Incorporation of Glucose-\(^{14}\)C, \textit{in vitro}, into Carbohydrates of Intestinal Mucosae of Sarcoma (Subcutaneous Yoshida-sarcoma)-bearing Rats

By

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Scrapings of intestinal mucosae from sarcoma (subcutaneous Yoshida-sarcoma)-bearing rats were incubated, \textit{in vitro}, with U-\(^{14}\)C-glucose, comparing with those from normal rats. The resulting \(^{14}\)C-labelled products were fractionated into three carbohydrate fractions: polysaccharide-containing fraction (PS); carbohydrate fraction eluted with 50% aqueous ethanol from the charcoal-absorbed substances (Et); carbohydrate fraction eluted with aqueous ethanol-ammonia from the charcoal-absorbed substances (Et-Am). Monosaccharide components in these carbohydrate fractions were separated by paper chromatography after acidic hydrolysis and the radioactivities incorporated into these sugars were determined.

The results showed that glucose-\(^{14}\)C incorporated much more into monosaccharide components of the three carbohydrate fractions from the sarcoma-bearing animals than those from normal rats. No remarkable difference was found on the ratios of radioactivities incorporated into the fractions of glucosamine and galactosamine and also on the analyzed three monosaccharides contents between normal and the sarcoma-bearing conditions.

The effects of sarcoma on the metabolism of hexosamine-containing carbohydrates were discussed.

The levels of circulating glycoproteins have been shown to rise in neoplastic diseases as well as a variety of other pathological conditions.¹⁻⁷ Although it is reported that liver is the major biosynthetic organ of plasma glycoproteins,⁷⁻¹⁹ the precise mechanisms by which such abnormalities are induced are still obscure. Except liver, little is known whether any change is induced in the metabolism of hexosamine-containing carbohydrates in other tissues located apart from the neoplasm.

In an attempt to learn what alterations are induced in the metabolism of such hexosamine-containing substances in tumor-bearing animals, the present
authors have been studying on the incorporation of glucose-$^{14}$C, in vitro, into mono-saccharide components of the three carbohydrate fractions in intestinal mucosae from sarcoma (subcutaneous Yoshida-sarcoma)-bearing rats, comparing with those from normal animals.

The results showed that glucose-$^{14}$C incorporated much more into sugar components of these carbohydrate fractions from the sarcoma-bearing animals than those from normal rats.

MATERIALS AND METHODS

**Animals:** Adult albino hybrid rats, weighing 190 g on an average, were used as the normal control. The animals were fed with commercial rat food purchased from Oriental Yeast Co., Ltd., Japan, ad libitum before use. Subcutaneous Yoshida-sarcoma in rats was induced by the subcutaneous implantation on the upper back region of the animals of 1–2 ml of saline-diluted ascites, containing approximately $1 \times 10^7$ cells of Yoshida-hepatoma. The animals noticed intraperitoneal metastasis of the tumor were excluded. The average weight of the sarcoma-bearing rats was 180 g.

**Materials:** Uniformly labelled-$^{14}$C-glucose, specific activity 7.1 mc per mM, was purchased from Daiichi Kagaku Co., Ltd., Japan. Other chemicals were of reagent grade.

**Determination of radioactivity of sugar components:** 3 ml of each of the three carbohydrate fractions to be described below were heated with an equal volume of 2N sulfuric acid in a boiling water bath for 5 hours. Sulfuric acid was removed as barium sulfate with barium hydroxide. The neutralized filtrate was concentrated to a small volume. To this solution was added a mixture of the carrier sugars: glucuronic acid, glucosamine sulfate, galactosamine sulfate, galactose, glucose, mannose, L-fucose and ribose, which were detected in the intestinal mucosae by the preliminary experiments. Although sialic acid was also detected, it was not examined in this experiment. Paper chromatography of the sugar mixtures was carried out in the descending way on Toyo filter paper No. 3, using a solvent mixture of ethylacetate-pyridine-water (2:1:2, v/v, upper layer) at 26–30°C for 12–14 hours according to the procedure of Masamune and Yosizawa. The both edges of the paper strip were cut asunder after drying in air, then sprayed with aniline-hydrogen-phthalate reagent. Each area corresponding to the position of each monosaccharide on the remaining paper strip was cut off and each component was eluted with water into an aluminum planchet. Radioactivity of each monosaccharide fraction was determined by a windowless gas-flow counter, from Nihon Musen Irigaku-kenkyusho, Japan.

**Analysis of sugar components:** Hexose and pentose were determined by PHR$_2$ method of Masamune and Sakamoto with thionalid-sulfuric acid reagent. Hexosamines were estimated according to the procedure of Yosizawa.
EXPERIMENTAL PROCEDURE

**Incubation:** Animals were anesthetized with ether and decapitated. Colon and small intestine were excised separately, opened by scissors, cleaned with cold water and then phosphate buffer (0.1 M, pH 7.4). Mucosa was peeled with glass plate. Four g of the wet colon scrapings for each experiment were incubated in a Warburg-type vessel, with an incubation mixture of 10 μmoles glutamine, 15 μmoles of cysteine, 20 μmoles of disodium ethylenediaminetetraacetate, 2 μc U-14C-glucose in 4ml phosphate buffer (0.1 M, pH 7.4), at 37°C for 1.5 hours. The reaction was terminated by the addition of 1 ml of 20% aqueous potassium hydroxide. Eight g of the wet scrapings from small intestine were incubated in the similar way as above with twice amounts of the incubation mixture except using 2 μc of U-14C-glucose.

**Fractionation of carbohydrates:** The above reaction mixture was dialyzed immediately after the addition of potassium hydroxide against five changes of each 2 l of distilled water in a Visking tube at 2-5°C for 4 days. The dialyzable fractions were passed through a column (2×15 cm) of Amberlite IRA 120 (H+). The effluent was concentrated to 10 ml under reduced pressure. The concentrated solution was then passed through a column (1.5×12 cm) of charcoal-celite (1: 1 w/w). The column was washed with water. The absorbed substances were eluted with 50% aqueous ethanol (400 ml), followed by the elution with 400 ml of aqueous ethanol-ammonia solution (99% ethanol: 28% ammonia: water=50: 10: 40, v/v). Both effluents were concentrated to 10 ml. The carbohydrate fraction eluted with 50% aqueous ethanol was designated as Et and the carbohydrate fraction eluted with aqueous ethanol-ammonia as Et-Am.

To the non-dialyzable fraction of the incubation mixture was added an equal volume of 10% trichloroacetic acid under stirring. After standing for 5 min, the resulting precipitate were centrifuged off and washed three times with 5% trichloroacetic acid. The supernatant and washing were combined and dialyzed against many changes of distilled water at 2-5°C for 3 days. The non-dialyzable fraction was concentrated under reduced pressure to 10 ml. This fraction was designated as PS.

RESULTS

**Incorporation of glucose-14C into monosaccharide components:** Radioactivities incorporated into PS (polysaccharide-containing fraction), Et (carbohydrate fraction eluted with 50% aqueous ethanol from the charcoal-absorbed substances) and Et-Am (carbohydrate fraction eluted with aqueous ethanol-ammonia from the charcoal-absorbed substances) are shown in Tables I and II. The data show the average value obtained from 8 experiments.
### TABLE I. Radioactivities (c.p.m./g wet weight of scrapings*)
Incorporated into the Sugar Components of the Carbohydrate Fractions from Rat Colonic Mucosa

<table>
<thead>
<tr>
<th>Condition</th>
<th>Carbohydrate fraction</th>
<th>PS↑</th>
<th>Et↑</th>
<th>Et-Am↑</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Monosaccharide fraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td>71</td>
<td>25,500</td>
<td>773</td>
<td></td>
</tr>
<tr>
<td>Hexosamine</td>
<td>275</td>
<td>5,470</td>
<td>421</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>369</td>
<td>4,000</td>
<td>606</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>159</td>
<td>6,420</td>
<td>992</td>
<td></td>
</tr>
<tr>
<td>Mannose</td>
<td>64</td>
<td>860</td>
<td>1,159</td>
<td></td>
</tr>
<tr>
<td>L-Fucose</td>
<td>41</td>
<td>140</td>
<td>280</td>
<td></td>
</tr>
<tr>
<td>Ribose</td>
<td>88</td>
<td>290</td>
<td>350</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1,067</td>
<td>42,680</td>
<td>4,541</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sarcoma-bearing</th>
<th>Monosaccharide fraction</th>
<th>PS↑</th>
<th>Et↑</th>
<th>Et-Am↑</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucuronic acid</td>
<td>190</td>
<td>29,130</td>
<td>993</td>
<td></td>
</tr>
<tr>
<td>Hexosamine</td>
<td>644</td>
<td>4,640</td>
<td>461</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>655</td>
<td>2,940</td>
<td>659</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>203</td>
<td>9,662</td>
<td>1,164</td>
<td></td>
</tr>
<tr>
<td>Mannose</td>
<td>93</td>
<td>1,030</td>
<td>1,361</td>
<td></td>
</tr>
<tr>
<td>L-Fucose</td>
<td>88</td>
<td>200</td>
<td>251</td>
<td></td>
</tr>
<tr>
<td>Ribose</td>
<td>126</td>
<td>340</td>
<td>328</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1,997</td>
<td>47,940</td>
<td>5,217</td>
<td></td>
</tr>
</tbody>
</table>

* 1 g of wet scrapings gave approximately 50 mg of dry substances.
† PS: Polysaccharide-containing fraction, Et: Carbohydrate fraction eluted with 50% aqueous ethanol from the charcoal-absorbed substances. Et-Am: Carbohydrate fraction eluted with aqueous ethanol-ammonia from the charcoal-absorbed substances.

### TABLE II. Radioactivities (c.p.m./g wet weight of scrapings*)
Incorporated into the Sugar Components of the Carbohydrate Fractions from Rat Small Intestinal Mucosa

<table>
<thead>
<tr>
<th>Condition</th>
<th>Carbohydrate fraction</th>
<th>PS↑</th>
<th>Et↑</th>
<th>Et-Am↑</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Monosaccharide fraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td>54</td>
<td>6,350</td>
<td>159</td>
<td></td>
</tr>
<tr>
<td>Hexosamine</td>
<td>226</td>
<td>1,320</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>186</td>
<td>2,450</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>66</td>
<td>2,450</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Mannose</td>
<td>39</td>
<td>250</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>L-Fucose</td>
<td>23</td>
<td>110</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Ribose</td>
<td>21</td>
<td>140</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>618</td>
<td>13,220</td>
<td>871</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sarcoma-bearing</th>
<th>Monosaccharide fraction</th>
<th>PS↑</th>
<th>Et↑</th>
<th>Et-Am↑</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucuronic acid</td>
<td>57</td>
<td>7,740</td>
<td>201</td>
<td></td>
</tr>
<tr>
<td>Hexosamine</td>
<td>357</td>
<td>1,810</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>318</td>
<td>2,850</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>96</td>
<td>2,190</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>Mannose</td>
<td>63</td>
<td>240</td>
<td>486</td>
<td></td>
</tr>
<tr>
<td>L-Fucose</td>
<td>33</td>
<td>140</td>
<td>157</td>
<td></td>
</tr>
<tr>
<td>Ribose</td>
<td>38</td>
<td>170</td>
<td>164</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>962</td>
<td>15,140</td>
<td>1,363</td>
<td></td>
</tr>
</tbody>
</table>

* † Signify the same as in Table I.
The total specific activities incorporated into the examined monosaccharide components of these three fractions from the sarcoma-bearing animals were found to be much more than those from the normal rats. The rate of incorporation of glucose-\textsuperscript{14}C into the sugar components of PS was small, but those of Et and Et-Am were fairly large.

Although all the examined monosaccharide fractions of PS from the sarcoma-bearing animals had much more radioactivities than those from normal rats, some monosaccharide fractions of Et and Et-Am did not show the same figures.

\textit{Ratio of radioactivities incorporated into the fractions of glucosamine and galactosamine}: Glucosamine and galactosamine were separated by paper chromatography according to the procedure of Yosizawa\textsuperscript{22} and the radioactivities of these amino-sugar fractions were determined. The ratios of the radioactivities incorporated into the fractions of glucosamine and galactosamine in the three carbohydrate fractions are shown in Tables III and IV. The data show the average value from 8 experiments.

As can be seen in Tables III and IV, no remarkable difference was observed.

\begin{table}[h]
\centering
\caption{Ratios of Radioactivities Incorporated into the Fractions of Glucosamine and Galactosamine of the Carbohydrate Fractions from Rat Colonic Mucosa}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Condition} & \textbf{Carbohydrate fraction} & \textbf{PS} & \textbf{Et} & \textbf{Et-Am} \\
\hline
Normal & Glucosamine & 1.2 & 1.0 & 2.3 \\
       & Galactosamine & 1.0 & 1.0 & 1.0 \\
Sarcoma-bearing & Glucosamine & 1.4 & 1.2 & 2.0 \\
           & Galactosamine & 1.0 & 1.0 & 1.0 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Ratios of Radioactivities Incorporated into the Fractions of Glucosamine and Galactosamine of the Carbohydrate Fractions from Rat Small Intestinal Mucosa}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Condition} & \textbf{Carbohydrate fraction} & \textbf{PS} & \textbf{Et} & \textbf{Et-Am} \\
\hline
Normal & Glucosamine & 2.0 & 0.91 & 0.56 \\
       & Galactosamine & 1.0 & 1.00 & 1.00 \\
Sarcoma-bearing & Glucosamine & 1.9 & 0.97 & 0.52 \\
           & Galactosamine & 1.0 & 1.00 & 1.00 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{†} Signify the same as in Table I.

\textsuperscript{**} Signify the same as in Table I.
between normal and the sarcoma-bearing conditions.

**Amounts of hexosamine, hexose and pentose in the three carbohydrate fractions:**
The amounts of hexosamine (as glucosamine), hexose (as galactose) and pentose (as ribose) in PS, Et and Et-Am are shown in Tables V and VI. The data show the average value from 8 experiments.

**DISCUSSION**

As to the utilization of glucose-^{14}C by intestinal mucosa, *in vitro*, Draper and Kent\(^{23}\) reported that glucose-^{14}C incorporated into mucosubstances of sheep colonic mucosa and the papain-digested ^{14}C-labelled mucosubstances consist of two nearly-related ^{14}C-labelled mucopolysaccharides. Acidic hydrolysis of the ^{14}C-
labelled mucopolysaccharides gave $^{14}$C-labelled sialic acid, galactose, glucosamine, galactosamine, fucose and possibly mannose. Wolf and his co-workers\textsuperscript{24-27} have been studying on mucopolysaccharides biosynthesis with colonic segments or homogenates dealing with the biochemical roles of vitamin A. The segments or homogenates of rats or pig colonic mucosa incubated with $^{14}$C-glucose gave radioactive mucopolysaccharide, from which $^{14}$C-glucosamine could be isolated after acidic hydrolysis. They showed that the $^{14}$C-labelled mucopolysaccharide resembled chondroitin sulfate. In addition, Wolf \textit{et al.}\textsuperscript{26} reported that mucopolysaccharide synthesis took place in cell particles similar to (or identical with) mitochondria in rat colonic mucosa, whereas in pig colon mucosal homogenates, the pH 5 enzymes\textsuperscript{27} were shown to play an important role in the biosynthesis. Investigations of the conversion of glucose into glucosamine in bovine colonic mucosa and into sialic acid by a soluble enzyme from sheep colonic mucosa have been reported by Pasternak\textsuperscript{28} and Kent and Draper,\textsuperscript{29} respectively. However, no investigation was reported as to the metabolism of hexosamine-containing carbohydrates in intestinal mucosa under neoplastic conditions.

The data obtained by the present experiments showed that glucose-$^{14}$C incorporated, \textit{in vitro}, much more into the monosaccharide components of the three carbohydrate fractions from the sarcoma-bearing animals than those from normal rats. The rate of incorporation of glucose-$^{14}$C into sugar components of PS was small, which coincided with the results of the other investigators.\textsuperscript{23-27} Fairly large incorporation rates were, however, observed in cases of Et and Et-Am. Since no remarkable difference was found on the ratios of radioactivities incorporated into glucosamine and galactosamine fractions and also on the contents of hexosamine, hexose and pentose between normal and the sarcoma-bearing conditions, the elevation of the rate of incorporation of glucose-$^{14}$C under the tumor-bearing condition seemed to be due to the stimulation of enzyme activities involved in the metabolism of these carbohydrates. Similar results were obtained with the rats bearing liver-tumor.\textsuperscript{30}

The levels of plasma glycoproteins have been shown to rise in a variety of pathological conditions.\textsuperscript{1-7} A number of studies has clearly implicated the liver as the major biosynthetic source of plasma glycoproteins.\textsuperscript{7-19} Kelley \textit{et al.}\textsuperscript{31} indicated that increases in serum mucoproteins might result as part of a reaction to stress. Catchpole\textsuperscript{32} reported that the increase in circulating glycoproteins arose from the ground substance at the site of invasion of the tumor. The glycoproteins were believed to arise from depolymerization of the ground substance with the resultant formation of small, water-soluble, and readily diffusible glycoproteins. According to an idea of Masamune,\textsuperscript{33} neoplastic cells secrete the substance(s), by which the function(s) of the normal cells might be changed.

Since the tissues examined in this experiments were located apart from the neoplastic region, the elevation of the incorporation of glucose-$^{14}$C into the
carbohydrates in the intestinal mucosae of the sarcoma-bearing rats must be due
to the indirect effect(s) of the sarcoma. Although the stress or the secreted sub-
stances from the neoplastic cells may activate or alter the metabolism of these
carbohydrates, the mechanisms involved remains to be elucidated.

Acknowledgments

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