Conversion of pyruvate into ketone bodies in rat hepatocyte suspensions

Caroline M. BATTERSBY, K. George M. M. ALBERTI and Loranne AGIUS

Department of Clinical Biochemistry and Metabolic Medicine, The Medical School, University of Newcastle, Newcastle upon Tyne NE2 4HH, U.K.

The contribution of pyruvate to ketogenesis was determined in rat hepatocyte suspensions by using [^{14}C]pyruvate. The rates of conversion of pyruvate into ketone bodies in hepatocytes from fed and 24 h-starved rats were 10 and 17 $\mu\text{mol/h}$ per g wet wt. respectively, and accounted for 50 and 29% of the total ketone bodies formed. In hepatocytes from fed rats, the addition of palmitate (0.25–1 mM) increased the rate of conversion of pyruvate into ketone bodies (80–140%), but decreased the relative contribution of pyruvate to total ketogenesis. In hepatocytes from starved rats, palmitate did not increase pyruvate conversion into ketone bodies.

INTRODUCTION

Ketone-body formation in liver increases with increasing [fatty acid], and it is generally accepted that the ketone bodies are derived entirely from acetyl-CoA formed from fatty acid oxidation. The contribution of acetyl-CoA derived from pyruvate to ketone-body formation is assumed to be negligible, because activation of the pyruvate dehydrogenase complex is generally associated with lipogenic conditions in which acetyl-CoA is channelled primarily into fatty acid synthesis via formation of citrate and because fatty acids are assumed to inactivate the pyruvate dehydrogenase complex (Wieland *et al.*, 1972; Denton *et al.*, 1981). Consequently, in ketogenic states the flux through pyruvate dehydrogenase in liver would be expected to be suppressed. Although inactivation of the pyruvate dehydrogenase complex by fatty acids is observed in various tissues, in liver preparations inhibition of the flux through pyruvate dehydrogenase by fatty acids is seldom observed. On the contrary, there is increasing evidence that, at physiological [pyruvate], fatty acids increase the flux through pyruvate dehydrogenase in perfused liver (Dennis *et al.*, 1978; Scholz *et al.*, 1978; Zwiebel *et al.*, 1982; Patel *et al.*, 1984) and in isolated hepatocytes (Demaugre *et al.*, 1984; Agius & Alberti, 1985).

Ketone-body formation in liver is determined by the rate of acetyl-CoA formation and by the mitochondrial free [oxaloacetate], which influences the partitioning of acetyl-CoA between formation of citrate and acetoacetyl-CoA. Fatty acids decrease the [oxaloacetate], thereby favouring the partitioning of acetyl-CoA towards acetoacetyl-CoA formation, as opposed to citrate (Siess *et al.*, 1982, 1984). If fatty acids also increase the flux through pyruvate dehydrogenase at physiological [pyruvate], this raises the possibility of a contribution of pyruvate to ketogenesis in the presence of fatty acids. The aim of this study was to determine the rate of conversion of pyruvate into ketone bodies and to quantify the contributions of pyruvate and palmitate to total ketogenesis in hepatocytes isolated from fed or starved rats.

MATERIALS AND METHODS

Collagenase (type I), bovine serum albumin (fraction V), enzymes, cofactors and pyruvate and palmitate (sodium salts) were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). [^{14}C]Palmitate was from Amersham International (Amersham, Bucks., U.K.). [$1\text{-}^{14}\text{C}$]-, [$2\text{-}^{14}\text{C}$]- and [$3\text{-}^{14}\text{C}$]-pyruvate were from New England Nuclear (Boston, MA, U.S.A.).

Hepatocytes were isolated by collagenase perfusion of the liver (Seglen, 1976) of male Wistar rats (180–240 g body wt.) either fed *ad libitum* or deprived of food for 24 h. The hepatocytes were preincubated (20 min) and washed in Krebs–Henseleit (1932) saline and suspended in saline supplemented with defatted (Chen, 1967) bovine serum albumin (10 mg/ml). Cell viability estimated by Trypan Blue (0.4%, w/v) exclusion was > 90%.

Hepatocytes were incubated with 0.5 mM- or 2 mM-pyruvate and 0, 0.25 mM-, 0.5 mM- or 1 mM-palmitate. Parallel incubations were performed with ($1\text{-}^{14}\text{C}$)-, [$2\text{-}^{14}\text{C}$]-, or [$3\text{-}^{14}\text{C}$]-pyruvate or [^{14}C]palmitate. The [^{14}C]pyruvate was divided into portions and freeze-dried. Sodium pyruvate solutions (200 or 50 mM) with added label ($1\text{-}^{14}\text{C}$, 0.4 Ci/mol; 2- or $3\text{-}^{14}\text{C}$, 0.2 Ci/mol) were prepared on the day of the experiment. Sodium [$2\text{-}^{14}\text{C}$]- and [$3\text{-}^{14}\text{C}$]-pyruvate were checked for [^{14}C]acetate contamination by h.p.l.c., with added acetate as carrier. There was no detectable (< 0.5%) acetate contamination in these preparations. Sodium palmitate was dissolved in water to a final concentration of 20 mM (without or with added [^{14}C]palmitate, 0.3 Ci/mol); it was then diluted in Krebs–Henseleit buffer containing 20 mg of defatted bovine serum albumin/ml to a [fatty acid] twice that in the final incubations.

Incubations (6×10^5 – 8×10^5 cells in 2 ml final volume) were in 25 ml Erlenmeyer flasks containing a Microfuge tube and gassed with O_2/CO_2 (19:1) in a shaking water bath at 37 °C. The incubations were terminated after 15 or 30 min with 0.3 M- HClO_4 . $^{14}\text{CO}_2$ was absorbed in Hyamine 10X-hydroxide [300 μl ; 10% (w/v) in methanol]. The radioactivity absorbed in Hyamine in blank

Table 1. Effects of [palmitate] on [^{14}C]pyruvate conversion into $^{14}\text{CO}_2$ in hepatocyte suspensions

Hepatocytes isolated from fed or 24 h-starved rats were incubated with 2 mM-pyruvate and [$1\text{-}^{14}\text{C}$]-, [$2\text{-}^{14}\text{C}$]- or [$3\text{-}^{14}\text{C}$]-pyruvate as label and the concentrations of palmitate indicated. Results are expressed as μmol of $^{14}\text{CO}_2$ formed/h per g wet wt. of hepatocytes. Values are means $\pm$ S.E.M. for five to nine experiments. Values that are significantly different (paired *t*-test, $P < 0.05$) from the corresponding ones without added palmitate are shown by *.

		$^{14}\text{CO}_2$ formed ($\mu\text{mol/h}$ per g wet wt.)					
Hepatocytes from...		Fed rats			Starved rats		
[Palmitate] (mM)	Pyruvate label...	[$1\text{-}^{14}\text{C}$]	[$2\text{-}^{14}\text{C}$]	[$3\text{-}^{14}\text{C}$]	[$1\text{-}^{14}\text{C}$]	[$2\text{-}^{14}\text{C}$]	[$3\text{-}^{14}\text{C}$]
0		81 $\pm$ 4	23 $\pm$ 2	15 $\pm$ 1	84 $\pm$ 8	22 $\pm$ 4	26 $\pm$ 7
0.25		106 $\pm$ 4*	15 $\pm$ 1*	21 $\pm$ 3*	104 $\pm$ 11*	21 $\pm$ 5	28 $\pm$ 7
0.5		116 $\pm$ 4*	13 $\pm$ 1*	21 $\pm$ 3*	114 $\pm$ 10*	17 $\pm$ 3	28 $\pm$ 7
1		132 $\pm$ 5*	12 $\pm$ 1*	23 $\pm$ 6*	123 $\pm$ 11*	18 $\pm$ 4*	29 $\pm$ 7

flasks, which contained the cell suspension and radio-labelled substrate but were not incubated, was less than 0.08, 0.8, 0.04 and 0.04% of the total radioactivity in the flask for [$\text{U}\text{-}^{14}\text{C}$]palmitate, [$1\text{-}^{14}\text{C}$]-, [$2\text{-}^{14}\text{C}$]- and [$3\text{-}^{14}\text{C}$]-pyruvate respectively. Palmitate did not cause non-enzymic decarboxylation of [$1\text{-}^{14}\text{C}$]pyruvate when incubated with pyruvate in the absence of cell suspension.

The total rate of formation of ketone bodies (acetoacetate + D-3-hydroxybutyrate) from endogenous substrates plus added palmitate and pyruvate was determined enzymically (Williamson *et al.*, 1962) in the neutralized perchlorate extracts.

Ketone-body formation from palmitate was determined radiochemically from the [$\text{U}\text{-}^{14}\text{C}$]palmitate incubations. After neutralization of the perchlorate extracts, 0.5 ml of sample was incubated with 0.1 M-Tris/HCl, 0.5 mM-NAD and 150 munits of 3-hydroxybutyrate dehydrogenase, pH 9.4, for 45 min for conversion of 3-hydroxybutyrate into acetoacetate. Acetoacetate carrier (20 μmol ; lithium salt) was then added, and the acetoacetate was precipitated as the Deniges salt (Hg-acetone complex) as described by Bates *et al.* (1969), and the radioactivity determined. Acetoacetate recovery in the precipitate was determined by using [$3\text{-}^{14}\text{C}$]acetoacetate. Rates of ketone-body formation from [$\text{U}\text{-}^{14}\text{C}$]palmitate were calculated by assuming 75% retention of label in the Hg-acetone complex from the [$\text{U}\text{-}^{14}\text{C}$]acetoacetate, since C-1 is lost during decarboxylation.

Ketone-body formation from pyruvate in the data presented in this study was determined radiochemically from the [$3\text{-}^{14}\text{C}$]pyruvate incubations. In preliminary studies, pyruvate incorporation into ketone bodies was determined by using both [$3\text{-}^{14}\text{C}$]- and [$2\text{-}^{14}\text{C}$]-pyruvate as label. For these determinations it was necessary to remove the [^{14}C]pyruvate by conversion into acetaldehyde before precipitation of the Deniges salt, because pyruvate was found to co-precipitate with acetoacetate (19%). After neutralization of the perchlorate extracts, 1 ml of sample was incubated in 100 mM-citrate buffer, pH 6.5, containing 2 mM-MgCl₂, 4 mM-thiamin pyrophosphate, 0.4 mM-NAD, 0.5 μunit of pyruvate decarboxylase (EC 4.1.1.1)/ml and 1 unit of lactate dehydrogenase (EC 1.1.1.27)/ml (final volume 2 ml) for 2 h for conversion of lactate and pyruvate into acetaldehyde. The acetaldehyde was then converted into alcohol (Bernt & Bergmeyer, 1968). The samples were then divided into

two portions; one was used for the enzymic conversion of hydroxybutyrate into acetoacetate and the other for the conversion of acetoacetate into hydroxybutyrate (the latter served as a blank to correct for non-acetoacetate radioactivity). Acetoacetate carrier was added to both samples and the Deniges salt precipitated. [^{14}C]Acetoacetate was determined from the difference in radioactivity in the two samples.

The incorporation of [$3\text{-}^{14}\text{C}$]pyruvate into the precipitated Hg-acetone complex was twice that from [$2\text{-}^{14}\text{C}$]pyruvate. Since [$2\text{-}^{14}\text{C}$]pyruvate labels the C-1 and C-3 positions of acetoacetate and [$3\text{-}^{14}\text{C}$]pyruvate the C-2 and C-4 positions, the relative incorporation of label from [$3\text{-}^{14}\text{C}$]- and [$2\text{-}^{14}\text{C}$]pyruvate is consistent with loss of half the radioactivity from [$2\text{-}^{14}\text{C}$]pyruvate during decarboxylation of the carboxyl carbon (C-1) of acetoacetate. Rates of ketone-body formation from [$3\text{-}^{14}\text{C}$]pyruvate were calculated by assuming that 2 mol of pyruvate are incorporated into 1 mol of ketone bodies.

Incubations were in triplicate, and rates were linear for at least 30 min. Rates of ketone-body formation are expressed as μmol formed/h per g wet wt. of hepatocytes.

Statistical analysis was by Student's paired *t*-test.

RESULTS AND DISCUSSION

Pyruvate oxidation

The formation of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]pyruvate represents pyruvate dehydrogenase and decarboxylation reactions after pyruvate carboxylation to oxaloacetate (Claus & Pilkis, 1977). Rates of $^{14}\text{CO}_2$ formation from [$1\text{-}^{14}\text{C}$]pyruvate (2 mM) were similar in hepatocytes from fed and 24 h-starved rats (Table 1) and comparable with those observed in perfused liver at similar [pyruvate] (Scholz *et al.*, 1978; Patel *et al.*, 1984). Palmitate (0.25–1 mM) increased $^{14}\text{CO}_2$ formation from [$1\text{-}^{14}\text{C}$]pyruvate in hepatocytes from fed (31–63%) and 24 h-starved (23–46%; Table 1) rats. When hepatocytes from fed rats were incubated with 0.5 mM-pyruvate, the rate of formation of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]pyruvate ($56 \pm 4 \mu\text{mol/h}$ per g) was increased by 46–78% with 0.25–1 mM-palmitate (results not shown). The flux through pyruvate dehydrogenase, determined from the $^{14}\text{CO}_2$ formed from [$1\text{-}^{14}\text{C}$]pyruvate, corrected for other decarboxylation reactions from the rates of formation of

$^{14}\text{CO}_2$ from $[3\text{-}^{14}\text{C}]$ pyruvate and the incorporation of $[1\text{-}^{14}\text{C}]$ pyruvate into glucose by a modification (Agius & Alberti, 1985) of the method of Claus & Pilkis (1977), was also increased in the presence of 0.25–1 mM-palmitate (35–76%). The total pyruvate metabolized (flux through pyruvate carboxylase plus flux through pyruvate dehydrogenase) determined radiochemically from the conversion of $[1\text{-}^{14}\text{C}]$ pyruvate into $^{14}\text{CO}_2$ plus $[^{14}\text{C}]$ glucose (Claus & Pilkis, 1977) was increased by 50–80% with palmitate. This radiochemical determination correlated with the enzymically determined decrease in pyruvate plus lactate in the medium (Agius & Alberti, 1985).

In perfused liver, fatty acids have a differential effect on the flux through pyruvate dehydrogenase in relation to [pyruvate] (stimulation below 0.5 mM- and inhibition at 5 mM-pyruvate: Dennis *et al.*, 1978; Scholz *et al.*, 1978; Zwiebel *et al.*, 1982; Patel *et al.*, 1984). We have not observed inhibition of pyruvate decarboxylation by palmitate at 2 mM-pyruvate. However the stimulation decreases with increasing [pyruvate]. The inhibition of $[1\text{-}^{14}\text{C}]$ pyruvate decarboxylation by fatty acids that has been observed in perfused liver (Dennis *et al.*, 1978; Scholz *et al.*, 1978; Patel *et al.*, 1984), but not in isolated hepatocyte suspensions (Demaugre *et al.*, 1984), may be due to O_2 limitation in perfused liver, because of the increased O_2 consumption caused by the fatty acids (Scholz *et al.*, 1978, 1984) and the increased respiration required to support gluconeogenesis (Tutwiler & Brentzel, 1982). O_2 limitation would result in a more reduced NADH/NAD⁺ couple, which may cause a decreased flux through the enzyme as well as inactivation of the enzyme by increased phosphorylation because of stimulation of the kinase. The differential effect of fatty acids on the flux through pyruvate dehydrogenase in perfused liver was associated with an increased and decreased activation state of the pyruvate dehydrogenase complex at low (0.05 mM) and high (5 mM) pyruvate concentrations respectively (Dennis *et al.*, 1978). This activation was probably due to inhibition of pyruvate dehydrogenase kinase by an increase in the intramitochondrial [pyruvate] (Dennis *et al.*, 1978), and the inactivation to a stimulation of the kinase by the increased NADH/NAD⁺ ratio. It is possible that in dilute hepatocyte suspensions, where O_2 limitation may be less of a problem than in perfused liver, the inhibitory effect of pyruvate on the kinase predominates over the redox effect, whereas the converse occurs in perfused liver.

In perfused liver, the stimulation by fatty acids of mitochondrial pyruvate metabolism at low [pyruvate] (Scholz *et al.*, 1978; Patel *et al.*, 1984) is probably due to increased pyruvate entry into mitochondria, either by increased pyruvate/hydroxyl transport because of a more alkaline matrix, or by increased pyruvate/acetoacetate transport (Zwiebel *et al.*, 1982; Patel *et al.*, 1982, 1984). Since in hepatocyte suspensions fatty acids increase mitochondrial pyruvate metabolism at concentrations (2 mM) higher than reported K_m values for the pyruvate translocator (Pande & Parvin, 1978; Denton & Halestrap, 1979), the proposition of increased pyruvate transport might seem unlikely. However, it remains possible that there are distinct monocarboxylate translocators with different specificities, for example a pyruvate/hydroxyl carrier with a low K_m for pyruvate and a pyruvate/acetoacetate carrier with a high K_m for pyruvate, if its primary function is acetoacetate efflux. K_m and $V_{\max}$ values for pyruvate determined in the absence of an

acetoacetate source (Pande & Parvin, 1978) may reflect the properties of the pyruvate/hydroxyl carrier, whereas, in the presence of an acetoacetate source, pyruvate transport may be mediated by an additional protein(s) with a consequently higher $V_{\max}$ and possibly also an increased K_m .

The formation of $^{14}\text{CO}_2$ from $[2\text{-}^{14}\text{C}]$ - and $[3\text{-}^{14}\text{C}]$ -pyruvate represents mainly citrate-cycle oxidation but also phosphoenolpyruvate carboxykinase and malic enzyme activity (Claus & Pilkis, 1977). $[2\text{-}^{14}\text{C}]$ - and $[3\text{-}^{14}\text{C}]$ -pyruvate are incorporated into citrate-cycle intermediates via oxaloacetate (pyruvate carboxylase) or acetyl-CoA (pyruvate dehydrogenase). Label incorporation via acetyl-CoA formation results in higher $^{14}\text{CO}_2$ formation from $[2\text{-}^{14}\text{C}]$ - than from $[3\text{-}^{14}\text{C}]$ -pyruvate. The decrease in $^{14}\text{CO}_2$ formation from $[2\text{-}^{14}\text{C}]$ pyruvate by palmitate in hepatocytes from fed rats is probably due to decreased label incorporation into citrate-cycle intermediates via $[^{14}\text{C}]$ acetyl-CoA because of dilution with acetyl-CoA derived from palmitate. The higher rate of formation of $^{14}\text{CO}_2$ from $[3\text{-}^{14}\text{C}]$ - but not $[2\text{-}^{14}\text{C}]$ -pyruvate in hepatocytes from 24 h-starved rats and the increase in $^{14}\text{CO}_2$ formation from $[3\text{-}^{14}\text{C}]$ pyruvate by palmitate in hepatocytes from fed rats (Table 1) are probably due to an increased flux through pyruvate carboxylase and gluconeogenesis (Agius & Alberti, 1985).

Fatty acids increase the incorporation of $[1\text{-}^{14}\text{C}]$ pyruvate (0.5 mM) into glucose, but not the incorporation of $\text{H}^{14}\text{CO}_3^-$ into $[1\text{-}^{14}\text{C}]$ pyruvate, which provides an estimate of pyruvate recycling through pyruvate carboxylase / phosphoenolpyruvate carboxykinase / pyruvate kinase or pyruvate carboxylase/malate dehydrogenase/malic enzyme (Agius & Alberti, 1985). Consequently pyruvate recycling probably does not contribute to the stimulation of $^{14}\text{CO}_2$ formation from $[1\text{-}^{14}\text{C}]$ pyruvate by fatty acids.

Rates of ketone-body formation

In the absence of added palmitate, ketone-body formation in hepatocytes from starved rats was 3 times that in cells from fed rats (Table 2). In hepatocytes from fed rats $[3\text{-}^{14}\text{C}]$ pyruvate conversion into ketone bodies ($9.8 \pm 1.4 \mu\text{mol}$ of pyruvate/h per g) accounted for 50% of the total ketone bodies formed (Table 2). In hepatocytes from starved rats, $[3\text{-}^{14}\text{C}]$ pyruvate conversion into ketone bodies ($17.0 \pm 3.4 \mu\text{mol}$ of pyruvate/h per g) was 70% higher than the corresponding rate in cells from fed rats. Depletion of glycogen in hepatocytes from fed rats did not change the incorporation of label, suggesting that the lower incorporation of pyruvate label in cells from fed rats was not due to dilution of the $[^{14}\text{C}]$ pyruvate with endogenously formed pyruvate. The higher rate of conversion of pyruvate into ketone bodies in cells from starved rats is probably related to the more reduced mitochondrial NADH/NAD⁺ ratio in hepatocytes from starved rats, which would favour the partitioning of acetyl-CoA towards acetoacetyl-CoA formation as opposed to citrate formation.

In the presence of palmitate (0.25–1 mM), the total rate of ketogenesis was increased by 30–80 and 30–90 $\mu\text{mol}/\text{h}$ per g in hepatocytes from fed and starved rats respectively (Table 2). Only in hepatocytes from fed rats, however, was the rate of incorporation of pyruvate (2 mM) into ketone bodies increased in the presence of palmitate (Table 2). The stimulation by palmitate of pyruvate conversion into ketone bodies (80–140%) was greater

Table 2. Ketogenesis from palmitate and pyruvate in hepatocyte suspensions

Hepatocytes isolated from fed or 24 h-starved rats were incubated with 2 mM-pyruvate and the concentrations of palmitate indicated, with either [3-¹⁴C]pyruvate or [U-¹⁴C]palmitate as label. Ketogenesis determined either enzymically ('Total') or from the incorporation of ¹⁴C into ketone bodies (see the Materials and methods section) is expressed as μmol of ketone bodies formed/h per g wet wt. Values are means $\pm$ S.E.M. for five to nine experiments. Values that are significantly different (paired *t* test, $P < 0.05$) from the values without added palmitate are shown by *.

Hepatocytes from...	Ketone bodies formed ($\mu\text{mol/h}$ per g wet wt.)					
	Fed rats			Starved rats		
	Total	From pyruvate	From palmitate	Total	From pyruvate	From palmitate
Ketone-body formation... [Palmitate] (mM)						
0	10 $\pm$ 1	5 $\pm$ 1		31 $\pm$ 6	9 $\pm$ 2	
0.25	39 $\pm$ 5*	9 $\pm$ 1*	27 $\pm$ 3	59 $\pm$ 10*	9 $\pm$ 1	47 $\pm$ 4
0.5	60 $\pm$ 7*	11 $\pm$ 2*	51 $\pm$ 2	102 $\pm$ 24*	10 $\pm$ 1	114 $\pm$ 19
1	92 $\pm$ 9*	12 $\pm$ 2*	84 $\pm$ 3	121 $\pm$ 13*	10 $\pm$ 1	124 $\pm$ 27

than the stimulation of ¹⁴CO₂ formation from [¹⁴C]pyruvate (Table 1), suggesting that, in addition to an increased flux through pyruvate dehydrogenase, there was an increased partitioning of pyruvate-derived acetyl-CoA towards acetoacetyl-CoA formation. In hepatocytes from starved rats the incorporation of pyruvate into ketone bodies was not increased further with added palmitate (Table 2).

When hepatocytes from fed rats were incubated with 0.5 mM-pyruvate, the rate of [3-¹⁴C]pyruvate conversion into ketone bodies (10 $\pm$ 3 μmol of pyruvate/h per g; mean $\pm$ S.E.M., $n = 6$) was similar to the rate at 2 mM-pyruvate (Table 2). However, the rate of [1-¹⁴C]-pyruvate conversion into CO₂ was lower (62 %) than at 2 mM-pyruvate. Palmitate (0.25 and 0.5 mM) increased the conversion of 0.05 mM-pyruvate into ketone bodies by 82 and 102% respectively ($P < 0.05$). The similar rate of pyruvate conversion into ketone bodies at 0.5 mM- and 2 mM-pyruvate may be due to a lower free [oxaloacetate] at the lower [pyruvate], resulting in an increased partitioning of acetyl-CoA towards formation of ketone bodies as opposed to citrate.

Relative contributions of pyruvate and palmitate to ketogenesis

At low [palmitate] (0–0.25 mM) the contribution of pyruvate to total ketogenesis was 23–50% in hepatocytes from fed rats and 15–29% in cells from starved rats. Although, in the latter hepatocytes the percentage contribution of pyruvate to total ketogenesis was lower because of the higher rate of total ketogenesis, the rate of conversion of pyruvate into ketones was higher in absolute terms. This suggests an increased partitioning of acetyl-CoA towards formation of acetoacetate, as opposed to citrate, and may be related to a more reduced mitochondrial redox state consequent to increased oxidation of endogenous fatty acid in hepatocytes from starved rats. At 0.5–1 mM-palmitate, the contribution of pyruvate to ketogenesis was 13–18% and 8–10% in hepatocytes from fed and starved rats respectively. The conversion of palmitate (0.5–1 mM) into acetyl-CoA determined from the incorporation of [U-¹⁴C]palmitate into ¹⁴CO₂ plus acid-soluble ¹⁴C-labelled metabolites,

assuming complete oxidation of palmitate to 8 acetyl units, was 220–270 and 260–320 μmol of acetyl units/h per g in hepatocytes from fed and starved rats respectively. The conversion of pyruvate into acetyl-CoA, determined from the conversion of [1-¹⁴C]pyruvate into ¹⁴CO₂ corrected for citrate-cycle and phosphoenolpyruvate carboxykinase activity (Claus & Pilkis, 1977), was 40–70 and 37–63 $\mu\text{mol/h}$ per g wet wt. for hepatocytes from fed and starved rats respectively. The higher rate of ketogenesis from palmitate (0.5–1 mM) than from pyruvate is in accordance with the higher rate of acetyl-unit formation from palmitate.

It has been suggested that pyruvate is converted into ketone bodies only at unphysiologically high [pyruvate] (Demaugre *et al.*, 1978). However, the present study shows that, although the rate of total pyruvate metabolism was 2-fold higher at 2 mM- than at 0.5 mM-pyruvate, as shown also in perfused liver (Scholz *et al.*, 1978; Patel *et al.*, 1984), the rate of conversion of pyruvate into ketone bodies was similar at 0.5 mM- and 2 mM-pyruvate, probably because of a lower free [oxaloacetate] at low [pyruvate], resulting in decreased partitioning of acetyl-CoA towards citrate. The higher rate of conversion of pyruvate into ketone bodies in the presence of exogenous palmitate in hepatocytes from fed rats is consistent with the findings by Siess *et al.* (1982, 1984) that the mitochondrial [oxaloacetate] decreases with increasing concentration of fatty acid. The failure of palmitate to stimulate pyruvate conversion into ketone bodies further in hepatocytes from starved rats may be due to the high rate of oxidation of endogenous fatty acids in the absence of added palmitate, resulting in a more reduced redox couple.

It is concluded that pyruvate contributes to ketogenesis at physiological pyruvate concentrations, and the rate of conversion of pyruvate into ketone bodies is determined by the rate of decarboxylation of pyruvate as well as by the partitioning of mitochondrial acetyl-CoA between citrate and acetoacetyl-CoA formation.

C.M.B. was in receipt of a Medical Research Council studentship. We also thank the British Diabetic Association for financial support.

REFERENCES

- Agius, L. & Alberti, K. G. M. M. (1985) *Eur. J. Biochem.* in the press
- Bates, M. W., Krebs, H. A. & Williamson, D. H. (1969) *Biochem. J.* **110**, 655–661
- Bernt, E. & Bergmeyer, H. U. (1968) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1506–1507, Academic Press, New York
- Chen, R. F. (1967) *J. Biol. Chem.* **242**, 173–181
- Claus, T. H. & Pilkis, S. J. (1977) *Arch. Biochem. Biophys.* **182**, 52–63
- Demaugre, F., Leroux, J.-P. & Cartier, P. (1978) *Biochem. J.* **172**, 91–96
- Demaugre, F., Buc, H. A., Cepanec, C., Moncion, A. & Leroux, J.-P. (1984) *Biochem. J.* **222**, 343–350
- Dennis, S. C., Debuysere, M., Scholz, R. & Olson, M. S. (1978) *J. Biol. Chem.* **253**, 2229–2237
- Denton, R. M. & Halestrap, A. P. (1979) *Essays Biochem.* **15**, 37–77
- Denton, R. M., McCormack, J. G. & Oviyasu, O. A. (1981) in *Short-Term Regulation of Liver Metabolism* (Hue, L. & van de Werve, G., eds.), pp. 159–174, Elsevier/North-Holland, Amsterdam
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Pande, S. V. & Parvin, R. (1978) *J. Biol. Chem.* **253**, 1565–1573
- Patel, T. B., Debuysere, M. S., Scholz, R. & Olson, M. S. (1982) *Arch. Biochem. Biophys.* **213**, 573–584
- Patel, T. B., Barron, L. L. & Olson, M. S. (1984) *J. Biol. Chem.* **259**, 7525–7531
- Scholz, R., Olson, M. S., Schwab, A. J., Schwabe, U., Noell, C. & Braun, W. (1978) *Eur. J. Biochem.* **86**, 519–530
- Scholz, R., Schwabe, U. & Soboll, S. (1984) *Eur. J. Biochem.* **141**, 223–230
- Seglen, P. O. (1976) *Methods Cell Biol.* **13**, 29–83
- Siess, E. A., Kientsch-Engel, R. I. & Wieland, O. H. (1982) *Eur. J. Biochem.* **121**, 493–499
- Siess, E. A., Kientsch-Engel, R. I. & Wieland, O. H. (1984) *Biochem. J.* **218**, 171–176
- Tutwiler, G. F. & Brentzel, H. J. (1982) *Eur. J. Biochem.* **124**, 465–470
- Wieland, O. H., Patzelt, C. & Löffler, G. (1972) *Eur. J. Biochem.* **26**, 426–433
- Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96
- Zwiebel, F. M., Schwabe, U., Olson, M. S. & Scholz, R. (1982) *Biochemistry* **21**, 346–353

Received 8 February 1985/13 June 1985; accepted 1 July 1985