

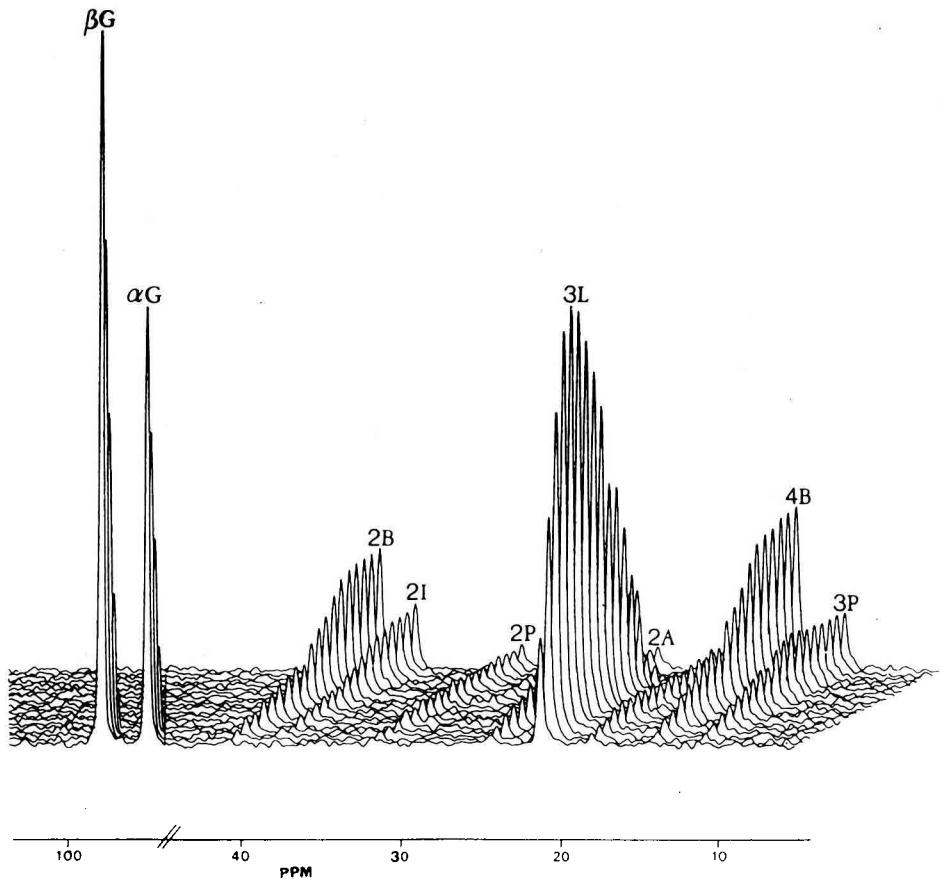
***In vivo*  $^{13}\text{C}$  NMR study of glucose and lactate catabolism by isolated hindgut bacteria.** J Stévani <sup>1</sup>, JP Grivet <sup>2</sup>, G Hannequart <sup>1</sup>, M Durand <sup>1</sup> (<sup>1</sup> INRA, Laboratoire de Nutrition et Sécurité Alimentaire, 78352 Jouy-en-Josas Cedex; <sup>2</sup> CNRS, Centre de Biophysique Moléculaire, 45071 Orléans Cedex 2, France)

$^{13}\text{C}$  Nuclear magnetic resonance (NMR) allows *in situ* study of the biochemical reactions taking place in live cells. NMR was applied in the present study to the investigation of the anaerobic

pathways of pig hindgut microflora metabolism, which are at present not well known.

Bacteria from the liquid phase were separated by differential centrifugation and suspended in NMR buffer (phosphate,  $\text{N}_2$  saturated). At time  $T_0$  ( $1\text{-}^{13}\text{C}$ ) glucose or ( $3\text{-}^{13}\text{C}$ ) lactate, 99.9% labelled, were injected in the thick bacterial suspension (to a final concentration of 40 or 20 mM, respectively). Cell incubations were made directly in a NMR spectrometer (Bruker AM 300) as described by Grivet *et al* (1989).

Glucose was quickly degraded and gave rise to a transient  $\text{C}_3$ -labelled lactate accumulation. As soon as the glucose was depleted, lactate



**Fig 1.** Example of spectra acquired during a kinetic run. Each trace corresponds to a 10-min recording period.  $\beta\text{G}$  : glucose  $\beta$ ;  $\alpha\text{G}$  : glucose  $\alpha$ ; 2B : butyrate  $\text{C}_2$ ; 2I : isobutyrate  $\text{C}_2$ ; 2P : propionate  $\text{C}_2$ ; 2C : acetate  $\text{C}_2$ ; 3L : lactate  $\text{C}_3$ ; 4B : butyrate  $\text{C}_4$ ; 3P : propionate  $\text{C}_3$ .

was fermented in short-chain fatty acids (SCFA) (fig 1).

Lactate directly added to the medium was also rapidly degraded into SCFA. Whatever the substrate, the calculation of the excess of the C<sub>3</sub> vs C<sub>2</sub> labelled propionate (Grivet *et al*, 1989) showed that this acid was produced in equal proportion by the succinate and acrylate path-

ways. By comparison, the latter explains only 24% of propionate production by rumen bacteria.

#### *Reference*

Grivet JP, Stévani J, Hannequart G, Durand M (1989) *Reprod Nutr Dev* 29, 83-88