Estimation of gluconeogenesis and glycogenolysis *in vivo* using tritiated water

Hepatic glucose output can be readily estimated at different stages of fasting by accepted procedures involving primed continuous infusion of [6-³H]glucose to steady state. In the long-term fasted state in which liver glycogen is essentially depleted, there is no difficulty in estimating gluconeogenesis, since this is equal to the endogenous glucose output. However, with shorter fasting periods, hepatic glucose output will be derived both from glycogenolysis and gluconeogenesis. Based on earlier studies *in vitro* with ³HHO [1], I describe an approach involving tritium incorporation onto C-6 of glucose *in vivo* to estimate relative contributions of gluconeogenesis and glycogenolysis to hepatic glucose output in the fasted state.

In previous experiments with isolated hepatocytes, in a medium containing ³HHO, tritium was incorporated extensively on to all six carbons of the glucose formed, when L-lactate, pyruvate or L-glutamine was the gluconeogenic substrate employed [1]. Tritium incorporation on the two positions on C-6 of glucose was nearly 90 % of the specific activity of the medium ³HHO. However, when glucose was formed in a ³HHO medium from glycogenolysis, under anaerobic conditions to prevent any gluconeogenesis, there was negligible tritium incorporation on C-6 of glucose, although extensive incorporation on to C-2 was found, as expected [2]. During gluconeogenesis from L-lactate, tritium was also extensively incorporated onto carbons 2, 3, 4 and 5, and this was true also when gluconeogenesis occurred from substrates that entered the gluconeogenic pathway at the triose-phosphate level, substrates such as fructose and dihydroxyacetone. When glucose was formed from fructose in hepatocytes from fasted rats, the tritium specific radioactivity on each hydrogen of C-6 of glucose was about 8 % of that of the medium ³HHO [1], and this incorporation was reduced to about 2 % when the phosphoenolpyruvate carboxykinase inhibitor, mercaptopicolinate, was added (my unpublished results). Thus lactate formation from fructose and a degree of gluconeogenesis from this lactate may have produced much of this C-6 labelling when fructose was the gluconeogenic substrate. It seems likely, therefore, that tritium incorporation on C-6 is largely confined to those gluconeogenic substrates that enter the pathway at the pyruvate level, or at the level of a Krebs cycle intermediate. Glycerol produced from lipolysis will also contribute carbon for gluconeogenesis, although this source has been estimated to be less than 10 % of the total hepatic glucose production [3,4]. As glycerol enters the gluconeogenic pathway at the triose-phosphate level, we assume negligible tritium incorporation on C-6 of glucose from this source *in vivo*. Glycerol gluconeogenesis must be independently determined from steady state infusion of ¹⁴C-labelled glycerol [3,4].

The difference between net hepatic glucose output and glycerol gluconeogenesis can be partitioned between glycogenolytic glucose production and non-glycerol gluconeogenesis. I am neglecting pentose cycle flux, which should be a small net outflow of carbon in the fasted, non-lipogenic and gluconeogenic state, where the stoichiometry of the cycle is 1 glucose 6-phosphate → 6 CO₂ [5]. To determine the extent of tritium incorporation from

³HHO on C-6 of glucose during gluconeogenesis *in vivo*, I use animals fasted for a sufficient time to deplete liver glycogen, and isolate plasma glucose approximately 2 h after intravenous injection of ³HHO. Mixing of ³HHO within the whole body should be complete enough within this time. The tritium specific radioactivity of the purified plasma glucose can be determined by periodate degradation according to Bloom [6], and the plasma water specific radioactivity measured by suitable dilution of an aliquot. By subtracting glycerol gluconeogenesis from the total hepatic glucose output, I obtain a factor for the average tritium incorporation on to each hydrogen atom of C-6 of glucose formed *in vivo* from non-glycerol gluconeogenesis. Then in experiments with shorter fasting periods, using again ³HHO preinjection, dividing the actual tritium specific radioactivity (normalized to that of the body ³HHO) by this factor gives the rate of non-glycerol gluconeogenesis. Glycogenolysis can be determined by difference, providing that glycerol gluconeogenesis and total hepatic glucose output are determined independently.

Plainly the approach as described above is totally inapplicable to human use. Even in animal studies, large amounts of ³HHO are required (about 10–100 mCi/100 g body wt.). However, humans tolerate without harm considerable amounts of ²H₂O, at least up to a final level of 0.2 atom% excess [7], and 0.5 atom% excess has also been employed [8]. Provided that the deuterium enrichment on C-6 of plasma glucose could be determined at these levels [9], the approach could be extended to human subjects.

The major advantage of the ³HHO method for the estimation of gluconeogenesis is that correction factors for the interactions with Krebs cycle, fatty acid and amino acid carbon are not required. These interactions have made attempts to estimate gluconeogenesis *in vivo* using ¹⁴C-labelled substrates rather difficult, and there is little rigour to date in most of the calculations based on such models of gluconeogenesis.

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