Correlation of Serum, Tumor, and Liver Serum Glycoprotein: N-Acetylneuraminic Acid Transferase Activity with Growth of the R3230AC Mammary Tumor in Rats and Relationship of the Serum Activity to Tumor Burden

Irene M. Evans, Russell Hilt, Michael Murphy, and H. Bruce Bosmann

Departments of Pharmacology and Toxicology (I. M. E., H. B. B.) and of Biochemistry (R. H., M. M.) and the University of Rochester Cancer Center (I. M. E., R. H., H. B. B.), University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

ABSTRACT

The observation that the activity of sialyltransferase (EC 2.4.99.1; serum glycoprotein-N-acetylneuraminic acid transferase) is often elevated in the serum of cancer patients necessitates an elucidation of the interrelationships of this serum enzyme with host tissues. Accordingly, the activity of this enzyme in serum, tumor, and liver was determined at various times after implantation of the R3230AC mammary carcinoma into Fischer rats. Results from samples obtained at numerous, sequential time points demonstrated that significant elevations in serum sialyltransferase enzyme activity occurred only in animals bearing large tumor burdens, i.e., greater than 20 g, or in animals with tumors present for longer than 21 days. In these tumor-bearing rats, the activity of sialyltransferase increased in liver tissue at 21 to 25 days concurrently with the increase in serum enzyme activity, suggesting that the liver may be a potential source of the serum enzyme. Sialyltransferase activity in tumor tissue was quite variable; the activity increased one week after tumor implantation and remained at the same level thereafter. When tumors were excised, the activity of the serum enzyme returned to control values within four days after surgery, suggesting that the half-life of serum sialyltransferase was two days. Serum enzyme levels were again elevated upon regrowth of the tumor. These results show that the serum sialyltransferase alters its activity in conjunction with changes in tumor burden.

INTRODUCTION

Glycosyltransferases transfer monosaccharides or oligosaccharides from "activated" nucleotide sugar substrates or lipid intermediates onto a growing protein, glycoprotein, or glycolipid molecule. The levels of several glycosyltransferase enzymes have been reported to be abnormal in cancer patients and in animals bearing tumors. It is possible that advantage might be taken of the alterations in these enzyme levels to monitor cancer patients for the determination of the extent of cancerous disease, the presence of metastases, and the clinical course of their disease.

Several investigators have reported elevations in serum activity of glycosyltransferases in both human (2, 3, 6, 11-14, 18, 20, 25-33, 35, 36, 44) and animal (5, 8, 10, 24, 36) tumor-bearing hosts. Some of these studies (25, 26, 37) suggest that, while cancer patients often have elevated serum galactosyl or sialyltransferase levels, some patients have levels of these enzymes that are only slightly elevated or even overlap normal control values. However, in one of these studies (25), non-overlapping elevated serum sialyltransferase levels were measured in breast cancer patients whose disease had progressed to metastasis, suggesting that measurement of serum sialyltransferase enzyme levels might be useful in diagnosing and monitoring patients with metastatic cancer.

In a detailed study of one patient with testicular cancer, Kessel et al. (29) demonstrated that serum sialyltransferase activity decreased when the patient entered remission and increased again with recurrence. In another study, Bauer et al. (3) suggested that fucosyltransferase enzyme levels might be useful in characterizing and staging tumor growth and in evaluating treatment efficacy. Similar findings have been reported by Ganzinger and Moser (18).

Although most investigators agree that transferase activities are often elevated in cancer patients, they differ regarding the extent of and conditions responsible for this elevation. Because of the limitations on human experimentation and because some animals show serum enzyme increases associated with the presence of tumors, it seemed logical that valuable information could be gained from studies of a suitable tumor-bearing animal model in which the relationship between the presence of a tumor and the increased activity of serum glycosyltransferases, as well as the factors influencing the amount of enzyme activity, could be determined.

Earlier studies in this laboratory demonstrated that serum sialyltransferase activity was increased in rats bearing transplantable or induced mammary tumors (9), results confirmed by Ip and Dao (24). The causes of the increased serum sialyltransferase activity, however, were not investigated. Is the enzyme released or "shed" from the growing tumor mass, as several investigators have suggested (6, 9, 37), or is the enzyme activity derived directly or indirectly from the liver or other organs, as others have suggested (10, 24)? We attempted to answer these and other questions through a detailed time analysis of sialyltransferase activity in the serum, liver, and tumor tissue of rats bearing the R3230AC mammary tumor. We found that, 21 days after tumors were transplanted, sialyltransferase activity was increased in both the serum and the liver of these rats, whereas the specific activity of the enzyme in the tumor was more variable and appeared to increase only during early stages of tumor growth. These results suggest that the liver of the host animal may be involved in causing the
increase in serum enzyme activity in animals bearing mammary tumors.

To determine the role of the tumor in causing elevations in serum sialyltransferase activity, we investigated the effects of complete or partial removal of the tumor from the host. Within 4 days of removal of the tumors, serum sialyltransferase activity returned to control levels; upon regrowth of the tumor, sialyltransferase activity was elevated again.

MATERIALS AND METHODS

Materials. CMP-[4,5,6,7,8,9-14C]sialic acid (235 mCi/mmol) was purchased from New England Nuclear, Boston, Mass. Fetuin was purchased from Grand Island Biological Co., Grand Island, N. Y., and sialic-free fetuin was prepared as described previously (16). Cyclophosphamide (Mead Johnson Laboratories, Evansville, Ind.) was obtained from the pharmacy at the University of Rochester School of Medicine and Dentistry.

The R3230AC Tumor. The R3230AC mammary tumor is a well-differentiated, slow-growing adenocarcinoma which is transplantaible in Fischer rats (22). For all experiments, tumors implanted 14 days previously were removed from Fischer hosts, dissected into 1-mm pieces in sterile 0.145 M NaCl, and injected in single or double implants into the submaxillary region of 80- to 90- to 5-week-old female Fischer rats with a sterile trocar needle technique (9). Animals were sacrificed beginning the next day (Day 1) and on succeeding days up to 45 days after tumor implantation. Samples of serum, liver, and tumor were collected and assayed as described below. Unsacrificed, tumor-bearing animals usually lived for 50 to 64 days after tumor implantation. The tumor rarely metastasizes from the axillary region (7), and no metastases were found upon gross and microscopic examination of liver and lung tissues from randomly selected animals.

Serum Collection. Blood was obtained by decapitation of animals or by cardiac puncture of animals lightly anesthetized with ether. After being allowed to clot, the blood was centrifuged at 2500 × g, and the serum was removed and assayed immediately.

Liver. The lower right lobe of the liver was removed and placed in ice-cold Tris buffer (0.1 M; pH 7.6; at 4°) for 30 min to allow the diffusion of trapped serum. Two g of liver tissue were excised from the lobe and homogenized in 10 ml of Tris buffer (pH 7.6; 4°) containing 1% Triton X-100 using a motor-driven pestle operated at maximum speed for 2 mm. Fifty ml of this homogenate, containing approximately 20 mg of protein, were then assayed for sialyltransferase activity as described below.

Tumor. Tumors were dissected, and necrotic tissue was discarded. Tumor samples were placed in Tris buffer (pH 7.6; 4° for 30 min) to remove trapped blood, removed, and homogenized in 0.1 M Tris buffer (pH 7.6; 4°) containing 0.1% Triton X-100. Fifty ml of this homogenate (approximately 20 mg of protein) were then assayed for sialyltransferase activity.

Sialyltransferase Assay. Serum sialyltransferase activity was assayed essentially as described previously (9). Each assay tube (total volume, 150 μl) contained: 50 μl of serum (2 to 4 mg of protein); 50 μl of desialylated fetuin (750 μg); 20 μl of 0.1 M Tris buffer (pH 7.6); 20 μl of 0.1 M MnCl2 and MgCl2; and 10 μl of CMP-[N-(acetyl-14C)]neuraminic acid (0.13 nmol; 150,000 cpm). Tubes containing the assay mixture were incubated for 20 min in a shaking water bath at 37°. The reaction was terminated by the addition of 1 ml of 1% phosphotungstic acid in 0.5 M HCl. Samples were centrifuged, and the precipitate was collected and then washed twice with 10% trichloroacetic acid and once with 95% ethanol/ether (2:1, v/v). Precipitates were dissolved in 0.2 ml of 0.1 M NaOH, neutralized with HCl, and counted in Aquasol (New England Nuclear, Boston, Mass.). Radioactivity was determined by counting samples in a Nuclear-Chicago scintillation counter. Counting efficiency for CMP-N-(acetyl-14C)]neuraminic acid was 65 to 75%. The activity of the enzyme using endogenous acceptors was determined by omitting the exogenous fetuin acceptor from the assay mixture. Control values to correct for nonenzymemediated radioactive transfer of N-acetyleneuraminic acid were obtained by incubating samples at 0°, substituting water for enzyme, substituting boiled enzyme extract, or immediately precipitating the assay mixture. With all these procedures, background activity and nonspecific binding were negligible (60 to 80 cpm/mg protein). Enzyme activity due to transfer of sialic acid to exogenous fetuin acceptor was calculated as the difference between the total activity with fetuin acceptor present minus the activity with only endogenous acceptors present and is expressed as cpm of radioactive N-acetyleneuraminic acid transferred per 20-min incubation period per mg of protein. Protein content of the serum sample was determined by the Lowry method as described previously (16).

Conditions of linearity for the serum, tumor, and liver homogenate-mediated enzyme reaction were met at the enzyme substrate concentration used for the 20-min incubation period. The amount of product formed in the reaction was observed to increase in a linear manner for up to 1 hr when 50 to 80 mg of protein (serum, liver, or tumor homogenate) were added to the assay mixture (data not shown). Optimal conditions for assay of the serum enzyme were met with regard to pH of buffer, ion requirements, and acceptor substrate concentration. The amounts of CMP-[14C]sialic acid used in the assay were limited by high cost and the nonavailability of commercial, nonradioactive CMP-sialic acid; the concentrations used, however, allowed the reactions to continue in a linear fashion for up to 1 hr. Since the concentration of CMP-sialic acid substrate used is well below the Km values reported for sialyltransferases (10-5 to 10-6 M), the enzyme activities measured may not be the Vmax rate. Reactions measuring activity of endogenous sialyltransferase activity, in which desialylated fetuin was not added, were also found to be linear for up to 2 hr, indicating that endogenous acceptors were not limiting.

The conditions utilized for assay of the serum enzyme are often, but not always, the same conditions of pH, ions, etc., that are optimal for the liver and tumor enzymes. However, since these enzymes showed broad and biphasic pH optima when determinations were run using tumor and liver tissue homogenate samples, the assay conditions were generally standardized to those maximal for detection of the serum enzyme. An exception was the addition of Triton X-100 at 0.1 and 1.0% for tumor and liver samples, respectively, since these tissue homogenates showed limited exogenous activity in the absence of detergent.

Hydrolysis and Breakdown of the CMP-N-acetyleneuraminic Acid Substrate. Breakdown of radioactive CMP-N-acetyl-

* I. Evans, R. Hill, and H. B. Bosmann, unpublished data.
neuraminic acid was determined by incubation of the substrate in the assay system described above, using identical conditions of temperature and time. The 200 μl of incubation medium were then precipitated with 500 μl of 95% ethanol to terminate hydrolysis. After centrifugation at 2500 x g to remove particulate material, 50 μl of the supernatant were spotted onto Whatman no. 1 chromatography paper. The samples were allowed to separate overnight in a solution of 1 M ammonium acetate:95% ethanol (3:7, v/v). Radioactive CMP-N-acetylneuraminic acid and N-acetyleneuraminic acid were used as standards. Chromatograms were cut up, and radioactivity was determined by liquid scintillation counting.

**Determination of Neuraminidase Activity.** Neuraminidase activity of control and tumor samples was determined using [3H]neuraminylactitol (New England Nuclear, Boston, Mass.; 52.2 Ci/mol; 245,000 cpm) as substrate. The incubation assay contained 10 μl of 0.1 M buffer (Tris buffer (pH 7.6) or citrate buffer (pH 4.3)), 10 μl of neuraminylactitol, and 80 μl of sample. The mixture was incubated for 2 hr since previous experiments had shown that enzyme activity remained linear for this period under the above conditions. The amount of breakdown of neuraminylactitol under these conditions without added homogenate was small (<250 cpm). The reaction was terminated by addition of 2 ml of glass-distilled water at 4°. An aliquot of the assay mixture was chromatographed on a Dowex AG 1-X8 column (inner diameter, 0.5 cm; height, 8 cm). After the sample was applied, free [3H]lactitol was eluted from the column by washing with 18 ml of glass-distilled water at 4°. The radioactivity of the eluate was determined by liquid scintillation counting.

**Animal Surgery.** After tumors had grown for 25 days, all or most of the tumor mass was excised and weighed. For tumor regrowth experiments, 1 to 2 g of nonnecrotic tumor tissue were left unresected to grow and again form a tumor mass. Regrowing tumors had reached a mass of 4 to 6 g by Week 1, 5 to 7 g by Week 2, and 25 to 30 g by Week 3. Sham-operated animals served as controls. Histological examination of tumor sections taken before and after regrowth showed similar morphological characteristics.

**Drug Treatment.** Animals with new implantations of R3230AC tumors or bearing R3230AC tumors for a duration of 25 days were treated with cyclophosphamide daily at 1 mg/100 g of body weight. Control animals (without tumors) received either cyclophosphamide or no treatment. The cyclophosphamide was prepared daily and injected into the rear leg muscles. Lower doses of cyclophosphamide were ineffective in reducing tumor burden, and higher doses killed too many of the tumor-bearing animals.

**RESULTS**

To investigate alterations in sialyltransferase activity, enzyme assays were conducted on serum and homogenates of liver and tumor tissues obtained from rats at various times after tumor implantation, for periods of up to 45 days. The wet weight of the growing tumor mass removed from rats on various days after implantation is shown in Chart 1. Tumor growth followed the general Gompertz pattern analyzed and described by Gompertz (45), in which an initial period of rapid growth is followed by a slower growth rate. The plateau growth period was accompanied by an increase in the amount of necrosis within the tumor mass, indicating that internal cells were dying and probably lysing. The rate of increase for the R3230AC tumor was nearly exponential up to 20 to 24 days after implantation. The density of the tissue (wet weight/mg protein) also remained constant. The pattern of growth observed in these experiments is consistent with that described previously for this tumor (7, 22).

Chart 2 shows the pattern of enzyme activity in the serum, liver, and tumor tissues of animals bearing R3230AC mammary tumors for periods of 1 to 45 days. Both serum and liver sialyltransferase activity increased significantly in animals receiving tumor implantations at least 21 days before sacrifice. Serum sialyltransferase activity in these rats was twice that found in age-matched controls and 3 to 4 times that of controls when the source of the enzyme was liver tissue. Enzyme activity in tumor tissue increased 1 week after tumors were implanted (tumor weight, 1.0 to 2.5 g), but after that, no further significant increase was observed (Chart 2).

These increases in both serum and liver tissue sialyltransferase activity were measured, using exogenous fetuin minus N-acetyleneuraminic acid as acceptor and correcting for sialyltransferase activity due to endogenous acceptors. When sialyltransferase activity was measured using endogenous acceptors, a similar pattern emerged; enzyme activity increased in serum and liver of animals bearing tumors for at least 21 days (Table 1). It is unclear whether the activities measured using endogenous serum or tissue acceptors or detected using our exogenously added fetuin minus N-acetyleneuraminic acid as acceptor were due to the same enzyme or to other molecular species (isozymes) of sialyltransferase.

To determine whether “activators” or “inhibitors” of sialyltransferase activity were present, mixing experiments were performed, using serum or liver preparations from control and tumor-bearing animals. The results (Table 2) demonstrate that the elevation in sialyltransferase activity in samples from tumor-bearing rats was not due simply to the presence of an activator or inhibitor since mixtures of control and experimental samples yielded additive results.
Several investigators (6, 9, 10, 24, 37) have proposed that the elevated levels of sialyltransferase activity in the serum of animals bearing tumors are due to either material and enzyme shed by the tumor directly into the serum or the stress the tumor places on the host, which could affect the biochemistry of the host organs. To determine whether a simple relationship exists between tumor weight or burden and serum sialyltransferase activity, we compared the wet weight of the tumor mass with levels of serum enzyme activity (Chart 3). The serum of animals bearing tumors weighing more than 15 g consistently shows an elevated level of sialyltransferase activity. The magnitude of the increase, which varied between 2- and 12-fold, did not seem to be directly related in a simple manner to the tumor burden; rather, elevations in enzyme activity seem to be detected after some unknown threshold event. Although increases in enzyme activity are consistently measured at large tumor burdens, much variability is found so that measured values are anywhere from 2- to 13-fold higher than those found in control animals. These results suggest that other factors besides tumor burden are important in determining serum levels of sialyltransferase.

Since other enzymes can cleave sialic acid from an acceptor molecule or hydrolyze the CMP-N-acetylneuraminic acid substrate molecule, and since alteration in the activity of these enzymes could affect our estimation of sialyltransferase activity in these tissues, the neuraminidase and CMP-N-acetylneuraminic acid hydrolyase activity of tissue samples was also determined. Neuraminidase activity was determined in tissue samples from animals bearing tumors that had been implanted 24 days before sacrifice. The serum of control and tumor-bearing rats had little or no neuraminidase activity as measured by ability to cleave the artificial substrate, neuraminlactitol (Table 3), but in the liver of the tumor-bearing animals, enzyme activity was twice that in the liver from age-matched, control animals. This finding suggests that the reported sialyltransferase activity could be underestimated since neuraminidase is capable of removing the transferred sialic acid from endogenous acceptor or exogenously added fetuin acceptor. Whether the enzyme activity detected using neuraminlactitol as substrate is capable of cleaving sialic acid from fetuin is not known. Neuraminidase activity in tumor tissue was approximately one-half that found in control liver tissue.

The breakdown of CMP-sialic acid (CMP-hydrolyase activity) was measured in serum, liver, and tumor samples obtained from controls or tumor-bearing rats. There were no significant differences in the amount of breakdown of substrate between serum or liver samples obtained from control animals and those from tumor-bearing animals (Table 4). The amount of substrate breakdown was significantly lower in samples obtained from nonnecrotic tumor tissue.

The effect of changes in tumor burden on serum sialyltransferase activity was investigated. Experiments were performed in which tumor tissue was surgically removed, the wound was closed, and the animal was tested for 1 to 4 days thereafter to determine serum sialyltransferase activity. Values obtained from animals bearing unresected tumors, animals in which tumors were surgically removed, sham-operated controls, and age-matched nontumor-bearing animals were compared (Chart 4). No change in serum sialyltransferase activity after surgery was detected 1 day postsurgery, and in only a few animals was there a slight, nonsignificant increase by Day 2. By Day 3, all tumor-bearing animals in which tumors were surgically excised showed a significant decrease in serum sialyltransferase activity; and by Day 4, the levels of enzyme activity in this group of animals had returned to control values.

The activity of the enzyme for endogenous acceptor molecules was also monitored. As can be observed in Table 5 and as reported previously (9), the activity of the enzyme using only endogenous acceptors was elevated in animals bearing tumors 20 to 25 days after implantation. Although there was a decline in enzyme activity after surgical removal of the tumor, the low values of enzyme activity detected and the relatively higher variation in experimental results make this serum assay with
Endogenous sialyltransferase activity at various times after R3230AC tumor implantation

Assays were performed in triplicate with tissue samples obtained from groups of from 2 to 10 control or tumor-bearing rats tested on at least 3 separate occasions.

<table>
<thead>
<tr>
<th>Days post-implantation</th>
<th>Range of tumor wt (g)</th>
<th>No. of animals</th>
<th>Serum (cpm/mg protein)</th>
<th>Liver (cpm/mg protein)</th>
<th>Tumor (cpm/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>Tumor</td>
<td>Control</td>
</tr>
<tr>
<td>1–5</td>
<td>&lt;1</td>
<td>30</td>
<td>56.1 ± 3.6</td>
<td>54.0 ± 4.4</td>
<td>1420 ± 61</td>
</tr>
<tr>
<td>6–11</td>
<td>1–3</td>
<td>26</td>
<td>61.0 ± 6.2</td>
<td>61.3 ± 3.6</td>
<td>1385 ± 157</td>
</tr>
<tr>
<td>11–15</td>
<td>3–7</td>
<td>18</td>
<td>64.3 ± 3.5</td>
<td>52.2 ± 3.1</td>
<td>1388 ± 60</td>
</tr>
<tr>
<td>16–20</td>
<td>7–21</td>
<td>10</td>
<td>69.5 ± 3.5</td>
<td>89.5 ± 10.3</td>
<td>1206 ± 102</td>
</tr>
<tr>
<td>21–25</td>
<td>15–32</td>
<td>12</td>
<td>52.1 ± 7.3</td>
<td>92.0 ± 2.7</td>
<td>1125 ± 45</td>
</tr>
<tr>
<td>26–30</td>
<td>20–44</td>
<td>15</td>
<td>59.3 ± 7.1</td>
<td>123.7 ± 9.5</td>
<td>1011 ± 91</td>
</tr>
<tr>
<td>31–40</td>
<td>30–52</td>
<td>8</td>
<td>68.2 ± 9.0</td>
<td>153.6 ± 113</td>
<td>1175 ± 113</td>
</tr>
</tbody>
</table>

*Mean ± SE.

Endogenous acceptors less useful than measurements of enzyme activity dependent on exogenous substrates.

To determine whether an elevation of serum sialyltransferase would occur with restoration of tumor burden, experiments were performed in which not all of the neoplastic tissue was removed. Instead, 1 to 2 g of tissue were left in situ to allow regrowth of the tumors. Chart 5 shows the data for serum sialyltransferase activity after 1, 2, and 3 weeks of tumor regrowth. After 2 weeks of tumor regrowth, the activity of sialyltransferase was increased and reached values comparable to those observed before surgery in animals bearing large tumor burdens. These values represent a 2- and 6-fold increase over the activity in control, nontumor-bearing animals, which is similar to the increase reported for animals bearing primary tumors of comparable size (9). Animals in which tumors were not surgically excised began to die on Day 32, and all were dead by Day 52.

In a few animals, the tumor was allowed to regrow for 4 weeks when the sialyltransferase activity was comparable to that in animals in which tumors had been allowed to regrow for 3 weeks (data not shown). Thus, after an initial increase in activity of the enzyme (after about 3 weeks of tumor growth when tumor weight was 20 to 35 g), activity of the serum enzyme reached a plateau with little or no further increase.

As another approach to investigate the relationship between tumor burden and serum sialyltransferase activity, animals were given implantations with R3230AC tumors and, 1 day later, were treated with daily doses of cyclophosphamide. As shown in Table 6, this treatment retarded tumor growth and prevented the elevations of serum sialyltransferase activity seen in untreated tumor-bearing rats. Control experiments in which cyclophosphamide was added directly to the assay tubes in vitro or in which control, nontumor-bearing animals were given cyclophosphamide demonstrated that the presence of the drug itself had no effect upon the enzyme activity as measured in the assay system described.

Administration of cyclophosphamide caused a 20 to 30% weight loss in both tumor-bearing and control, nontumor-bearing rats. No other toxic effects were observed in these animals upon necropsy. Treatment of animals bearing advanced tumors (duration, 25 days or more) with cyclophosphamide did not...
Table 3

Neuraminidase activity in serum, liver, and tumor homogenates
All tissue samples were from animals receiving tumor implantations 24 days before neuraminidase determination or from age-matched controls.

<table>
<thead>
<tr>
<th>Homogenate</th>
<th>pH 7.2</th>
<th>pH 4.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (4)</td>
<td>0</td>
<td>114</td>
</tr>
<tr>
<td>Animals with tumors (4)</td>
<td>0</td>
<td>105</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (4)</td>
<td>1774</td>
<td>4819</td>
</tr>
<tr>
<td>Animals with tumors (4)</td>
<td>3106</td>
<td>9379</td>
</tr>
<tr>
<td>Tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor homogenate (4)</td>
<td>809</td>
<td>2647</td>
</tr>
</tbody>
</table>

a Numbers in parentheses, number of animals monitored.

Table 4

Breakdown of CMP-sialic acid after incubation with serum, liver, or tumor homogenates
All tissue samples were from animals receiving tumor implantations 24 days before hydrolyase determination or from control animals.

<table>
<thead>
<tr>
<th>Homogenate</th>
<th>AcNeu (cpm)</th>
<th>CMP-AcNeu (cpm)</th>
<th>% of breakdown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (4)</td>
<td>2455</td>
<td>4658</td>
<td>0.35</td>
</tr>
<tr>
<td>Rats with tumors (4)</td>
<td>2474</td>
<td>4808</td>
<td>0.34</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (4)</td>
<td>1813</td>
<td>4585</td>
<td>0.28</td>
</tr>
<tr>
<td>Rats with tumors (4)</td>
<td>1604</td>
<td>4256</td>
<td>0.27</td>
</tr>
<tr>
<td>Tumor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor tissue (4)</td>
<td>772</td>
<td>5107</td>
<td>0.13</td>
</tr>
</tbody>
</table>

AcNeu, N-acetylneuraminic acid; CMP-AcNeu, CMP-N-acetylneuraminic acid.

Table 5

Endogenous serum sialyltransferase activity on days following tumor removal
Activity of enzyme (cpm/mg protein) using endogenous serum acceptors. The experiment was performed as described in Chart 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1a</th>
<th>Day 2a</th>
<th>Day 3a</th>
<th>Day 4a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58 ± 8.8</td>
<td>79 ± 14.2</td>
<td>56 ± 17.4</td>
<td>79 ± 9.3</td>
</tr>
<tr>
<td>Tumor (not operated)</td>
<td>193 ± 26.9</td>
<td>154 ± 25.4</td>
<td>137 ± 9.01</td>
<td>157 ± 21.5</td>
</tr>
<tr>
<td>Tumor (sham operated)</td>
<td>192 ± 35.5</td>
<td>167 ± 14.4</td>
<td>155 ± 19.0</td>
<td>151 ± 14.8</td>
</tr>
<tr>
<td>Tumor (removed on Day 0)</td>
<td>138 ± 12.3</td>
<td>105 ± 22.9</td>
<td>79 ± 5.8</td>
<td>65 ± 4.8</td>
</tr>
</tbody>
</table>

a Mean ± S.E.
(24) have shown similar acceptor requirements between the serum and liver enzyme activities in animal hosts, leading them to suggest that the serum enzyme might be wholly or partially derived from liver tissue.

In our present investigation, we have used unfraccionated serum or liver or tumor homogenate as our enzyme source and fetuin lacking sialic acid as an acceptor molecule. Since fetuin contains sialic acid linked α-2→3 and α-2→6 to galactose (1, 41) as well as linked α-2→6 to N-acetylgalactosamine (42), the unpurified homogenate enzyme source may measure several asialofetuin sialyltransferase enzyme activities. The enzyme assays reported in this paper were run at the same pH in the hope of facilitating comparisons between the serum, tumor, and liver enzyme activities. Since a prior report (23) has shown that 2 different enzyme activities may exist in liver tissue and be differentially stimulated by pH 6.0, 7.0, and 8.0, we measured the sialyltransferase activity of serum, liver, and tumor samples from animals that had had tumors implanted up to 6 weeks previously, using enzyme assays performed at pH 6.0, 7.0, or 8.0. These experiments gave the same results shown in Chart 2; i.e., animals that had tumors implanted 3 weeks prior to sacrifice showed elevated activity of sialyltransferase in their serum and liver tissue (data not shown). The same increase in enzyme activity in liver and serum of animals bearing tumors implanted 3 weeks previously was detected when CMP-N-acetyl-[14C]neuraminic acid of lower specific activity was substituted in the assay (no data shown).

Our earlier studies (8) showed that the activity of sialyltransferase and other glycosyltransferase enzymes was elevated in malignant breast tissue as compared to premalignant or normal breast tissue. Subsequent studies by others (see discussions in Refs. 12, 24, and 44) have reported elevated and reduced as well as similar enzyme levels when neoplastic tissue is compared to its normal counterpart. Since tumor cells in culture secrete enzyme into the media (44), and since secretion has been proposed as a mechanism for formation of the serum enzyme, it seems important to measure the rate of enzyme release as well as the amount of residual enzyme in the tumor tissue.

Podolsky et al. (37) examined the electrophoretic properties of galactosyltransferase found in the serum of rats bearing tumors derived from BHK cells and found 2 electrophoretically distinct activities. Control animals possessed only one serum galactosyltransferase activity which comigrated with a galactosyltransferase derived from rat liver. The other activity had the same electrophoretic mobility as did enzyme obtained from the tumor tissue. These data would suggest that the normal serum enzyme may be derived from the liver; however, the increased activity found in animals bearing tumors may be a mixture of enzymes derived from both liver and tumor cells. The present results show that elevations in serum and liver sialyltransferase activity occurred at about the same time after tumor implantation, raising the possibility that growth of the tumor at a site distant from the liver had exerted an effect on the biochemistry of this organ.

It is well known that the presence of a tumor effects many changes in its host (see reviews in Refs. 4 and 40). The livers of tumor-bearing animals display biochemical changes in the activity of many hepatic enzymes (4, 10, 19, 21, 39, 40, 43, 46). Animal studies using a variety of transplantable tumors have demonstrated changes in serum activity of various enzymes and other molecules, some of which are largely tumor derived, whereas other changes result from response of the organ systems of the host to the presence of the tumor burden (4, 15, 38–40).

The results presented here and those reported previously (9, 10) are compatible with the hypothesis that the liver is synthesizing increased amounts of enzyme and secreting it into the blood plasma. However, since enzyme levels were measured only in the liver itself, there is no firm evidence that increased enzyme activity in the blood was due to increased release of the enzyme by liver cells. Although attempts were made to remove blood from the livers, it cannot be ruled out that “trapped” serum is the source of the elevated sialyltransferase activity found in liver. Alternatively, the sialyltransferase enzyme may be cleared from the serum by liver cells which thus accumulate the enzyme. Although the tumor tissue did not show significant increases in enzyme activity per mg of tissue, the large mass of tumor tissue present 2 to 3 weeks after implantation was capable of producing and secreting sufficient amounts of enzyme to account for the increased serum activity. Identification of the tissue source of the serum enzymes will

### Table 6

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Av. wt (g)</th>
<th>Av. tumor wt (g)</th>
<th>Exogenous activity</th>
<th>Endogenous activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>142 ± 8.2</td>
<td>416 ± 25</td>
<td>101 ± 23</td>
</tr>
<tr>
<td>Control (no tumor + CYP)</td>
<td>4</td>
<td>103 ± 5.5</td>
<td>399 ± 52</td>
<td>51 ± 14</td>
</tr>
<tr>
<td>Tumor (+ CYP)</td>
<td>10</td>
<td>105 ± 5.0</td>
<td>473 ± 29.7</td>
<td>132 ± 17</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* CYP, cyclophosphamide.
require isolation and biochemical characterization of sialyltransferases from various tissues, e.g., liver and tumor.

Surgical excision of the tumor or chemotherapy resulted in a significant decrease in the activity of serum sialyltransferase. Within 2 to 3 days, decreases in serum levels of enzyme were detected, and enzyme activity returned to control (nontumor) values by 4 days. From these results, we estimated that the half-life of the elevated activity of serum sialyltransferase was 24 to 36 hr. It is interesting to note that Bauer et al. (3), studying serum fucosyltransferase activity in cancer patients whose primary tumors had been surgically removed, estimated the serum half-life of fucosyltransferase to be about 2 days. Our experiments also showed that, when growth of the primary tumor was controlled by chemotherapy, no elevation in serum sialyltransferase activity was detected, suggesting that size or growth rate of the tumor may be a determining factor in changes in sialyltransferase activity. The length of time that the tumor was present appeared to be less important since serum sialyltransferase activity was not elevated in animals treated with cyclophosphamide even though the tumor was present for 6 to 8 weeks.

Other reports have also suggested that growth rate of the tumor may be a critical factor in elevating serum sialyltransferase activity. Bosmann et al. (10) and Ip and Dao (24) found higher elevations in rats bearing rapidly growing tumor than in animals bearing slower-growing tumors. It is possible that cyclophosphamide treatment limited the growth of the tumor to such an extent that elevations in serum sialyltransferase activity did not ensue.

One case report has been published (29) in which a longitudinal study was conducted to measure serum glycosyltransferase levels at the time of diagnosis, during the remission period induced by chemotherapy, and after the patient suffered a relapse. Levels of plasma sialyltransferase, which had been elevated 6-fold over control values, decreased to a level only slightly higher than normal during the remission period but increased to levels 6 to 7 times normal when cancer recurred. Thus, there was a close correlation between the sialyltransferase measured in serum samples, the clinical course of the disease, and the quantity of malignant cells present. Bauer et al. (3) also reported sharp decreases in serum fucosyltransferase activity in 2 patients after removal of colon carcinoma and in breast cancer patients after successful therapy; patients whose tumors were incompletely removed or who had numerous metastatic sites exhibited only slight decreases in serum fucosyltransferase activity.

The implications of these findings for cancer detection and treatment are obvious. The next step will be to investigate the nature of the changes in glycoprotein-synthesizing enzymes through the use of animal tumor models.

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REFERENCES

34. Kim, Y. S., Perdomo, J., and Whitehead, J. S. Glycosyltransferases in human

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Correlation of Serum, Tumor, and Liver Serum Glycoprotein: $N$-Acetylneuraminic Acid Transferase Activity with Growth of the R3230AC Mammary Tumor in Rats and Relationship of the Serum Activity to Tumor Burden

Irene M. Evans, Russell Hilf, Michael Murphy, et al.


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