

Use of stable isotopes naturally occurring in dietary compounds for the study of the metabolism of glycoprotein sugars and its nutritional regulation in the rat. C Rambal¹, C Pachiaudi², S Normand², JP Riou², P Louisot¹, A Martin¹ (¹INSERM U 189, Faculté de Médecine Lyon-Sud, BP 12, 69921 Oullins Cedex; ²INSERM U 197, Faculté de Médecine Alexis Carrel, Rue Guillaume Paradin, 69008 Lyon, France)

Metabolic or nutritional studies using stable isotopes are being carried out on healthy human subjects. The metabolism of glycoprotein sugars and their nutritional regulation in man may be studied *via* dietary compounds naturally enriched in ¹³C. As a prerequisite, we have tested the practicability of this technique in the rat *via* the study of glycoproteins in the serum and in particular in the intestine, in which the existence of nutritional regulation was demonstrated.

Rats were fed for 1 wk on a semi-synthetic diet containing wheat starch (1.08692% ¹³C) as the only sugar source, then for 1 wk on the same diet containing corn starch (1.10042% ¹³C). At the end of the wheat starch diet (basal values) and at various intervals after the change in diet, rats were killed and intestinal mucosa was harvested and homogenized. Macromolecules were precipitated, delipidated and submitted to acidic hydrolysis. Hydrolysates were purified by ion exchange. Neutral sugars were reduced and acetylated. Alditol acetates were

separated by gas chromatography and analyzed by coupled isotope ratio mass spectrometry, which allows single-step determination of sugar amount and its isotopic enrichment.

Determinations are possible on glycoprotein sugars from 200 mg intestinal mucosa or 1 ml serum. Analytical variation for isotopic enrichment is \approx 3% in the same sample. From different samples, variations were higher but enrichment of 0.001% above basal values were found to be significant. One-way variance analysis indicated that the evolution in isotopic enrichment of intestine and serum glycoproteins were significant.

For riboses enrichment increased linearly with time over a 1-wk period. For glucose there was a peak at 12 h after change in diet, then a decrease from 40 to 68 h. Enrichment increased again up to 158 h.

For glycoprotein sugars results were different: for fucose and galactose a significant enrichment appeared at 12 h after change in diet to reach a plateau between 40 and 85 h. Then it increased up to 158 h. By contrast, mannose enrichment increased only from 68 h after change in diet. In serum glycoproteins, enrichment after 1 wk was of the same order of magnitude than that in intestinal glycoproteins.

Maximum enrichment was never higher than 30% of maximum theoretical enrichment (calculated from the difference in ¹³C contents between corn and wheat). However, the results demonstrate that the technique is sensitive enough to determine significant enrichment in glycoprotein sugars *via* the use of naturally ¹³C enriched dietary compounds.