>None</td>
<td>None</td>
<td>82</td>
<td>83</td>
</tr>
<tr>
<td>CMP-NANA (1 mM)</td>
<td>CTP</td>
<td>CTP</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>UDP-N-acetylglucosamine (20 μM)</td>
<td>None</td>
<td>None</td>
<td>31</td>
<td>50</td>
</tr>
<tr>
<td>UDP-N-acetylglucosamine (20 μM)</td>
<td>NAD⁺</td>
<td>NAD⁺</td>
<td>39</td>
<td>64</td>
</tr>
<tr>
<td>UDP-N-acetylglucosamine (1 mM)</td>
<td>None</td>
<td>None</td>
<td>75</td>
<td>79</td>
</tr>
<tr>
<td>UDP-N-acetylglucosamine (1 mM)</td>
<td>NAD⁺</td>
<td>NAD⁺</td>
<td>91</td>
<td>96</td>
</tr>
<tr>
<td>UDP-galactose (7 μM)</td>
<td>None</td>
<td>None</td>
<td>23</td>
<td>37</td>
</tr>
<tr>
<td>UDP-galactose (7 μM)</td>
<td>ATP</td>
<td>ATP</td>
<td>46</td>
<td>51</td>
</tr>
<tr>
<td>UDP-galactose (0.1 mM)</td>
<td>None</td>
<td>None</td>
<td>69</td>
<td>72</td>
</tr>
<tr>
<td>UDP-galactose (0.1 mM)</td>
<td>ATP</td>
<td>ATP</td>
<td>71</td>
<td>76</td>
</tr>
</tbody>
</table>

<sup>a</sup> Concentration was 1 mM when present.
<sup>b</sup> Percentage of the total radioactivity recovered from the chromatogram, which ranged from 50 to 70% of the added counts.
ststantial protection of the substrate and maximum activity of the enzyme were achieved.

In serum, pyrophosphatase levels were low; nevertheless, these nucleotides stimulated the activity of glycosyltransferases to some extent. This stimulation was large for N-acetylglucosaminyltransferase.

Mixing experiments with a few samples of normal and malignant ovarian homogenates and sera demonstrated that the effect of pyrophosphatas could be completely eliminated by the addition of these nucleotides, and no other inhibitor or activator of the glycosyltransferases was present in the crude tissue extracts or in the serum samples.

Composition of Incubation Media for the Assay of Glycosyltransferases. Incubation conditions and the composition of the reaction mixture are presented in Table 1. Under these conditions, the reaction rates were linear and were dependent only on the amount of added enzymes. Consumption of donor nucleotide sugar was within 20 to 30% of the added amount. With these concentrations of acceptors (Table 1), the reaction did not plateau but increased very slowly with additional acceptors. Endogenous activities (i.e., activities in the absence of added acceptors) were kept to a minimum by using low concentrations of enzyme proteins and varied as follows: galactosyltransferase, 0.03 to 0.1% in tissues and sera; sialyltransferase, 2 to 5% in tissues and sera; fucosyltransferase 1, 7 to 13% in tissues, 4 to 5% in sera; fucosyltransferase 2, 20 to 30% in tissues and 30 to 40% in sera; N-acetylglucosaminyltransferase 1, 13 to 31% in tissues, 9 to 13% in sera; and N-acetylglucosaminyltransferase 2, 28 to 37% in tissues and 8 to 11% in sera.

Galactosyl and the 2-N-acetylglucosaminyltransferases had absolute requirement for Mn^{2+}; sialyl- and 2 fucosyltransferases were not stimulated by Mn^{2+}, Mg^{2+}, or Mn^{2+} plus Mg^{2+}. EDTA inhibited the activity of 2 fucosyltransferases by about 30 to 40% suggesting that some endogenous divalent cation may be involved in fucose transfer reactions. No such effect of EDTA (up to 25 mM) was observed for sialyltransferase.

All the glycosyltransferases have rather broad pH optima (6 to 7.5); the maximum activities were obtained near neutral pH, except for galactosyltransferase (pH 6.2).

The amount and nature of nucleotides used as pyrophosphatase inhibitors are important. Concentrations of nucleotides higher than 1 to 2 mM were inhibitory for all the glycosyltransferases. Even at low concentrations 2 fucosyltransferases were inhibited by guanosine nucleotides, whereas galactosyl and N-acetylglucosaminyltransferases were inhibited by uridine nucleotides.

Triton X-100 (0.5%) stimulated the activity of glycosyltransferases in the malignant and normal ovarian tissues by 2- to 3-fold, suggesting that these enzymes are membrane bound. Enzymes present in the serum were either unaffected or only marginally stimulated by the presence of 0.5% triton X-100 in the reaction mixture.

Glycosyltransferases in the Homogenates from Normal and Malignant Ovarian Tissues. In order to compare the role of glycoprotein synthesis in normal and malignant ovarian tissues, glycosyltransferases were assayed in homogenates prepared from about 20 patients with ovarian epithelial carcinoma. Description of these patients has been presented in Table 3. The normal ranges of the enzymes in the ovary were established by assay of about 20 histologically confirmed normal samples (different patients). The upper limit of the normal range was set at 2 S.D. higher than the normal mean. Results shown in Table 4 indicate that only galactosyl- and sialyltransferases were high in all the specimens analyzed. Among other transferases, fucosyltransferase 1 was high in 86%, fucosyltransferase 2 was high in 45%, N-acetylglucosaminyltransferase 1 was high in 53%, and N-acetylglucosaminyltransferase 2 was high in 10% of the tissues examined. It should, however, be noted that the mean specific activities of all the glycosyltransferases in malignant tissues were higher than normal values. Mixing experiment data ruled out the possibility that the differences in the activities of these enzymes in normal and malignant tissues were caused by the presence of activators or inhibitors of glycosyltransferases in the crude homogenate preparations.

Glycosidases in the Homogenate from Normal and Malignant Ovarian Tissues. In order to compare the extent of glycoprotein degradation in the normal and malignant ovarian tissues 4 glycosidases were measured in these homogenate preparations. Results presented in Table 5 show that a significant elevation of N-acetyl-β-D-glucosaminidase was found in 85% of the tumor samples, followed by N-acetyl-β-D-galactosaminidase (63%), β-D-galactosidase (50%), and α-L-fucosidase (35%). Mean values of all the glycosidases were, however, high in malignant tissues compared with normal.

It has been suggested that at least a part of the glycosyltransferases are associated with cellular plasma membrane as ectoenzyme (14–16, 34). Plasma membrane constituents are shed, both in vivo and in vitro, into the surrounding media as a result of the turnover of these membranes (11). Since cancer cells multiply and turn over more rapidly than do normal cells, it is conceivable that some of these constituents will be found in the host sera. Alternately, leakage of the enzyme from either intact or lysed tumor cells into the systemic circulation will also result in the elevation of glycosyltransferases in the patient’s sera (6). Any such elevation can thus be used as a biochemical indicator of malignancy.

Glycosyltransferase Activities in the Sera of Normal Controls and Cancer Patients. Normal levels of the glycosyltrans-
ferases in the sera were first established in 30 healthy donors ranging in age from 22 to 77 years with a median age of 43. The blood group distribution in this control group of women was as follows: A\textsuperscript{Rh+}, 9; A\textsuperscript{Rh-}, 1; B\textsuperscript{Rh+}, 4; O\textsuperscript{Rh+}, 13; and O\textsuperscript{Rh-}, 3. The median age of the cancer patients was 57 years, slightly higher than the control group, and the distributions of the major blood groups were similar (Table 3). Among these groups of patients, 25 had advanced disease (Stages III and IV), 4 had early stage disease (Stages I and II), while one had a recurrent borderline malignancy. The upper limit of normal was again set at 2 S.D. higher than the normal mean. Results summarized in Table 6 show that only the level of galactosyltransferase was consistently elevated in the sera of all the ovarian cancer patients. Two N-acetylglucosaminyltransferases were elevated in 80 to 90% of these patients. Although sialyltransferase was high in all the tumor tissues, only 20% of the patients had an elevation of this enzyme in the serum. On the other hand, only 10% of the tumor samples had high activity for N-acetylglucosaminyltransferase 2, but 80% of the patients' sera had enhanced enzymatic activity. Similarly, no correlation of corresponding values was found when a comparison between tissue and serum levels was made for fucosyltransferase 1 or N-

### Table 4
Glycosyltransferases in homogenates from normal and malignant ovariies
Incubation conditions have been described in Table 1 and in "Materials and Methods". Endogenous values were subtracted.

<table>
<thead>
<tr>
<th>Transferase</th>
<th>Normal</th>
<th>Malignant</th>
<th>% high in tumor*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fucosyl-1-b</td>
<td>Range 35-176</td>
<td>Mean ± S.D. 96 ± 45</td>
<td>20</td>
</tr>
<tr>
<td>Fucosyl-2-b</td>
<td>20-65</td>
<td>36 ± 11 20</td>
<td>15</td>
</tr>
<tr>
<td>Sialyl-b</td>
<td>161-279</td>
<td>230 ± 42 15</td>
<td>45</td>
</tr>
<tr>
<td>Galactosyl-b</td>
<td>0.6-1.8</td>
<td>1.1 ± 0.3 22</td>
<td>100</td>
</tr>
<tr>
<td>N-Acetylglucosaminyl-1-b</td>
<td>359-850</td>
<td>519 ± 85 16</td>
<td>53</td>
</tr>
<tr>
<td>N-Acetylglucosaminyl-2-b</td>
<td>285-574</td>
<td>384 ± 71 16</td>
<td>10</td>
</tr>
</tbody>
</table>

* Higher than normal mean + 2 S.D.

### Table 5
Glycosidases in normal and malignant ovarian tissues
Assay conditions have been described under "Materials and Methods."

<table>
<thead>
<tr>
<th>Glycosidases</th>
<th>Normal</th>
<th>Malignant</th>
<th>% high in tumor*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>Range 0.09-0.45</td>
<td>Mean ± S.D. 0.22 ± 0.08</td>
<td>20</td>
</tr>
<tr>
<td>N-Acetyl-α-galactosaminidase</td>
<td>0.025-0.19</td>
<td>0.09 ± 0.05 16</td>
<td>63</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>0.06-0.17</td>
<td>0.10 ± 0.04 16</td>
<td>50</td>
</tr>
<tr>
<td>a-L-Fucosidase</td>
<td>0.02-0.11</td>
<td>0.06 ± 0.03 17</td>
<td>35</td>
</tr>
</tbody>
</table>

* Higher than normal mean + 2 S.D.

### Table 6
Glycosyltransferases in the serum
Incubation conditions have been described in Table 1 and under "Materials and Methods."

<table>
<thead>
<tr>
<th>Transferases</th>
<th>Control group</th>
<th>Cancer patients*</th>
<th>Cured patients*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fucosyl-1-b</td>
<td>Range 77-471</td>
<td>Mean ± S.D. 256 ± 110</td>
<td>124 ± 38 0</td>
</tr>
<tr>
<td>Fucosyl-2-b</td>
<td>36-104 55 ± 16</td>
<td>240-231 93 ± 39 60</td>
<td>57 ± 20 1</td>
</tr>
<tr>
<td>Sialyl-b</td>
<td>1.94-9.02 4.83 ± 1.57</td>
<td>4.86-17.91 7.73 ± 2.41 20</td>
<td>5.68 ± 1.77 1</td>
</tr>
<tr>
<td>Galactosyl-b</td>
<td>19-39 29 ± 6</td>
<td>43-78 51 ± 6 100</td>
<td>28 ± 4 0</td>
</tr>
<tr>
<td>N-Acetylglucosaminyl-1-b</td>
<td>6-12 8.2 ± 1.4</td>
<td>7-23 14 ± 3.7 90</td>
<td>8.1 ± 1.0 0</td>
</tr>
<tr>
<td>N-Acetylglucosaminyl-2-b</td>
<td>0.46-1.31 0.82 ± 0.29</td>
<td>0.79-2.51 1.55 ± 0.33 80</td>
<td>0.68 ± 0.11 0</td>
</tr>
</tbody>
</table>

* Detailed descriptions of these patients are presented in Table 3.

* These patients (n = 10) had no clinical evidence of disease for 6 to 12 months prior to assay.

* Higher than normal mean + 2 S.D.

* pmol/hr/ml serum.

* nmol/hr/ml serum.
acetylglucosaminyltransferase 1.

Glycosyltransferases were also determined in a group of 10 ovarian cancer patients who had no clinical evidence of disease for at least 6 to 12 months prior to the assay. Data in Table 6 for this group indicate that except for fucosyltransferase 2 and sialyltransferase, the serum levels of these enzymes never exceeded the upper limit of the normal range. The mean values of fucosyltransferase 1 and N-acetylglucosaminyltransferase 2 were even lower than the normal value.

No significant effect of age, blood group, sex, or ethnic origin of the donors was noted on their serum glycosyltransferase activity. Higher median age and the slightly different distribution of blood group in the cancer patients, compared to the normal group, could not cause such a marked difference in serum enzyme levels.

Identification of the Monosaccharide Transferred to the Acceptor. It was felt necessary to determine whether the monosaccharide was transferred unaltered to the macromolecular acceptor from its nucleotide sugar. Besides, contamination of the nucleotide sugar may give misleading results. For example, ovalbumin can act as an acceptor for both galactose and N-acetylglucosamine. Since we had to use high concentrations of UDP-N-acetylglucosamine for the assay of N-acetylglucosaminyltransferase 1 to obtain measurable radioactivity, slight contamination of the labeled substrate by labeled UDP-galactose may result in substantial incorporation of galactose into ovalbumin. This may be the explanation for the elevation of N-acetylglucosaminyltransferase 1 in a high percentage of serum samples from cancer patients. This, however, cannot explain the elevation of N-acetylglucosaminyltransferase 2, since RNase A cannot act as an acceptor for galactose.

Products of the glycosyltransferases were hydrolyzed either by acid or by enzymatic methods, and the liberated monosaccharides were analyzed by paper chromatography. Results with the tumor extracts are presented in Chart 1. Essentially, a single peak of radioactivity was obtained in each case in the expected positions. In chromatograms for N-acetylglucosaminyltransferases 1 and 2, respectively, 20 and 40% of the radioactivity were present in the origin. In these 2 cases, hydrolysis was enzymatic which probably was not complete even in 200 hr.

Qualitatively similar results were obtained when enzymes from normal ovarian extracts or sera, from either cancer patients or controls, were used as the enzyme source.

Identification of the Macromolecular Products of Glycosyltransferases by SDS-Polyacrylamide Gel Electrophoresis. The products of all the glycosyltransferases were nondialyzable and are precipitated by phosphotungstic acid. The SDS-polyacrylamide gel electrophoresis profile obtained with the serum of a cancer patient is shown in Chart 2. The radioactivity was not released under denaturing conditions, and more than 80% of phosphotungstic acid-precipitable radioactivity could be recovered from the macromolecular regions of the gels. Commercial fetuin (Chart 2, third from top) contains multiple bands of proteins. During preparation of acceptors from fetuin, further degradation takes place. These degradation products are, however, able to accept monosaccharides from nucleotide-sugar donors. The radioactive peaks coincided only with the strongly stained protein bands for fetuin derivatives. Ovalbumin and RNase A were essentially free of contaminating proteins. The radioactive peak obtained by using ovalbumin as acceptor coincided with the major protein band, but products with RNase A showed 3 to 4 additional peaks, beside the major peak, which occupied the same position as RNase A. Radioactive peaks preceding the position of bromophenol blue (downward arrows) were probably due to residual labeled nucleotide-sugars, which had similar mobility under these conditions.

In this serum sample, endogenous activity (i.e., activity in the absence of added acceptor) for fucosyltransferase 2 was 35% of that in the presence of fetuin (—NANA). Interestingly, no other peak of radioactivity was obtained on electrophoresis. The situation was the same for other glycosyltransferases with the exception of N-acetylglucosaminyltransferase 2. Similar SDS-polyacrylamide gel electrophoresis patterns were obtained using homogenates from normal and malignant ovaries as well as normal sera. All these observations suggest that the endogenous activity was either due to insignificant incorporation into several glycoproteins or that it did not take place at all in the presence of exogenous acceptor in large excess.

DISCUSSION

Alterations of the levels of glycosyltransferases have been
Glycoprotein Metabolism in Ovarian Carcinoma

Fucosyltransferase 2, which is responsible for linking fucose with galactose by (1 → 2) linkage, had high activity in 45% of the specimens. When N-acetylgalcosaminyltransferase was assayed using ovalbumin as acceptor (N-acetylgalcosaminyltransferase 1), the transfer of N-acetylgalcosamine to the peripheral mannose and N-acetylgalcosamine residues of ovalbumin. N-Acetylgalcosaminyltransferase 2 has been assayed using RNase A as acceptor. This enzyme builds the first carbohydrate-peptide bond of the glycoprotein by linking N-acetylgalcosamine with asparagine (23). The levels of N-acetylgalcosaminyltransferases 1 and 2 were elevated, respectively, in 53 and 10% of the tumor samples. Designation of these enzymes should, however, be considered tentative, since rigorous structural investigation of the products has not been performed. The activities of enzymes responsible for transfer of monosaccharides to the terminal nonreducing end of the glycoconjugates were higher in the malignant tissues, whereas those building the core regions were not. Fucosyltransferase 2 is an exception to this generalization, but its activities are weak also in other human sources (36), suggesting less frequent occurrence of glycopeptides in which fucose is linked to galactose by (1 → 2) linkage.

Conflicting results were also reported from different laboratories on the alterations of glycosidases in the various human tumor tissues (8, 10, 25, 26). In ovarian carcinoma, significant elevation of N-acetylglucosaminidase was observed in 85% of the tumor samples followed by N-acetylgalactosaminidase (63%) and β-galactosidase (50%). The mean values of these enzymes in the neoplastic tissue are high in every case compared to controls, suggesting greater turnover of glycoproteins in tumors.

One should use caution in comparing these data with solid tumors since a number of factors may effect the results. These are heterogeneity of the cell population, presence of cells in different cell cycle phases, contamination by some normal and necrotic tissue materials, and the presence of macrophages and other leukocytes.

Elevation of various glycosyltransferases in the sera and ascitic fluids of animals bearing tumors (9, 31) as well as in the plasma and sera of cancer patients (4, 18, 19, 21, 24, 39) has been reported from a number of laboratories. Weiser et al. (39) found that on the average the total serum galactosyltransferase activity in cancer patients was only slightly higher than that of the control subjects. However, by discontinuous polyacrylamide gel electrophoresis, they detected an isoenzyme of galactosyltransferase in the sera of 43 of 58 patients with various types of cancers. Among that group, only one had ovarian cancer, and she was negative for the cancer-associated isoenzyme (39). In the sera of patients with metastatic breast cancer, fucosyl- and galactosyltransferases showed considerable overlap between the controls and cancer patients. Marked elevation of sialyltransferase, however, was observed in 48 patients with metastatic breast cancer (19). In our earlier study (6), serum galactosyltransferase was assayed in 11 patients with ovarian epithelial carcinomas; all of them had significantly high levels (2 S.D. higher than normal mean) of this enzyme. In this group of 30 patients, similar consistent elevation of the galactosyltransferase in the serum was observed. In contrast to breast cancer patients (19), no marked elevation of sialyltransferase was found in the ovarian cancer patients.

Chart 2. SDS-polyacrylamide gel electrophoresis pattern of the products of glycosyltransferases. Results obtained with a serum from a cancer patient are presented. Details of the procedures are included under "Materials and Methods." Photograph of the stained gels are included; they are (from the top) RNase A, ovalbumin, native fetuin, fetuin (−NANA), and fetuin (−NANA, galactose).

In all the ovarian epithelial tumors examined, we found a significant elevation of the activities of galactosyl- and sialyltransferases in comparison with normal tissue. Levels of fucosyltransferase 1, which transfers fucose to the N-acetylgalcosaminyl residue of fetuin (−NANA, galactose), were high in 86% of the tumors. Fucosyltransferase 2, which is responsible for linking fucose with galactose by (1 → 2) linkage, had high activity in 45% of the specimens. When N-acetylgalcosaminyltransferase was assayed using ovalbumin as acceptor (N-acetylgalcosaminyltransferase 1), the transfer of N-acetylgalcosamine to the peripheral mannose and N-acetylgalcosamine residues of ovalbumin. N-Acetylgalcosaminyltransferase 2 has been assayed using RNase A as acceptor. This enzyme builds the first carbohydrate-peptide bond of the glycoprotein by linking N-acetylgalcosamine with asparagine (23). The levels of N-acetylgalcosaminyltransferases 1 and 2 were elevated, respectively, in 53 and 10% of the tumor samples. Designation of these enzymes should, however, be considered tentative, since rigorous structural investigation of the products has not been performed. The activities of enzymes responsible for transfer of monosaccharides to the terminal nonreducing end of the glycoconjugates were higher in the malignant tissues, whereas those building the core regions were not. Fucosyltransferase 2 is an exception to this generalization, but its activities are weak also in other human sources (36), suggesting less frequent occurrence of glycopeptides in which fucose is linked to galactose by (1 → 2) linkage.

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A comparison between tissue and serum levels showed a correlation of corresponding values for only galactosyltransferase. It is possible that sialyl- and fucosyltransferase 1, which have high tissue content, are either not released into the serum, are inactivated, or are eliminated from the serum at a rapid rate. N-Acetylglucosaminyltransferases have high activity in a small percentage of tissue samples, but their serum levels were high in 80 to 90% of the ovarian cancer patients. These enzymes are probably stable in the serum and are only slowly eliminated from the system.

At least a part of the galactosyltransferase resides on the outside of the cell surface as an ectoenzyme, where it may be responsible for the biosynthesis and repair of the carbohydrate portion of the membrane glycoconjugate (34). One mechanism for the appearance of this enzyme in the serum may be by the shedding of the plasma membrane constituents into the systemic circulation of the host (11, 14-16). Alternately, it may be leaked from intact tumor cells due to overproduction or released from the lysed tumor cells (6).

Kim et al. (27) proposed that serum galactosyltransferase originates from the liver and that an abnormal rise in the level of this enzyme in the serum may be due to hepatocellular damage. Among this group of 30 patients, 4 had early stage disease without liver involvement. Also, in a number of patients with advanced disease, the liver was not involved at the time of laparotomy. Levels of other serum constituents such as total bilirubin, alkaline phosphatase, lacti dehydrogenase, etc., varied during treatment, but their values rarely correlated with the elevation of galactosyltransferase in sera of the cancer patients.

In progress is the isolation of the different isoenzymes of this enzyme in the serum. This hopefully will eventually lead to the development of a diagnostic test for ovarian carcinoma based on the assay of galactosyltransferase.

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REFERENCES


Glycosyltransferase and Glycosidase Activities in Ovarian Cancer Patients

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