First-pass hepatic uptake and utilization of glucose in the rat

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First-pass hepatic retention of glucose had previously been measured indirectly from the appearance of ingested labelled glucose into the systemic circulation. To determine the accuracy of the procedure, results obtained by this indirect method were compared with those of direct measurement of hepatic retention of labelled glucose given by instantaneous injection into the portal vein. In the rat, the indirect procedure gave a value of $13.7 \pm 2.3\%$. In the direct method, [^{14}C]glucose was injected intraportally together with [^3H]sucrose as a marker of extracellular distribution. Hepatic content of both labels was maximal immediately after administration; the content of sucrose fell to basal values by 15 s, indicating that the injected bolus had passed through the liver; the content of [^{14}C]glucose continued to fall for 90 s. The difference in tissue retention between glucose and sucrose, representing intracellular glucose, was constant from 90 to 180 s, and indicated a first-pass retention of $13 \pm 0.7\%$. Thus the indirect procedure gives a reliable estimate of hepatic uptake and retention of glucose. Comparison of the time courses of hepatic content of [^{14}C]glucose, [$2\text{-}^3\text{H}$]glucose and [^3H]sucrose indicated that 50% of portal-vein glucose enters the hepatic cells; subsequently 15% traverses the glucose/glucose 6-phosphate futile cycle, 22% is released without undergoing metabolic change and 13% is retained for metabolic purposes.

INTRODUCTION

The rate at which the liver removes glucose from the load supplied by the portal vein and hepatic artery (hepatic glucose extraction, first-pass hepatic retention) has been measured by two techniques, both indirect. In the first, labelled glucose given orally is absorbed and transported via the portal circulation to the liver; its rate of appearance in the systemic circulation integrated over time provides a measure of the total amount of the oral load which appears in the circulation, and the difference between this value and the ingested load is the amount retained in a single passage through the liver. In the second method, hepatic extraction is calculated as the net splanchnic removal of orally administered labelled glucose, measured by the arterial-hepatic-vein catheterization technique. These approaches have given quite variable results. By the first method, values of 16% (Steele *et al.*, 1968) and 10% (Abumrad *et al.*, 1982) have been obtained in the dog, and values of 8% (Radziuk *et al.*, 1978) and 26–33% (Hall *et al.*, 1980) in man. The second method gave a value of 3% in man (DeFronzo *et al.*, 1983).

Both methods are indirect, and, although the assumptions on which they are based seem reasonable, neither procedure has been validated. The purpose of the present work was to compare the first of these indirect procedures with a direct measure of hepatic glucose retention, by using the procedure of Oldendorf (1970) for measurement of metabolite uptake by the brain and adapted by Pardridge & Jefferson (1975) to the study of liver uptake of amino acids and sugars. In addition we have studied the immediate fate of the glucose taken up by the liver cells.

METHODS

Adult male rats weighing 225–260 g were given standard laboratory chow and water *ad lib*. For the direct

measurement of liver uptake, they were injected with phentolamine (0.2 mg intramuscularly) to inhibit glycolysis, and anaesthetized 30 min later with sodium pentobarbital intraperitoneally; the abdomen was opened and the portal vein exposed. Normal rat serum (0.1 ml) containing 0.5 μCi of D-[U- ^{14}C]glucose and ^3H -labelled compounds as indicated in the Results section were injected rapidly as a bolus into the portal vein. At a precise time after the injection, a portion of the left lateral lobe of the liver was rapidly excised with scissors (previously chilled in liquid N_2) and then frozen in liquid N_2 and weighed. In some experiments a sample of blood was immediately taken from the inferior vena cava and chilled for later measurement of glucose. The remainder of the liver was then excised, blotted and weighed. At a later time the initial liver sample was weighed, brought to 0 °C, and homogenized in an equal volume of 1 M-HClO₄ at 0 °C. The protein-free supernatant was titrated to pH 6.5–7 with KOH and the insoluble KClO₄ removed by centrifugation for 10⁵ g·min at 4 °C. The supernatant was made up to 10 ml, and samples were taken for measurement of radioactivity in a liquid-scintillation counter with automatic external standardization for quench correction. The liver uptake index for D-[^{14}C]glucose was measured by reference to $^3\text{H}_2\text{O}$ or L-[^3H]glucose. The uptake index is defined as

$$\frac{^{14}\text{C d.p.m.}/^3\text{H d.p.m. in liver}}{^{14}\text{C d.p.m.}/^3\text{H d.p.m. of injected material}} \times 100$$

(Pardridge & Jefferson, 1975). In experiments involving glucose feeding, 5 ml of 60% (w/v) glucose solution was given by stomach tube 60 min before anaesthesia; control animals received 5 ml of water. Total glucose retention in the liver was calculated from the radioactivity per g of liver sample and the total liver weight (sample + residual liver mass).

For the indirect measurement of hepatic retention, animals were injected with phentolamine and anaestheti-

zed with Nembutal. [^3H]Glucose in 0.9% NaCl ($5\ \mu\text{Ci}/\text{ml}$) was infused into the right jugular vein for the duration of the experiment (90 min), at a rate of 1 ml/h. From 60 to 80 min an additional infusion containing $5\ \mu\text{Ci}$ of [^{14}C]glucose in 1 ml of saline was given; this was delivered at a rate of 1 ml/h into the portal vein, by using a fine (28-gauge) needle so as not to obstruct the normal portal blood flow. From 60 to 85 min blood samples (0.3 ml) were taken at 5 min intervals from a catheter previously inserted through the left femoral vein into the inferior vena cava; these were used for measurement of glucose and glucose radioactivity.

The rate of appearance of unlabelled glucose in the circulation was calculated from the concentrations of glucose and [^3H]glucose by using the formula of Steele (1959). The rate of appearance of [^{14}C]glucose in the systemic circulation was calculated as described by Steele *et al.* (1968) for orally administered glucose, with correction of the ^{14}C specific radioactivity for recycling (Reichard *et al.*, 1963); the results are expressed as per cent of the total infused label/min. The difference between the amount of glucose infused over the 20 min period and the total amount appearing in the systemic circulation is taken as the amount retained during a single passage through the liver (Steele *et al.*, 1968).

Since the calculation of the rate of appearance of glucose is dependent on the apparent volume of distribution of the labelled glucose, this was determined for the rat in preliminary experiments using 3-*O*-methylglucose, a sugar which undergoes membrane transport like glucose but which cannot be metabolized. 3-*O*-[$\text{Me-}^3\text{H}$]Methyl-D-glucose ($5\ \mu\text{Ci}$) in saline was infused intraportally over a period of 30 min at a rate of 1 ml/h. Blood samples taken through a catheter in the vena cava were deproteinized and assayed for radioactivity; for each sample the apparent volume of distribution was estimated from the amount of 3-*O*-methylglucose infused from zero time and the plasma concentration. 3-*O*-[^3H]methylglucose was distributed rapidly, giving an

Fig. 1. Volume of distribution of 3-*O*-[^3H]methylglucose infused into the portal vein

This is calculated as: d.p.m. infused from zero time/plasma concentration (d.p.m./ml). Results are means $\pm$ S.E.M. for five experiments.

apparent volume of distribution of 230 ml/kg body wt. after 5 min, and an equilibrium value of 245 ml/kg by 10 min (Fig. 1). The value obtained at 5 min was subsequently used in estimating the rates of entry into the systemic circulation of unlabelled glucose and of portally infused [^3H]glucose.

Glucose concentration in plasma was measured with a Beckman glucose analyser. For measurement of radioactivity by liquid-scintillation counting, plasma was deproteinized (Somogyi, 1952), and samples of the protein-free supernatant were dried at 65°C , redissolved in 1 ml of water and mixed with scintillation fluid (Formula 963; New England Nuclear). This procedure served to remove $^3\text{H}_2\text{O}$ from samples in experiments in which [^3H]glucose was administered.

RESULTS

Transhepatic circulation and hepatic retention of glucose

[^{14}C]Glucose and [^3H]sucrose (an extracellular marker) were injected intraportally and liver samples were taken at intervals up to 3 min. As shown in Fig. 2, the sucrose content of the liver fell rapidly, so that by 15 s only 5% of the injected dose remained; it then fell more slowly, with no indication of a secondary increase owing to recirculation. This pattern confirms the observations of Pardridge & Jefferson (1975), and indicates that passage through the liver was essentially complete by 15 s. Subsequently there was a slow disappearance of residual extracellular fluid. The tissue content of glucose was considerably greater than that of sucrose, the difference indicating the extent of intracellular transport: at 10 s this was approx. 50%. The intracellular content of the glucose label fell from 10 to 90 s, indicating outward transport. Subsequently the total liver content of ^{14}C fell slowly, but the intracellular label was constant at $13 \pm 0.7\%$ (mean $\pm$ S.E.M.) for the next 90 s. To determine the early fate of the intracellular glucose, [^{14}C] glucose and [^3H]glucose were injected intraportally; measurements were made of the tissue content of ^{14}C and of non-aqueous ^3H . As early as 5 s after injection the content of ^3H was significantly

Fig. 2. Time course of hepatic content of D-[^{14}C]glucose and [^3H]sucrose injected as an instantaneous bolus into the portal vein

Points are means $\pm$ S.E.M. for eight to ten animals each. Δ , Difference between glucose and sucrose.

Fig. 3. Hepatic retention of [^{14}C]glucose and [$2\text{-}^3\text{H}$]glucose injected as a bolus into the portal vein.

Points are means $\pm$ S.E.M. for the numbers of animals indicated in parentheses. Δ , Difference between ^{14}C and ^3H .

lower than that of ^{14}C ; subsequently the values for the two isotopes approached each other, and by 60 s they did not differ significantly (Fig. 3).

Liver uptake index

The uptake of D-glucose, measured 20 s after the injection of label, was 3–4-fold greater than that of L-glucose and identical with that of water (Table 1). The uptake was apparently not influenced by the plasma glucose concentration or by the absorption of glucose from the gastrointestinal tract. Total glucose retention in the liver after a single passage (20 s) measured in 16 animals was $46 \pm 7\%$ (mean $\pm$ S.E.M.) of the injected dose. The mean uptake index relative to L-glucose was 3.47; since L-glucose is confined to the extracellular space, the fraction of the injected glucose held intracellularly was $2.47/3.47 \times 46\%$, or 33%, in good agreement with the value of 37% at 20 s seen in Fig. 2, in which sucrose was the extracellular marker.

Indirect measure of first-pass hepatic retention of glucose

The mean values for glucose entry into the circulation are shown in Fig. 4. The entry of portally infused glucose fell to unmeasurable values immediately after the

Fig. 4. Rates of appearance in the systemic circulation of unlabelled glucose and of portally infused [^{14}C]glucose

The latter was given for 20 min; its rate of appearance is given as percentage of total infused label/min. Results are means $\pm$ S.E.M. for seven experiments. The total recovery of portally infused glucose in the systemic circulation was $86.3 \pm 2.3\%$ (mean $\pm$ S.E.M.); the first-pass retention was therefore $13.7 \pm 2.3\%$.

infusion was stopped; this is in accord with the direct measurement of liver glucose retention (above) in demonstrating that glucose to be released from the intracellular space is not retained in the liver longer than 90 s. The first-pass retention averaged $13.7 \pm 2.3\%$ (mean $\pm$ S.E.M.).

DISCUSSION

The hepatic cell membrane is freely permeable to water, so that movement of water into the cell is flow-limited

Table 1. Hepatic uptake of glucose from the portal vein in the rat

Animals in Groups 1–3 were given 5 ml of 60% glucose or water, by gavage, 60 min before the experiment. Results shown are means $\pm$ S.E.M. ($n = 12$ in each group), P values for the differences between means. Abbreviation: LUI, liver uptake index, calculated as

$$\frac{[^{14}\text{C}]/[^3\text{H}] \text{ in liver}}{[^{14}\text{C}]/[^3\text{H}] \text{ injected}} \times 100$$

Group	Experimental condition	Phentolamine	Tracers	Plasma glucose		LUI of D-glucose	
1	Glucose	—	D- ^{14}C]Glucose + L- ^3H]glucose	187 ± 8	$P < 0.01$	371 ± 23	$P > 0.1$
	Water	—		152 ± 6		341 ± 21	
2	Glucose	+	D- ^{14}C]Glucose + L- ^3H]glucose	151 ± 13	$P < 0.01$	330 ± 23	$P > 0.01$
	Water	+		94 ± 3		315 ± 31	
3	Glucose	+	L- ^{14}C]Glucose + $^3\text{H}_2\text{O}$	157 ± 19	$P < 0.05$	105 ± 2	$P > 0.1$
	Water	+		110 ± 5		99 ± 2	
4	Fed	+	D- ^{14}C]Glucose + L- ^3H]glucose	113 ± 13	$P > 0.05$	342 ± 22	$P > 0.05$
	Starved 24 h	+		95 ± 3		385 ± 22	

rather than membrane-limited (Pardridge & Jefferson, 1975). L-Glucose and sucrose are essentially excluded from the intracellular space, especially during short exposure times. In agreement with previous results, we have found that the rate and extent of entry of D-glucose into the cells are the same as those of water and 3–4-fold greater than those of L-glucose or sucrose. The liver uptake index of 3.47 with respect to L-glucose is identical with the value that can be calculated from the data of Pardridge & Jefferson (1975).

From 90 to 180 s after intraportal injection, the intracellular hepatic content of labelled glucose was virtually constant, at approx. 13%. The experimental procedure used here is analogous to a pulse-chase experiment, with endogenous portal glucose providing the 'chase'; the intracellular label retained after 90 s was not displaced by unlabeled glucose and was therefore no longer 'free' glucose. This study therefore provides an estimate of first-pass hepatic uptake and utilization of 13%.

Estimation of first-pass retention by the indirect procedure is dependent on the value of the apparent volume of distribution of the portally administered tracer, since this is not in a steady state. The volume of distribution has been estimated in the dog at about 130 ml/kg (Cowan & Hetenyi, 1964). It is apparent, however, that the value will depend on the length of the interval between entry of tracer into the plasma and the sampling of plasma for measurement of tracer concentration; the greater the interval, the greater the volume. In addition, the volume of distribution is dependent on the animal size; the smaller the animal, the less the time required for equilibration within the final volume. Thus it was necessary to measure the apparent volume of distribution in the rat under the conditions to be used within the main experiment. It was found that by 5 min the volume was 93% of the equilibrium value; the latter, 245 ml/kg, was reached by 10 min. The 5 min value was subsequently used in the estimation of the rates of entry of glucose into the plasma.

The estimate for first-pass retention by the indirect method of 13.7% is in close agreement with that obtained by the direct method and with those obtained by Williams *et al.* (1968) and by Topping *et al.* (1983), using a non-recycling perfusion of isolated liver. Thus the indirect method gives a reliable estimate of first-pass uptake.

The intraportal injection experiments also indicate the early fate of glucose taken up by the liver. The difference in retention between glucose and sucrose was due to

intracellular glucose; it decreased from 10 to 90 s, owing to outward transport from the cells. After this time the intracellular content was constant. The 15% difference in tissue content between ^{14}C and ^3H from C-2 of glucose within 10 s after injection indicates that a large portion of the intracellular glucose was rapidly phosphorylated and isomerized, with conversion of ^3H to water. This estimate is identical with that of Topping *et al.* (1983), based on liver perfusion. The subsequent elimination of the difference between ^3H and ^{14}C indicates loss of ^{14}C without an accompanying loss of ^3H ; we suggest that this was due to outward transport of [^{14}C]glucose which had undergone isomerization, and thus reflects the extent of the glucose $\rightarrow$ glucose 6-phosphate $\rightarrow$ glucose futile cycle. It is concluded that about 50% of the portal glucose entered the hepatocytes (Fig. 2), that within 1 min 15% of the glucose traversed the futile cycle and was released from the cells (Fig. 3), 13% was retained (Fig. 2) and (by difference) 22% was released without having been phosphorylated.

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