Gluconeogenesis from acetone in starved rats

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1. To non-anaesthetized rats starved for 3 days, [U-¹⁴C]acetone, NaH¹⁴CO₃, L-[U-¹⁴C]lactate, [2-¹⁴C]acetate or D-[U-¹⁴C]- plus D-[3-³H]-glucose was injected intravenously. 2. From the change in the plasma concentration of labelled acetone versus time after the injection, the metabolic clearance rate of acetone was calculated as 2.25 ml/min per kg body wt., and its rate of turnover as 0.74 μmol/min per kg. 3. The extent and time course of the labelling of plasma glucose, lactate, urea and acetoacetate were followed and compared with those observed after the injection of labelled lactate, acetate and NaHCO₃. 4. The labelling of plasma lactate was rapid and extensive. 5. Some 1.37% of the ¹⁴C atoms of circulating glucose originated from plasma acetone, compared with 44% originating from lactate. 6. By deconvolution of the Unit Impulse Response Function of glucose, it was shown that the flux of C atoms from acetone to glucose reached a peak at about 100 min after injection of labelled acetone. In comparable experiments the transfer from lactate reached a peak at 14 min after the injection of labelled lactate. 6. It was concluded that acetone is converted into lactate to a degree sufficient to account for the labelling of plasma glucose and is thus a true, albeit minor, substrate of glucose synthesis in starved rats.

INTRODUCTION

Acetone in plasma has been generally regarded to be an unmetabolizable waste product on the route to excretion by the lungs and kidneys. This view was first challenged by Price & Rittenberg (1950), who found that in long-term experiments rats convert [¹⁴C]acetone into ¹⁴CO₂ and 'active acetate'. Various amounts of radioactivity were also found in excreted urea, hepatic glycogen, cholesterol and some amino acids in tissue protein. Mourkides *et al.* (1960) found that mice oxidize labelled acetone to CO₂. From the distribution of the label in alanine and glutamate in tissue protein, they conjectured that acetone gives rise to both pyruvate and acetate in the liver. In lactating cows Black *et al.* (1972) demonstrated the incorporation of ¹⁴C atoms from [2-¹⁴C]acetone into glucose. The radioactivity incorporated was small and independent of the concentration of acetone in blood. Black *et al.* (1972) conjectured from the distribution of the ¹⁴C atoms in glucose that acetone labels hepatic oxaloacetate via both acetyl-CoA and other routes. The latter contribute to net glucose synthesis. Coleman (1980) demonstrated that mice do convert [2-¹⁴C]acetone into lactate. The enzyme system catalysing this conversion is a NADPH-requiring oxygenase in the liver. More recently Casazza *et al.* (1984) have identified two possible pathways of lactate formation from acetone. The common first step in both is the formation of acetol in the liver. By the 'methylglyoxal' pathway acetol is converted into methylglyoxal, which is further metabolized by glyoxalase to D-lactate, a precursor of glucose. Another, as yet unclarified, route from methylglyoxal to glucose is also likely to exist. A fraction of acetol is released into the circulation and is converted by extrahepatic tissues into propanediol. The 'propanediol' pathway leads to L-lactate in two steps. The first, catalysed by alcohol dehydrogenase, yields L-lactaldehyde, which is subsequently converted by aldehyde dehydrogenase into L-lactate, and returned to the liver.

The possibility of glucose formation from acetone in

man has been raised by Reichard and his associates (Reichard *et al.*, 1979; Owen *et al.*, 1982). In fasted men ¹⁴C from acetate was found in plasma glucose and protein, but not in non-esterified fatty acids, acetoacetate, β-oxobutyrate and 'anionic compounds'. In diabetics 20–80% of acetone was exhaled, the percentage being positively correlated with the plasma concentration of acetone. Plasma glucose became labelled to a very widely varying degree: 6 h after the injection of [2-¹⁴C]acetone, no radioactivity was found in plasma glucose in five out of eight patients. In the other three patients ¹⁴C in glucose accounted for 14, 20 and 31% of all radioactivity in plasma. Again non-esterified fatty acids, ketone bodies and 'anionic compounds' remained unlabelled. The lack of label in ketone bodies seems to exclude significant labelling of hepatic acetyl-CoA and is at variance with the hypotheses of Black *et al.* (1972) and Mourkides *et al.* (1960) based on experiments in different species. It was not specified whether lactate is part of the 'anionic compounds'.

Since the labelling of 'active acetate', presumably acetyl-CoA, by [¹⁴C]acetone has been claimed, the transfer of ¹⁴C atoms to glucose may be by 'metabolic exchange' in the hepatic oxaloacetate pool (Krebs *et al.*, 1966). It is also possible that the appearance of ¹⁴C atoms in glucose is by CO₂ fixation after the oxidation of labelled acetone. In either case acetone would not be a substrate of net glucose synthesis. Answers to these questions cannot be obtained from the experiments in which the distribution of label in amino acids (Mourkides *et al.*, 1960) or glucose (Black *et al.*, 1972) was studied, since the suggested pathways of the transfer were, and to some extent still are, unsubstantiated.

The purpose of the experiments described below was to establish to what extent the transfer of ¹⁴C atoms from [U-¹⁴C]acetone to glucose is by metabolic exchange or CO₂ fixation, as compared with pathways of net synthesis. For this purpose the transfers of ¹⁴C atoms from acetone, lactate, NaH¹⁴CO₃ and acetate to plasma glucose, urea, acetoacetate and lactate were compared.

In particular, answers to the following questions were sought. (1) What is the overall contribution of plasma acetone to the carbon atoms of plasma glucose? (2) Do plasma glucose, lactate, urea and acetoacetate become labelled after the injection of [U-¹⁴C]acetone? (3) If so, what is the extent and time course of the labelling? (4) How does the labelling of these compounds compare with those observed after the injection of labelled lactate, acetate and bicarbonate?

METHODS AND MATERIALS

Animals

Experiments were carried out on non-anaesthetized male Sprague-Dawley rats. The animals had access to water and food pellets (26% protein, 9% fat, 62% carbohydrate, 3% fibre, on a dry-weight basis) and were housed at 19–21 °C with a 12 h (07:00–19:00) light cycle. Then 3 days before the experiments a plastic cannula (Clay Adams PE-50) was introduced into the carotid artery. After surgery, food, but not water, was withdrawn until the experiment 3 days later.

Experimental design

In the first series of experiments, carried out on seven rats, 55.4–66.6 μ Ci of [U-¹⁴C]acetone was injected via the cannula. After repeated thorough rinsing with 0.9% NaCl and 0.9% NaCl/heparin (20 units/ml), five blood samples were withdrawn, at 10, 50, 120, 240 and 360 min after the injection of the tracer. The plasma concentrations of both labelled and unlabelled glucose, lactate, urea, acetone were determined in all samples, and that of acetoacetate only in the first and last samples.

In the second series of experiments (11 rats), 14.4 μ Ci of L-[U-¹⁴C]lactate, or 10.4 μ Ci of [2-¹⁴C]acetate, or 108.6–117.0 μ Ci of NaH¹⁴CO₃, was injected and blood samples were withdrawn at 4, 12, 30, 80, 130 and 190 min later. The plasma concentrations of labelled and unlabelled glucose, lactate, urea were determined in all samples, and those of acetoacetate in the first and last samples only.

In the third series of experiments, carried out on five rats, 10 μ Ci of D-[U-¹⁴C]glucose and 25 μ Ci of D-[3-³H]glucose were injected simultaneously. Blood samples were withdrawn at 4, 12, 25, 40, 80, 115 and 155 min after the injection. The plasma concentrations of unlabelled and labelled glucose (with respect to both labels) were determined in all samples.

Chemical analysis

The concentration of glucose was determined in 0.01 ml of plasma with a Beckman II Glucose Analyzer, and that of lactate as described by Hohorst (1963). Plasma urea was determined as described by Faulkner & King (1970), plasma acetoacetate as described by Williamson *et al.* (1962) and plasma acetone as described by Peden (1964).

Radioactive glucose was isolated from deproteinized plasma by the method of Dunn *et al.* (1957), lactate as described by Reilly (1974), urea (as ¹⁴CO₂) as described by Hetenyi *et al.* (1982) and plasma acetoacetate by the method of Mayes & Felts (1967) as modified by Wastney *et al.* (1984). Radioactivity in acetone was estimated as the difference in radioactivity before and after evaporation at 80 °C of effluent from the glucose column. By this

method the mean recovery of labelled acetone was 98%. Radioactive samples were counted in a Nuclear-Chicago Mark II liquid-scintillation spectrometer.

Specific radioactivity (SA) was expressed in units of d.p.m./ μ g of C, and the concentration of radioactive metabolites (*C**) as d.p.m./ml of plasma. *M** is the injected amount of tracer (expressed in units of d.p.m. $\times 10^6$).

Calculations

Curve-fitting was carried out by standard least-squares techniques. In the first series of experiments a single exponential function was fitted to the SA-versus-time curve for plasma acetone, and correction for the deviation from this curve for *t* < 50 min was made as described by Corney & Heath (1970). The ratio of integrals (from 0 to infinity) of the SA-versus-time curve of glucose to that of the injected labelled precursor was taken as the fraction of glucose carbon arising from the C atoms of the precursor (Shipley & Clark, 1972). This calculation disregards possible metabolic exchange of C atoms in intermediate pools (Krebs *et al.*, 1966) and underestimates the true contribution (Hetenyi, 1981; Hetenyi & Ferrarotto, 1983). The time course of the transfer of label from acetone or lactate to glucose was calculated by deconvolution, taking the *C*/M**-versus-time curve obtained in the third series of experiments as the Unit Impulse Response Function and the *C*/M**-versus-time curve of glucose as the output function after the injection of labelled acetone or lactate (Hetenyi, 1981).

The plasma clearance rate of a metabolite was calculated as $M^*/\int_0^\infty C^* dt$, and its turnover rate (*R_T*) as the product of the clearance rate and the plasma concentration.

Materials

[U-¹⁴C]Acetone (44 Ci/mol), NaHCO₃ (9.2 Ci/mol), L-[U-¹⁴C]lactate (> 100 Ci/mol), [2-¹⁴C]acetate (45–60 Ci/mol), D-[3-³H]glucose (10–20 Ci/mol) and D-[U-¹⁴C]glucose (15 Ci/mol) were purchased from New England Nuclear Co. (Lachine, P.Q., Canada). All other chemicals were obtained from Fisher Scientific Co., Toronto, Canada, and were of the highest grade of purity available.

RESULTS AND DISCUSSION

The mean body weight of the rats was 430 $\pm$ 6 g, and the mean concentration of glucose in their plasma 7.10 $\pm$ 0.33 mmol/l. In the 18 rats of the first and second groups the mean concentrations of lactate, urea and acetoacetate were 1.27 $\pm$ 0.14, 6.17 $\pm$ 0.41 and 0.45 $\pm$ 0.034 mmol/l respectively. The mean plasma concentration of acetone in the seven rats of the first group was 0.38 $\pm$ 0.02 mmol/l.

In Fig. 1 curves of specific radioactivity versus time for plasma acetone, glucose, lactate, urea and acetoacetate after the injection of [U-¹⁴C]acetone are shown. From the curve for plasma acetone and the injected amount of radioactive acetone (d.p.m.), the plasma clearance and the turnover rate of acetone were calculated as 2.25 ml/min per kg and 0.74 μ mol/min per kg respectively. The ratio of the integral (from 0 to ∞) of the curve for SA of glucose versus time to that for acetone was

Fig. 1. Specific radioactivity (SA) of plasma acetone, lactate, acetoacetate, glucose and urea (d.p.m./ μ g of C) after the intra-arterial injection of [U-¹⁴C]acetone at zero time

The specific radioactivities are normalized with respect to the injected amount of labelled acetone, M^* (d.p.m. $\times 10^6$). $\blacklozenge$, Acetone; $\bullet$, lactate; $\blacksquare$, glucose; $\circ$, urea; $\blacktriangle$, acetoacetate (AcAc).

calculated to be 0.0137, indicating that 1.37% of the C atoms in plasma glucose originated from muscle; the rapid and strong labelling of plasma lactate indicates the presence of the propanediol pathway of acetone metabolism (Casazza *et al.*, 1984) in the intact animal. Lactate nevertheless may have been produced by the methylglyoxal pathway too and either utilized in, or released by, the liver, possibly as a mixture of D- and L-lactate. In rats D-lactate is converted into L-lactate and glucose (Giesecke *et al.*, 1981). Therefore the possible contribution of the methylglyoxal pathway to the production of plasma lactate and consequently to gluconeogenesis cannot be excluded on the basis of the results of our experiments.

The labelling of plasma acetoacetate indicates the transfer of C atoms from labelled acetone to hepatocellular acetyl-CoA (Hetenyi & Ferrarotto, 1983). This confirms the conclusion reached by Price & Rittenberg (1950) about the conversion of acetone carbon into 'active acetate'. The labelling early in time is, however, weak when compared with the labelling of acetoacetate observed after the injection of labelled acetate or lactate. The higher specific radioactivity observed 360 min after the injection of labelled acetone is likely to be the result of the transfer of label from pyruvate/lactate by the pyruvate dehydrogenase reaction.

In Fig. 2, the specific-radioactivity-versus-time curves of plasma glucose, lactate, urea and acetoacetate are shown after the injection of L-[U-¹⁴C]lactate or [2-¹⁴C]acetate or NaH¹⁴CO₃. The ratio of the integral of the curve for SA of glucose versus time to that for lactate

Fig. 2. Specific radioactivity (SA) of plasma lactate, glucose, urea and acetoacetate (d.p.m./ μ g of C) after the intra-arterial injection of L-[U-¹⁴C]lactate (a), [2-¹⁴C]acetate (b) or NaH¹⁴CO₃ (c) at zero time

The specific radioactivities are normalized with respect to the injected amount of tracer, M^* (d.p.m. $\times 10^6$). Symbols as in Figure 1.

after the injection of labelled lactate was calculated as 0.44; thus an estimated 44% of the carbon atoms of circulating glucose arose from plasma lactate. The early labelling of acetoacetate was stronger with either [2-¹⁴C]acetate (which primarily labels acetyl-CoA) or L-[U-¹⁴C]lactate (which labels acetyl-CoA by the pyruvate dehydrogenase reaction) than that observed after the injection of labelled acetone. This observation again indicates that the labelling of acetoacetate after injection of labelled acetone is largely secondary.

Tables 1 and 2 summarize the ratios of specific radioactivities at selected points in time. The SA(glucose)/SA(lactate) ratio in the plasma of rats injected with [U-¹⁴C]lactate is higher than in those injected with [U-¹⁴C]acetone. This finding is compatible with the hypothesis that lactate is labelled primarily and that it gives rise to the labelling of glucose.

Taking the specific radioactivity of plasma urea to

Table 1. Ratio of specific radioactivity of glucose to that of lactate or urea after the injection of L-[^{14}C]lactate (three experiments), [^{14}C]acetate (four experiments), [^{14}C]acetone (seven experiments) or $\text{NaH}^{14}\text{CO}_3$ (four experiments) at selected times (min) after the intra-arterial injection of the tracer

Mean values are shown with the S.E.M. in parentheses.

Time (min)	Tracer injected...	Sp. radioactivity glucose/lactate				Sp. radioactivity glucose/urea			
		Lactate	Acetate	NaHCO_3	Acetone	Lactate	Acetate	NaHCO_3	Acetone
10–12		0.26 (0.10)	0.31 (0.08)	1.15 (0.36)	0.07 (0.01)	16.7 (2.8)	5.2 (1.28)	0.39 (0.04)	3.8 (0.30)
40–50		0.31 (0.12)	0.46 (0.07)	0.70 (0.20)	0.10 (0.02)	5.7 (1.3)	2.6 (0.31)	0.43 (0.01)	4.3 (0.24)
120–130		—	0.15 (0.02)	0.22 (0.08)	0.07 (0.01)	4.2 (0.84)	1.5 (0.13)	0.26 (0.02)	3.1 (0.12)

Table 2. Ratio of specific radioactivity of glucose or lactate to that of acetoacetate after the injection of L-[^{14}C]lactate (three experiments), [^{14}C]acetate (four experiments), [^{14}C]acetone (experiments) or $\text{NaH}^{14}\text{CO}_3$ (four experiments) at 4 or 10 min after the intra-arterial injection of the tracer

Mean values are shown with the S.E.M. in parentheses.

Time (min)	Tracer injected...	Sp. radioactivity glucose/acetoacetate				Sp. radioactivity lactate/acetoacetate			
		Lactate	Acetate	NaHCO_3	Acetone	Lactate	Acetate	NaHCO_3	Acetone
4 min		0.91 (0.46)	0.13 (0.02)	7.56 (1.31)	—	9.1 (1.67)	0.89 (0.27)	9.63 (5.65)	—
10 min		—	—	—	0.24 (0.02)	—	—	—	3.74 (0.44)

equal that of the hepatocellular bicarbonate/ CO_2 pool (Hetenyi *et al.*, 1982), the high SA(glucose)/SA(urea) ratio observed after the injection of labelled acetone compared with the same ratio after the injection of $\text{NaH}^{14}\text{CO}_3$ argues against CO_2 fixation from oxidized acetone being a major pathway in the transfer of label from acetone to glucose.

The magnitude of the ratios SA(glucose)/SA(acetoacetate) and SA(lactate)/SA(acetoacetate) observed after the injection of [^{14}C]acetone are lower than those found after the injection of labelled lactate, but higher than those found after the injection of labelled acetate. This indicates that acetone labels glucose by contributing ^{14}C atoms to both acetyl-CoA (i.e. by metabolic exchange) and to lactate (i.e. possible net synthesis).

In the third series of experiments the composite C^*/M^* -versus-time curve of plasma glucose after the injection of D-[^{14}C]glucose had the form of

$$C^*(t)/M^* = 5893 \exp(-0.06516t) + 4963 \exp(-0.009267t)$$

This is the Unit Impulse Response Function of plasma glucose and was used to determine by deconvolution the time course and extent of the rate of influx of ^{14}C atoms from precursors into glucose as the fraction of the injected amount (M^*)/min. The time courses of the influx of label into glucose are shown on Fig. 3. Comparison of the two curves shows that the transfer from lactate is not only more extensive but also faster than from acetone. The former curve reaches a peak at 14 min and the latter

Fig. 3. Incorporation of ^{14}C atoms into plasma glucose from injected L-[^{14}C]lactate or [^{14}C]acetone

The concentration of labelled glucose in 1 ml of plasma, (C^* , d.p.m./ml) at time t , normalized with respect to the injected tracer (M^* , d.p.m. $\times 10^6$) is plotted against time (min) from the injection of the tracer. The tracer was injected at zero time.

at about 100 min after the injection of the respective precursor.

In the same experiments the turnover rate of glucose was calculated in each rat from the curves for specific radioactivity of [^3H]glucose versus time. The average turnover rate for the five animals was 35.67 ± 2.36 $\mu\text{mol/min per kg}$ (6.42 ± 0.42 mg/min per kg).

In summary: in the 3-day-starved rat, plasma acetone appears to be a precursor of net glucose synthesis. The pathway of glucose formation leads through, and can be quantitatively accounted for by, the formation of C_3 compounds. Nevertheless, even in starved rats, acetone is only a minor contributor to circulating glucose. Even if correction is made for the metabolic exchange of C atoms in the hepatic oxaloacetate pool (Krebs *et al.*, 1966; Hetenyi, 1981; Hetenyi & Ferrarotto, 1983), it could not account for more than 2.5% of the C atoms in plasma glucose. Acetone therefore is not a significant pathway of glucose formation from fatty acids during starvation.

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