Effects of specific dietary sugars on the incorporation of $^{13}$C label from dietary glucose into neutral sugars of rat intestine and serum glycoproteins

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Although theoretically all glycoprotein sugars can be derived from glucose, it may be hypothesized that specific dietary sugars could be preferential substrates for glycoprotein synthesis. To test this hypothesis, groups of rats received either continuously (continuous-labelling experiment) or for a single nutritional period (pulse-labelling experiment) a $^{13}$C-rich diet containing either maize starch or artificially labelled $[^{13}$C$]$glucose. Some groups of rats were also provided during a single nutritional period with low amounts (20–200 mg/animal) of low-$^{13}$C dietary sugars (mannose, galactose, fucose or fructose). If specific dietary sugars were preferentially incorporated into glycoproteins instead of glucose-derived labelled sugars, a decrease would be expected in the intestinal or serum glycoprotein-sugar $^{13}$C enrichment monitored by gas chromatography–isotope-ratio mass spectrometry (GC-IRMS). Contrary to this hypothesis the results showed no significant decrease with any of the specific dietary sugars. Furthermore, with dietary low-$^{13}$C mannose or galactose, a significant increase in $^{13}$C enrichment of glycoprotein-sugars was observed compared with some other nutritional groups. Moreover, in the pulse-labelling experiment, dietary mannose and galactose induced similar patterns of $^{13}$C enrichment in intestinal and serum glycoprotein-sugars. Therefore, although specific dietary sugars do not appear to be preferential substrates for glycosylation under conditions and doses relevant to current concepts of nutrition, regulatory roles of some specific dietary sugars in relation to glycoprotein-sugar metabolism might be hypothesized. These findings could lead to similar studies using stable-isotope methodology in man which could have practical consequences, especially in parenteral nutrition where glucose is the only sugar provided to the metabolism.

Glycosylation: Dietary sugars: Stable-isotope technique: GC-IRMS

Oligosaccharide chains of glycoproteins play a crucial role in various cellular structures and functions (West, 1986). The biosynthesis of these chains is now well documented (Kornfeld & Kornfeld, 1985), but the physiological regulation of such a complex pathway is far from being completely understood. As an element of this regulation we have recently reviewed the influence of dietary factors (Biol et al. 1992). The general hypotheses for such studies were that dietary components might exert regulatory effects at different sites of the glycosylation pathway and that glycosylation might utilize some dietary components as substrates or energy sources. Theoretically, all the glycoprotein sugars can be derived from glucose through metabolic pathways described many years ago. However, specific dietary sugars could be preferentially incorporated into glycoproteins and/or could exhibit

* For reprints.
regulatory roles in the glycosylation processes. The first aim of the present study was to test these hypotheses in the animal and the second to validate the use of stable isotopes to trace glycoprotein-sugar metabolism with a view to future studies in man.

Previous studies have demonstrated, exclusively in animals, that specific exogenous radioactive sugars (especially hexosamine and fucose) are efficiently incorporated, without significant interconversion, into glycoproteins of various tissues (Coffey et al. 1964; Shetlar et al. 1964; Capps et al. 1966; Bekesi & Winzler, 1967; Robinson, 1967, 1968; Bocci & Winzler, 1969), with the exception of galactosamine which is largely converted to glucosamine (White et al. 1965). However, a large proportion (up to half the administered dose) is recovered either in the urine or in expired CO₂ (Robinson, 1968). In addition, some of these studies have shown also that addition of substantial amounts of the same non-radioactive sugar as a carrier reduced the incorporation of the tracer into glycoproteins and increased its excretion (Coffey et al. 1964; Bocci & Winzler, 1969). Most of these studies used injections of the tracer (intravenous or intraperitoneal) and few administered it orally. Moreover, in most of the studies the nutritional status, particularly that relating to minor dietary sugars, was not controlled.

In contrast to the previous studies and to test the hypothesis of preferential incorporation of specific dietary sugars, we have chosen to use stable-isotope labelling of glucose, the most abundant dietary sugar. Low doses (relevant to current concepts of nutrition) of specific dietary sugars were added transitorily to a well-defined semi-synthetic diet. If these sugars were preferentially incorporated into glycoproteins, a significant decrease in glycoprotein-sugar labelling would be expected. If such a metabolic regulation does exist, this might have some practical consequences in nutrition, especially in total parenteral nutrition where glucose is the only carbohydrate substrate provided for metabolism.

Before application to man, validation of such experimental design and methodology in animal models is required. We used either starch naturally enriched in stable isotopes or artificially-labelled glucose and have followed the ¹³C enrichment in glycoprotein-sugars using the combination gas chromatography–isotope-ratio mass spectrometry (GC–IRMS). This technique has been demonstrated to be sensitive enough for this purpose in the animal (Rambal et al. 1992). Stable-isotope methodology is used largely in humans for the study of protein (Garlick et al. 1989), fatty acid (Goodman & Brenna, 1992; Brossard et al. 1994) or glucose metabolism (Tissot et al. 1990; Normand et al. 1992). The results reported here indicate that it would be interesting also for the clinical study of glycosylation regulation.

MATERIALS AND METHODS

Materials

Diet components were obtained from ICN Nutritional (Costa Mesa, CA, USA). ¹³C-enriched glucose was obtained from Isotec (Miamisburgh, OH, USA). The components, particularly starch and cellulose, were controlled for their absence of contamination with specific minor sugars, before and after hydrolysis and analysis by GC.

Animals

Male rats of the Sprague–Dawley strain (IFFA-CREDO, L’Arbresle, France), weighing 140–160 g at the beginning of the experiment, were housed under controlled conditions (temperature 21°C, daily cycle of 12 h light–12 h darkness) in cages containing six or eight animals. They were fed for 1 week on a semi-synthetic diet containing ¹³C-reduced wheat starch as the dietary source of glucose to obtain a stable level of isotopic enrichment. Amounts and ¹³C enrichment of wheat starch and other diet components of this isotope-equilibration diet are given in Table 1 (constant components).
**Continuous-labeling experiment**

At the end of the isotope-equilibration period a control group continued on the wheat-starch equilibration diet, whereas different test groups of three rats received for a single 12 h overnight feeding period (beginning at time 0) a diet in which wheat starch was replaced by naturally $^{13}$C-enriched maize starch, containing 0, 1, 5 or 10 g $^{13}$C-reduced mannose/kg at the expense of starch (Fig. 1(a)). In two independent experiments, two levels of $^{13}$C-enrichment of mannose were used: mannose A ($^{13}$C 1.08637 %) in the first experiment and mannose B ($^{13}$C 1.07985 %) in the second experiment. After this 12 h nutritional period all the test groups were fed continuously on the same maize-starch diet without mannose. For each test or control group, three animals were killed by decapitation just before the diet change (time 0 or basal) or at various intervals after time 0 (12, 40, 68 and 86 h). Weight gain and food and water intakes were regularly monitored. There was no significant difference between the nutritional groups for these variables throughout the experiment; the mean food intake per animal during the overnight feeding period with dietary mannose was 22 (SE 3) g.

**Pulse-labeling experiment**

At the end of the isotope-equilibration period with the wheat-starch diet, different groups of four rats received for a nutritional period of 5 h (between 06.00 and 11.00 hours, Fig. 1(b)) the wheat-starch diet to which was added either only artificially enriched pure glucose (total $^{13}$C from glucose and wheat starch 1.3782 %) or the enriched glucose and 2 g/kg of one of the following low-$^{13}$C sugars: mannose (either mannose C or mannose D), fucose, fructose or galactose, whose $^{13}$C enrichments are given in Table 1 (variable components). At the end of this nutritional period, all the groups were again fed on the wheat-starch diet without

### Table 1. Composition of diets fed to rats during the isotope-equilibration period and continuous- and pulse-labeling experiments

<table>
<thead>
<tr>
<th>Constant components</th>
<th>(g/kg diet)</th>
<th>Atom % $^{13}$C*</th>
<th>Variable components†</th>
<th>(g/kg diet)</th>
<th>Atom % $^{13}$C*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>1.08247</td>
<td>Continuous-labeling experiment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>670</td>
<td>1.08592</td>
<td>Mannose A 1–10</td>
<td>1.08637</td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td></td>
<td>1.08592</td>
<td>Mannose B 1–10</td>
<td>1.07985</td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td></td>
<td>1.08552</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipids§</td>
<td>50</td>
<td>1.08058</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamins†</td>
<td>19.5</td>
<td>1.09927</td>
<td>Fucose 2</td>
<td>1.08592</td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>19.5</td>
<td>1.08585</td>
<td>Fucose 2</td>
<td>1.00000</td>
<td></td>
</tr>
<tr>
<td>Minerals¶</td>
<td>39</td>
<td>1.10098</td>
<td>Galactose 2</td>
<td>1.08248</td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>2</td>
<td>1.07867</td>
<td>Mannose C 2</td>
<td>1.08690</td>
<td></td>
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<tr>
<td>Pulse-labeling experiment</td>
<td></td>
<td></td>
<td>Mannose D 2</td>
<td>1.09790</td>
<td></td>
</tr>
<tr>
<td>Glucose†</td>
<td>2</td>
<td>&gt; 99</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Atom % $^{13}$C = ($^{12}$C/($^{12}$C + $^{13}$C)) × 100.
† Artificially enriched glucose in the pulse-labeling experiment and variable components for the different nutritional groups were introduced into the diet at the expense of starch.
§ Lipids were a 1:1 (w/w) mixture of peanut and sunflower oils (Lesieur Cotelle, Boulogne, France). All the other products were obtained from ICN Nutritional Biochemicals (Cleveland, OH, USA).
| Vitamin mixture (AIN 76A) contained (mg/kg mixture): retinyl palmitate 32, D-tocopherol acetate 400, cholecalciferol 5, thiamine hydrochloride 12, riboflavin 12, pyridoxine hydrochloride 14, nicotinic acid 0.06, calcium D-pantothenate 0.02, folic acid 4, b-iodin 0.4, cyanocobalamin 0.02, menadione 0.01, sucrose 19.46 g.
| Mineral mixture (AIN 76) contained (g/kg mixture): calcium phosphate dibasic 20, sodium chloride 2.96, potassium citrate monohydrate 8.8, potassium sulphate 2.08, magnesium oxide 0.96, manganese carbonate 0.14, ferric citrate 0.24, zinc carbonate 0.064, cupric carbonate 0.012, potassium iodate 0.0004, sodium selenite 0.0004, chromium potassium sulphate 0.022, sucrose 4.72.
Continuous-labelling experiment

Maize starch + 0, 1, 5 or 10 g mannose A or B/kg
Wheat starch
1-week isotope-equilibration period
Sampling time (h)
0 12 40 68 86

(b) Pulse-labelling experiment

Wheat starch + 2 g [13C]glucose/kg with or without 2 g/kg fructose, fucose, galactose, mannose C or D

Wheat starch
Wheat starch
1-week isotope-equilibration period
Sampling time (h)
0 4 5 8 10 12 27 32

Fig. 1. Scheme to show the design of the continuous- and pulse-labelling experiments.

either enriched glucose or one of the other sugars. Isotopic enrichments were determined at 4, 5, 8, 10, 12, 27 and 32 h after the beginning of the nutritional period for the rats fed on the diet containing only [13C]glucose and at 32 h for the rats fed on diets also containing a low-[13C] sugar. There was no significant difference between the nutritional groups in weight gain and food intake throughout the experiment. During the 5 h nutritional period, mean food intake was 9 (SE 2) g.

Sampling
The small intestine of each animal was individually processed. The intestines were removed, thoroughly washed with cold NaCl (9 g/l), weighed and opened. The mucosa was removed by scraping and was diluted in distilled water (5 ml/g wet tissue) for glycoprotein analysis. The mucosa was homogenized by using ten strokes of a Potter-Elvejhem homogenizer and the homogenate was immediately frozen and stored at −20° until required for analysis.

Gas chromatography–isotope-ratio mass spectrometry of the alditol acetates of glycoprotein-neutral sugars
The procedure for the preparation of the alditol acetates of glycoprotein-sugars for analysis by GC-IRMS has been improved and described in detail by Rambal et al. (1992). Briefly, glycoproteins in 2 ml intestinal homogenate or in 1 ml serum were precipitated with cold trichloroacetic acid, the lipid removed by the method of Folch et al. (1957), and hydrolysed in 1 M-HCl for 2 h at 100°. After the addition of 100 μg xylose as an internal standard and purification by ion-exchange chromatography the samples were lyophilized. The alditol acetates were prepared according to Blakeney et al. (1983) and Henry et al. (1983).
were separated by GC (Hewlett-Packard 5890 A) on a Supelo 2380 capillary column (30 m × 0.32 mm ID) coupled to an isotope-ratio mass spectrometer (SIRA 12; VG ISOGAS, Middlewich, Cheshire). Results were obtained as δ^{13}C_{\%o}, after calibration with the international standard PDB and correction for 17O content (Craig, 1957), according to the equation:

\[ \delta^{13}C_{\%o} = \left( (^{13}C/^{12}C) \text{ sample} - (^{13}C/^{12}C) \text{ standard} \right) \times 10^3/(^{13}C/^{13}C) \text{ standard}. \]

In order to eliminate isotope dilution due to the naturally-labelled C of acetic anhydride used for sugar derivatization the results were expressed in atom percent excess (APE δ^{13}C):

\[ \text{APE } ^{13}C = \text{ atom }\% \text{ sample at time } t - \text{ mean value of atom }\% \text{ at time } 0. \]

Atom% (no. of atoms of ^{13}C/100 C atoms) was calculated according to the following equation:

\[ \text{atom }\% = (100R(0.001\delta_{\%o} + 1))/(1 + R(0.001\delta_{\%o} + 1)), \]

where R is the ^{13}C/^{12}C of the international standard (PDB; R 0.0112372).

Each analysis was performed in duplicate. Under the operating conditions used the sensitivity was as high as 0.001 % ^{13}C enrichment and the reproducibility was 3 % (Rambal et al. 1992). Results are expressed as means with their standard errors for n independent values and analysed by analysis of variance (ANOVA) followed by Newman–Keuls test (used at P < 0.05).

RESULTS

13C enrichment of glycoprotein-neutral sugars during the continuous-labelling experiment

The ^{13}C enrichment of intestinal glycoprotein-neutral sugars is shown as a function of time and dietary mannose concentrations (Fig. 2) for fucose, galactose and mannose. There was no change in ^{13}C enrichment for any of the sugars in control animals fed continuously on the wheat-starch diet. With the maize-starch diet without mannose, isotope enrichment for fucose and galactose (Fig. 2(a and b)) significantly increased from 12 h, compared with time 0 values, and then plateaued between 40 and 86 h. In contrast, the isotope enrichment of mannose (Fig. 2(c)) began only at 68 h and was not further increased at 86 h.

In the first experiment the transitory addition of mannose A (^{13}C 1.08637 %) to the maize-starch diet resulted in an enhancement of the isotope enrichment in glycoprotein-sugars. This increase was highly significant at 40 h for glycoprotein-fucose and -mannose at 1 and 5 g mannose/kg diet, at 40 h for glycoprotein-galactose at 1 g mannose/kg diet, at 68 h for the three glycoprotein-sugars at the three dietary mannose concentrations, at 86 h for glycoprotein-mannose and -fucose at the three dietary mannose concentrations and only at 1 g mannose/kg diet for glycoprotein-galactose. Corresponding dietary mannose intakes averaged 22, 110 and 220 mg/animal at dietary mannose concentrations of 1, 5 and 10 g/kg respectively. For 1 g mannose/kg diet the effect reached a maximum at 40 h for the three glycoprotein-sugars then it decreased, but it remained constant up to 86 h for the two other concentrations.

In a second similar experiment, dietary mannose B with a lower ^{13}C enrichment (1.07985 %) was added to the diet. No significant difference was observed between the nutritional groups for ^{13}C enrichment of glycoprotein-sugar and for kinetic variables (data not shown).

The levels of glycoprotein-sugars were more variable than the ^{13}C enrichment and there was no systematic trend towards modifications of these levels. At 40 h in the first experiment (Fig. 3), for which the isotopic effect was maximum, diet had no significant effect on the levels of glycoprotein-fucose and -mannose. For glycoprotein-galactose the diet containing 1 g mannose/kg induced a slight but significant increase in levels.
Fig. 2. $^{13}$C-enrichment (atoms % excess; APE) kinetics of intestinal glycoprotein-neutral sugars, glycoprotein-fucose (a), glycoprotein-galactose (b), glycoprotein-mannose (c), in the continuous-labelling experiment. (+), Control rats fed on the wheat-starch diet throughout the experiment. The other groups consumed maize starch between time 0 and 86 h, with various concentrations of dietary mannose A (g/kg) given for a single nutritional period between time 0 and 12 h: (○) = 0; (△) = 1; (◇) = 5; (□) = 10. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from the values obtained with 0 g mannose/kg diet: * $P < 0.05$. For details of diets and procedures, see Table 1 and pp. 445–447.

$^{13}$C enrichment of glycoprotein-neutral sugars after pulse-labelling

The results for intestinal glycoproteins are shown in Fig. 4(a) and for serum glycoproteins in Fig. 4(b). In the intestine a significant peak of $^{13}$C enrichment was observed for the three sugars at 4 h after the beginning of the nutritional period when the rats were not fed a specific dietary sugar. Thereafter, $^{13}$C enrichment decreased and reached a plateau between 10 and 32 h. As in continuous-labelling experiments, isotope enrichment was more marked for glycoprotein-fucose and -galactose than for glycoprotein-mannose. For serum glycoproteins, enrichments of galactose and mannose exhibited superimposable patterns. At 5 h, $^{13}$C enrichment was not significantly different from that at any other time interval, so that the existence of an enrichment peak at time 4 h, as found in the intestine, was not established. The amount of fucose in rat serum glycoproteins was too low to obtain reliable values for $^{13}$C enrichment.
Fig. 3. Sugar contents of intestinal total glycoproteins at time 40 h in the continuous-labelling experiment, as a function of dietary mannose concentration: (O), fucose; (△), galactose; (◇), mannose. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those of other dietary mannose concentrations for the same glycoprotein-sugar: * $P < 0.05$. For details of diets and procedures, see Table 1 and pp. 445-447.

Fig. 4. 13C-enrichment (atoms % excess; APE) kinetics of (a) intestinal and (b) serum glycoprotein-neutral sugars in the pulse-labelling experiments for control rats fed on a diet without specific dietary sugars: (O), fucose; (◇), galactose; (△) mannose. Values are means with their standard errors represented by vertical bars. For details of diets and procedures, see Table 1 and pp. 445-447.

The effects of the addition of low-13C sugars to the diet on the 13C enrichment of glycoprotein-sugars at 32 h are shown for the intestine (Fig. 5(a)) and for the serum (Fig. 5(b)). One-way analysis of variance gave highly positive $F$ values and subsequent comparisons between groups indicated consistent significant variations. Although there was a trend towards an increase in 13C enrichment of glycoprotein-sugar with dietary mannose D or galactose and to a decrease with dietary mannose C, there was no significant difference from the control for any specific dietary sugar. However, the 13C enrichment
obtained with dietary mannose D ($\% \, ^{13}\text{C} \, 1.0979$) was significantly higher than that with dietary mannose C ($\% \, ^{13}\text{C} \, 1.0869$) for the three glycoprotein-sugars for both the intestine and the serum. Similarly, for the intestine and the serum, glycoprotein-sugar enrichment was consistently significantly higher with dietary mannose D than with dietary fucose, although dietary fucose ($\% \, ^{13}\text{C} \, 1.1000$) was slightly more enriched than dietary mannose D ($\% \, ^{13}\text{C} \, 1.0979$). In some cases the effect of dietary galactose was also significant when compared with the effect of other dietary sugars. Dietary galactose induced significantly higher enrichment for intestinal glycoprotein-fucose when compared with dietary fucose or dietary mannose C, and for intestinal glycoprotein-galactose when compared with the diet containing mannose C (Fig. 5(a)). For serum glycoprotein-mannose and -galactose (Fig. 5(b)) the galactose-containing diet induced significantly higher enrichments than the diets containing fructose, fucose or mannose C. It is noteworthy that the $^{13}\text{C}$-enrichment patterns were very similar for the glycoprotein-neutral sugars obtained from both the intestinal and serum glycoproteins.
Table 2. The effects of specific dietary sugars on glycoprotein sugar levels (nmol/mg protein) at 32 h in the pulse-labelling experiment in which rats received a $^{13}$C-rich diet for a single nutritional period*

(Mean values with their standard errors for four separate duplicate determinations)

<table>
<thead>
<tr>
<th>Dietary sugar</th>
<th>Control</th>
<th>Fructose</th>
<th>Fucose</th>
<th>Galactose</th>
<th>Mannose C</th>
<th>Mannose D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Intestinal glycoproteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannose</td>
<td>20.9</td>
<td>2.7</td>
<td>18.6</td>
<td>3.1</td>
<td>23.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Galactose</td>
<td>10.2</td>
<td>0.9</td>
<td>9.3</td>
<td>3.6</td>
<td>134*</td>
<td>1.3</td>
</tr>
<tr>
<td>Serum glycoproteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannose</td>
<td>32.0</td>
<td>2.8</td>
<td>27.0</td>
<td>3.3</td>
<td>32.9</td>
<td>3.9</td>
</tr>
<tr>
<td>Galactose</td>
<td>88.5</td>
<td>6.0</td>
<td>154.4</td>
<td>4.2</td>
<td>132.2</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* Values with the same superscript letter were significantly different ($P < 0.05$).

In contrast to the significant differences in the $^{13}$C enrichment the amounts of glycoprotein-sugars were not significantly modified by the various diets. The results reported in Table 2 indicate that the only significant difference was found for intestinal glycoprotein-mannose which was significantly higher with dietary galactose than with dietary fucose. The effect of dietary mannose on glycoprotein-galactose content observed in the first continuous-labelling experiment (Fig. 3) was not repeated in this experiment.

**DISCUSSION**

Contrary to the starting hypothesis the addition to the diet of specific sugars at the low doses found in foodstuffs did not decrease $^{13}$C incorporation from dietary glucose into glycoprotein-sugars. After a brief analysis of the results of label incorporation from dietary glucose in control rats the discussion will focus on this surprising observation.

During the re-introduction of the wheat-starch diet after the isotope-equilibration period the enrichment of glycoprotein-sugars remained unchanged (Fig. 2). This finding indicates first that feeding a low-$^{13}$C diet for 1 week is sufficient to establish isotope equilibrium in an animal previously fed on a standard commercial diet devoid of maize and, second, that despite its complexity the glycosylation process does not induce detectable isotope discrimination in relation to incorporation of free monosaccharides into glycoproteins.

In control rats, differences in enrichment values were observed for intestinal glycoprotein-sugars in the two types of experiments (continuous or pulse-labelling): glycoprotein-fucose and -galactose were more enriched than glycoprotein-mannose. This finding might result from either differential turnover of glycoprotein-sugars (Tauber et al. 1989) or a difference in kinetics between O-glycoproteins and mannose-containing N-glycoproteins. In contrast, in serum glycoprotein-sugars the same pattern of enrichment and kinetics was found for mannose and galactose since these sugars are essentially incorporated together in secreted N-glycoproteins. After intraperitoneal radioactive fucose injection, fucose incorporation into intestinal glycoproteins exhibited two peaks, a precocious peak about 40 min after injection and a delayed peak 3 h after injection (Coffey et al. 1964; Bekesi & Winzler, 1967). The second peak was interpreted as a recycling of radioactive sugars from other organs. The plateau obtained in the present study (Fig. 4) might support the same interpretation.
of sugar recycling, since the enrichment remains at a constant level despite a continuous renewal of intestinal mucosa and the replacement of $^{13}$C-labelled diet by low-$^{13}$C wheat-starch diet.

In the first continuous-labelling experiment only the effect of low-$^{13}$C dietary mannose was studied, because this sugar was not previously studied using radioactive techniques and because mannose can be converted either to fucose by a specific pathway (Ginsburg, 1961) or to other sugars by phosphomannose isomerase (EC 5.3.1.8). The results reported were unexpected since the ingestion of minute amounts of dietary mannose (about 20 mg per animal with 1 g mannose/kg diet) induced a significantly higher $^{13}$C enrichment than the ingestion of the maize-starch diet alone, reaching a maximum 40 h after the beginning of the nutritional period. Thus, at this level of ingestion dietary mannose is not preferentially incorporated into glycoproteins. However, the increase in $^{13}$C enrichment was not observed in two situations: at the highest (10 g/kg) dietary concentration (mannose A) and when dietary mannose had a very low $^{13}$C content (mannose B).

Thus, an hypothesis was put forward to explain this discrepancy between the two experiments: the $^{13}$C enrichment increase could suggest a regulatory role for minute amounts of dietary mannose on glycoprotein-sugar metabolism; this role could have been masked in the two previous situations (high mannose intake and very low $^{13}$C content) which, after incorporation of the low-$^{13}$C mannose into glycoproteins, diluted the label provided by glucose.

This hypothesis was tested in a pulse-labelling experiment using mannose with different $^{13}$C contents and testing some other dietary sugars. Since the effect of dietary mannose was maximum on the second day for the continuous-labelling experiment, the effect of specific dietary sugars was studied at a similar interval after the end of the nutritional period. In this experiment it was expected that the decrease in glycoprotein-sugar labelling induced by preferential incorporation of specific sugars would have been particularly clear with artificially-enriched dietary glucose, since the difference in $^{13}$C content between dietary glucose (artificially-enriched glucose + glucose from starch) and other specific dietary sugars was about 0.3%. When compared with the control, no dietary sugar significantly modified $^{13}$C enrichment, but there was a trend towards a decrease in $^{13}$C enrichment with the mannose C and a trend towards an increase with the mannose D. One-way analysis of variance gave highly significant $F$ values and further statistical comparisons indicated significant differences between some groups for all the glycoprotein-neutral sugars in the intestine and in the serum: between dietary mannose C and D and between dietary fucose ($^{13}$C 1.1%) and the richest mannose D ($^{13}$C 1.0979%). In some cases the effect of dietary galactose was also significant. All these results confirm those of the first continuous-labelling experiment. First, they indicate that specific dietary sugars are not preferential substrates for glycosylation, although it might be argued that rats were adapted for 1 week to a diet devoid of these sugars so that their entry into glycoprotein metabolism could be impaired. Second, they confirm that increases in $^{13}$C enrichment cannot be observed if the $^{13}$C content of dietary mannose is too low, diluted by the label provided by glucose. Third, the differences in $^{13}$C enrichment between some treatment groups, with little or no modification in glycoprotein-sugar levels, might also support the hypothesis of possible regulatory effects of some specific dietary sugars, especially galactose and mannose, on glycoprotein metabolism.

Interpretation of these results remains difficult at the present time, but the surprising observation that the $^{13}$C-enrichment patterns in the nutritional groups are very similar for all the glycoprotein-sugars in the serum and the intestine suggests general metabolic effects common for glycoprotein-sugars. One hypothesis might be to suggest a channelling from glucose to glycoprotein-sugars, regulated by a specific sugar acting at the level of
GLYCOSYATION AND SPECIFIC DIETARY SUGARS

Glycoprotein-sugar turnover by modifications of sugar-nucleotide or glycoprotein biosynthesis or breakdown or both. A second, non-exclusive, hypothesis is that regulation could be exerted at the level of endogenous pools of sugars for glycoprotein metabolism. Indeed, in the two types of experiments (continuous- or pulse-labelling) the $^{13}$C content of glucose present in the diet is markedly higher than the $^{13}$C content determined in glycoprotein-neutral sugars. Since $^{13}$C content corresponds to a specific activity, this fact might suggest that the label is diluted in endogenous sugar pools.

The specific sugars were added transitorily to the diet for a single nutritional period; thus, due to the low amount ingested and the delayed effect (maximum about 28 h after a specific sugar is withdrawn from the diet), some enzymic modifications could be suspected rather than direct effects of specific sugars on enzymic activities. Direct effects of sugars on the expression of some genes has already been reported for glucosamine and glucose in cell cultures (McClain et al. 1992) and for dietary mannose in vivo (Miyamoto et al. 1993), although in this last case the amount of dietary mannose (550 g/kg diet) was not relevant to nutrition. Since the amounts of specific sugars are not controlled in standard laboratory animal diets, it could be one of the sources of natural variations reported in experiments performed on glycoprotein metabolism at different times in different laboratories.

A general problem arises in relation to the absorption kinetics of minor dietary sugars, since preferential incorporation can take place only if specific sugars are absorbed. The intestinal absorption rate of these sugars is low compared with that of glucose and represents about one-tenth of glucose absorption (Wood & Cahill, 1963). However, mannose and fucose absorption in rodents is important, since only 5-9 and 13-24% of an ingested dose of mannose and fucose respectively are recovered in the faeces (Bocci & Winzler, 1969; Brydon et al. 1987). A proportion of specific dietary sugars must be in the form of various glycans which theoretically are not digested in the small intestine and could be degraded in the caecum. However, glycan-derived sugars might constitute a useful source, as reported for radioactive fucose and glucosamine bound to proteins (Robinson, 1967, 1968; Bocci & Winzler, 1969). The effect of microflora on such specific sugars or glycans has never been studied, but the microflora is not responsible for the different kinetics of the tracer between injection and ingestion (Robinson, 1968).

Experimental conditions for application to man can be deduced from these results. The use of natural labelling would induce the consumption of very large amounts of maize starch to obtain significant labelling of glycoproteins, since in the present study rats ate about 70 g starch/kg body weight. From preliminary human experiments using ingestion of 100 g maize starch in 30 min, natural labelling appears to be insufficient. No enrichment was detected in glycoprotein-sugars up to 6 h after ingestion, despite early glucose $^{13}$C appearance in the plasma (Normand et al. 1992). Future studies in man will necessitate the use of artificially-enriched $^{13}$C compounds at doses which remain to be determined. In the present study, $^{13}$C-labelled-glucose consumption by rats was about 90 mg/kg body weight diluted to about 30 g/kg body weight by glucose from starch. A similar dilution could be used in man. Information about mechanisms for the metabolic effects reported in the present paper will require more refined studies in animals, but the results also encourage the investigation of such effects in man.

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