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SOME OBSERVATIONS ON THE USE OF D-GLUCOSE-2-^{3}H AS A TRACER IN TURNOVER STUDIES IN MERINO SHEEP

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ABSTRACT

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Bolus injection of a mixture of D-glucose-2-^{3}H and -(U)-^{14}C into each sheep enabled a turnover rate to be calculated for each isotope and to be compared in the same animal. No statistically significant differences were found in pool size, or glucose space. The total entry rates calculated from the D-glucose-2-^{3}H results were found to be significantly higher ($P > 0,95$ paired t test) than those calculated from the D-glucose -(U)-^{14}C results, the average difference being 18%. This difference indicated that the extent of glucose recycling was somewhat less than that reported for monogastric mammals (30%—Katz & Dunn, 1967) and similar to that found by Judson & Leng (1972) in sheep.

Glycogen was isolated from skeletal, cardiac and smooth muscle as well as from the liver and the ^{3}H: ^{14}C ratios determined. These ratios were found to be significantly higher than those reported for monogastric mammals such as the rat or dog.

INTRODUCTION

Our interest lies in the intermediary metabolism of glucose, its precursors and catabolites as well as the hormonal control of this metabolism in the whole animal. The tracer turnover techniques that have been developed to follow such interactions under physiological conditions are well suited for our studies. All the earlier results have been obtained using ^{14}C labelled substrates and Black (1969) recently questioned this practice in the measurement of whole body glucose turnovers. He postulated a significant recycling of carbon during the catabolism of glucose, which would lead to an underestimation of glucose turnover. Significantly higher glucose turnovers were obtained in the rat when ^{3}H-glucose was injected instead of ^{14}C glucose.

In particular, 2-^{3}H-glucose appeared to yield a turnover rate corrected for carbon recycling due to both the Cori Cycle as well as glycogenolysis (Katz & Dunn, 1967). This was ascribed to the early irreversible loss of ^{3}H from the glucose molecule shortly after tissue uptake mediated by the isomerase reaction (glucose - 6 - phosphate → fructose - 6 - phosphate) described by Rose & O'Connell (1961). This was later confirmed in further experiments using rats (Dunn, Chenoweth & Schaeffer, 1969; Katz & Rognstad, 1969, Hetenyi & Mak, 1970) as well as dogs (Issekutz, Allen & Borkow, 1972).

When Judson & Leng (1972) compared the glucose turnover rates in sheep injected or infused with ^{14}C or ^{3}H labelled glucose they found no significant differences. They postulated that there was little recycling of carbon during glucose catabolism in sheep and that either ^{3}H or ^{14}C may be used as a label in glucose turnover studies.

In order to verify these results in our experimental sheep and to test the validity of the bolus injection technique these experiments were repeated. Moreover, since glycogen has been found to play an important role in the glucose metabolism of our sheep (Procos, J., Vinnik, A. Van der Walt, J. G. 1969) it was decided that the labelling patterns in this metabolite should be investigated at the same time.

MATERIALS AND METHODS

Experimental procedure

Seven adult Merino wethers were housed in single pens and fed 1 600 g lucerne hay (ca. 16% crude protein) daily (800 g at 08h00 and 800 g at 16h00). On the day prior to an experiment the left jugular vein of each sheep was catheterized with a Braunule plastic needle which was fitted with a stainless steel 3 way tap. The assembly and catheter were flushed and filled with heparin-saline (500 I.U./ml) to prevent blockage. The sheep were fed as usual on the day of the experiment and a bolus injection of the combined tracer glucose was given at 10h30. Four sheep received an injection of 200 μCi D-glucose-2-^{3}H plus 150 μCi D-glucose-(U)-^{14}C while the remaining three received 162 μCi D-glucose-2-^{3}H plus 235 μCi D-glucose-(U)-^{14}C. Jugular blood was sampled (10 ml) at regular intervals for the next 2 hours after which the sheep were anaesthetized with an overdose of Euthatal† and tissue samples rapidly removed for glycogen analysis.

Chemical analysis

Each 10 ml blood sample was immediately added to 10 ml cold 1N perchloric acid and well mixed. After centrifugation in the cold at 10 000 × *g* for 10 minutes, 10 ml of the supernatant was neutralized with 0,85 ml of 8 N potassium hydroxide and allowed to stand for 30 minutes. The resulting suspension was centrifuged at 15 000 × *g* for 20 minutes. A 9 ml portion of the supernatant was acidified with 5 drops of glacial acetic acid and taken to dryness on a steambath under a stream of dry nitrogen. The residue was transferred to a 5 ml volumetric flask using 0,3 N acetic acid.

Aliquots of 1 ml were eluted through columns (0,9 × 30 cm) of Sephadex G 10* equilibrated against 0,3 N acetic acid to separate glucose and lactate (Riley, 1968). Instagel scintillation fluid** was added to the fractions, and the ^{14}C and ^{3}H content estimated using a Packard Tricard Series 3 000 liquid scintillation spectrometer according to the method of De Wachter & Fiers (1967). The glucose and lactate concentrations were determined with the Blood Sugar, GOD-Perid-Method and the Lactate, U-V Method test kits respectively***.

Tissue glycogens were extracted with boiling 30% potassium hydroxide. After alcohol precipitation the glycogens were hydrolysed with sulphuric acid to glucose, which was further analysed as described above.

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† Maybaker

* Pharmacia

** Packard Instrument Co.

*** Boehringer Mannhein C.M.B.H.

Results

The parameters listed in Table 1 were calculated from the isotope dilution data using the methods outlined by Katz & Dunn (1967) and White, Steel, Leng & Luick (1969). Only the first exponential phase of the dilution curve was analysed further and the method of least squares was used to obtain the straight line of best fit (See Fig. 1 for a typical example). The paired t-test was used to evaluate the differences found in the half time, the pool size, the total entry rate and the glucose space. No statistically significant differences were noted in the glucose pool or space. The 18% difference between the total entry rates calculated from the ^{14}C or ^{3}H data was found to be significant at the $P>0,95$ level which indicated that some glucose carbon was being recycled during the course of the experiment.

TABLE 1 Some parameters of glucose metabolism calculated from turnover data obtained from the simultaneous injection of U-^{14}C and 2-^{3}H glucose

Glucose injected	Half time min	Pool† mg/kg^{0,75}	Total entry rate mg/min/kg^{0,75}	Space* % body mass
U-^{14}C...	74±33	334±88	3,32±0,76	16,9±3,35
2-^{3}H....	63±19	335±67	3,91±0,78	17,1±2,64

Data are given as the mean of 7 values ± standard deviation
* Average metabolic mass of sheep = 18,65±3,4 kg^{0,75}
† Average plasma glucose = 72,4±4,6 mg/100 ml. plasma

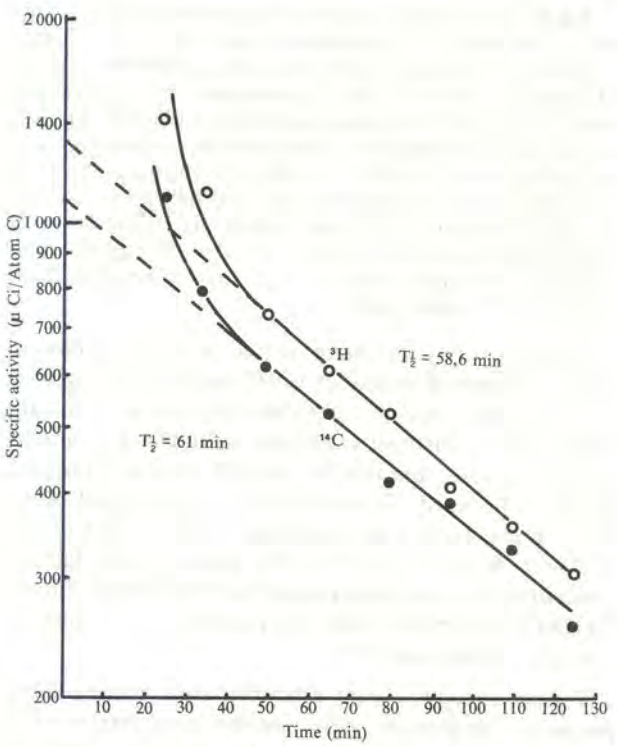

FIG. 1 Dilution curve of blood glucose after bolus injection of 2-^{3}H and U-^{14}C glucose showing best line fit to first exponential phase

The ^{3}H: ^{14}C ratios presented in Table 2 were calculated according to Katz & Dunn (1967) and Dunn *et al.*, (1969). These ratios, obtained from sheep, when compared to the ratios found in rats by Katz & Dunn (1967), would seem to indicate large differences in the pattern of glucose metabolism. The final and midpoint ratios calculated for blood glucose in the sheep reveal that the irreversible loss of ^{3}H from the glucose pool is only slightly faster than that of ^{14}C (final blood glucose ratio 0,858). This is a contrast to the situation in the rat where ^{3}H was rapidly lost to the body water pool (final blood glucose ratio 0,254). The high ratio found in sheep skeletal muscle glycogen emphasizes the relatively greater retention of ^{3}H in the glucose pool.

TABLE 2 ^{3}H: ^{14}C ratios found in various metabolites after simultaneous injection of U-^{14}C and 2^{3}H glucoes

Metabolite	^{3}H: ^{14}C ratio sheep	^{3}H: ^{14}C ratio rat*
Blood Glucose..Final..	0,858±0,089	0,254±0,038†
Blood Glucose—Midpoint.....	0,923±0,041	0,79±0,04
Blood Lactate.....	0,475±0,114	—
Liver Glycogen.....	0,406±0,035	0,306±0,088
Skeletal Muscle Glycogen.....	0,747±0,129	0,094±0,039
Cardiac Muscle Glycogen.....	0,408±0,072	0,072±0,04
Involuntary Muscle Glycogen.....	0,04±0,02	—

All ratios have been adjusted relative to an injection ratio of 1
* Data derived from Katz & Dunn (1967)
† Calculated for a 125 minute duration

Discussion

The relatively low rate of glucose recycling found in these experiments compares favourably with that reported by Judson & Leng (1972) for sheep (19%). The values obtained by Katz & Dunn (1967), Hetenyi & Mak (1970) in rats and Issekutz, *et al.* (1972) in dogs indicated that monogastric mammals had considerably higher recycling rates of about 30–40%. The values obtained for the parameters glucose space (17%), total entry rate (3,3–3,9 mg/min/kg^{0,75}) and pool size (334 mg/kg^{0,75}) compare favourably with those reported by others (Judson & Leng., 1972; White *et al.*, 1969; Dunn *et al.*, 1969, Bergman, Brockman & Kaufman, 1974).

The relatively high standard deviation here reported can be ascribed to the twice daily feeding regime (White *et al.*, 1969).

The high final ^{3}H: ^{14}C ratio found in the blood glucose (0,858) of sheep is the result of a relatively greater retention of ^{3}H rather than a greater loss of ^{14}C. According to Rose & O'Connell (1961) the value of this final ratio is controlled by 4 factors influencing the phosphohexose isomerase reaction. In the first place there is an isotope discrimination effect against the ^{3}H glucose leading to an initial retention of label. The relative contribution of the pentose cycle to glucose oxidation also influences the final ratio in that the glucose, as it by-passes the isomerase reaction, does not lose any H. However, little or no pentose cycle activity has been found in ruminant muscle tissue (Raggi, Hansson, Simesin, Kronfeld & Luick, 1961).

The relative rate of the isomerase reaction will also influence the final ratio. Nonetheless, it is considered to be high in most tissues and non-ratelimiting and so its importance is likely to be minimal. Large differences have been reported in the transfer: exchange ratios for isomerase enzymes isolated from various sources (Rose & O'Connell, 1961; Katz & Rognstad, 1969). On this basis it would seem reasonable to postulate that sheep muscle isomerase would have a transfer: exchange ratio different to that reported for rabbit muscle isomerase and closer to that found in rat epididymal fat pads (Katz & Rognstad, 1969).

The overall fate of glucose in the sheep is largely undetermined. However, the known values of 33% of the total production being oxidised and less than 1% being converted to fat are considerably lower than those for monogastric animals (Lindsay, 1969). If the rate of glucose uptake by sheep skeletal muscle is lower than in monogastric mammals then the ^{3}H: ^{14}C ratio will be further elevated. It would seem reasonable to ascribe the high ^{3}H: ^{14}C ratio found in sheep skeletal muscle to a combination of low uptake and altered isomerase enzyme kinetics. The cardiac muscle glycogen ^{3}H: ^{14}C ratio of 0,408 could reflect a greater uptake of glucose or an isomerase transfer: exchange ratio different to that in skeletal muscle. The ratio of 0,04 found in involuntary muscle glycogen indicates a glucose metabolism similar to rat skeletal and cardiac muscle.

That skeletal muscle is the major site of glucose catabolism is suggested by the high ^{3}H: ^{14}C ratio (0,475) found in blood lactate. This high ratio would seem to preclude the use of 2-^{3}H-glucose to measure glucose turnover rates corrected for Cori Cycle activity. Although there is little uptake of glucose by the ruminant liver (Bergman, Katz & Kaufman, 1970) the liver glycogen showed incorporation of ^{3}H(^{3}H: ^{14}C = 0,406) probably resynthesized mainly from the labelled lactate (^{3}H: ^{14}C ratio = 0,475) absorbed from the blood.

In summary, the utilization of the doubly labelled (^{3}H and ^{14}C) glucose bolus injection technique demonstrated that, although some recycling of glucose carbon occurred during the experiment, the magnitude of this recycling was small compared to that found in the monogasts. This finding will justify the use of glucose labelled with either isotope for the determination of the glucose metabolic parameters listed above, while simultaneously investigating another related metabolite labelled with the alternative isotope.

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