Mechanism of Liver Glycogen Repletion In Vivo by Nuclear Magnetic Resonance Spectroscopy

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24 h before study to deplete liver glycogen. At 8 a.m. on the day of study, mg/g body wt, under light ether anesthesia which lasted <2 min. The glucose was enriched by 50% with [1-^{13}C]glucose. Four rats were sacrificed at 1 h after glucose ingestion and four after 3 h. A second group of eight rats received a lower dose of glucose, 1 mg/g body wt, that was 99% enriched with [1-^{13}C]glucose. Four rats were sacrificed at 1 h post glucose and four after 2 h. In all studies tracer amounts of [1-^{14}C]glucose were added to the ingested glucose to monitor constancy of the plasma glucose specific activity. Following glucose ingestion, the animals were allowed

Samples were heated to 340°K to shift the HDO resonance upfield from

The ^{13}C-fractional enrichment of plasma C-1 glucose, C-3 alanine, and C-3 lactate were determined from ^{1}H-NMR spectra using a homonuclear double resonance spin echo difference sequence (9, 10). A 10-s relaxation delay was used for the glucose and a 15-s delay for lactate and alanine determinations. During the relaxation delay, the HDO resonance was saturated with a 50 mW single radio frequency field (8). During the spin echo τ delay (136 ms for glucose, 68 ms for lactate and alanine), single frequency ^{1}H-decoupling was applied at 50 mW. All

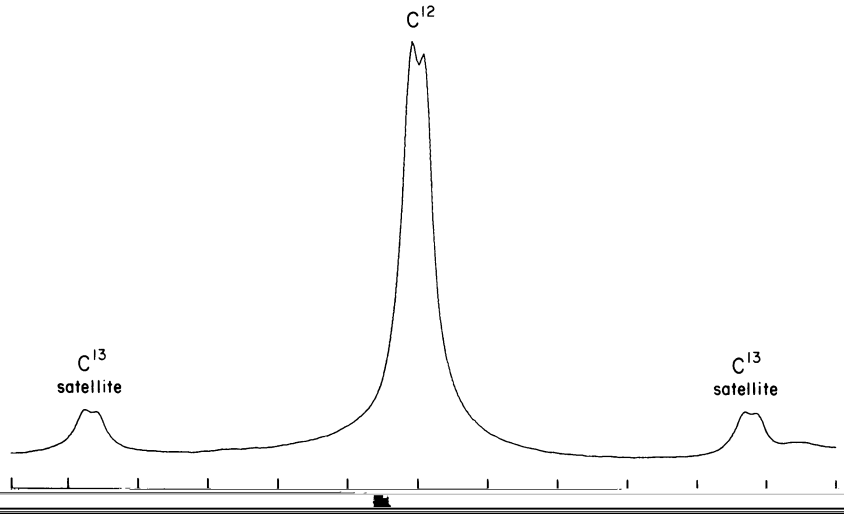

Figure 2. Representative ^{1}H-NMR spectrum of the C-1 position of the glucosyl unit obtained from hydrolyzed hepatic glycogen.

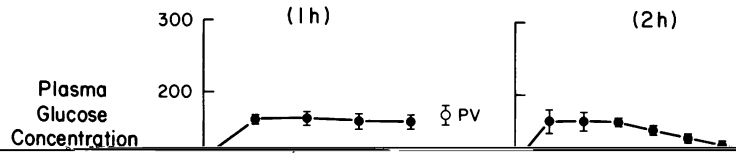

Figure 4. Time course of change in mixed ve-

was derived from alanine plus lactate. These represent minimum values because labeled oxaloacetate (OAA) derived from these gluconeogenic precursors is diluted by unlabeled OAA derived from the TCA cycle (11). In fed rats it has been determined that this dilution factor is 1.38 (12). Using this value the contribution of alanine/lactate to glycogen synthesis can be estimated to be 10 and 28% in high- and low-dose groups, respectively.

isomerase level to glycogen synthesis was also calculated from

lead to an underestimation of the amount of glucose that was directly incorporated into glycogen. The activity of the pentose cycle has been examined both in vitro (18) and in vivo (19) and found to represent no more than 10% of the total glucose flux in both circumstances. Furthermore, other workers (4, 20) found that the ratio of the specific activities of [^{14}C]glucose in liver glycogen to administered [^{14}C]glucose were nearly identical

support the assumption that the activity of the pentose cycle in

discrepancy is not clear but may be related to differences in the diet, age of the rats, method of glucose administration (bolus vs. continuous infusion), lighting conditions, or actual length of fast. Newgard et al. (4) suggested that the dose of glucose might play an important role in determining the pathway via which glycogen is replenished following feeding. Their results suggested

untenable assumption, especially under the hyperinsulinemic conditions of our study.

Glycogen synthesis from the indirect pathway, involving glucose conversion to triose-phosphates and subsequent conversion to glycogen, was also calculated and found to represent 3 and 1% of hepatic glycogen synthesis in the high- and low-

a minimum of between 10 and 28% of the glycogen synthesized:

and that (*d*) the three pathways through which the labeled flux

exchange in the calculation of the rate of gluconeogenesis in rats. *Biochem. Med.* 29:372–378.