The Fuel of Respiration of Rat Kidney Cortex

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1. In kidney-cortex slices from the well-fed rat, glucose (5 mM) supplied 25–30% of the respiratory fuel; in the starved state, the corresponding value was 10%. These results are based on measurements of the net uptake of glucose and of the specific radioactivity of labelled carbon dioxide formed in the presence of [U-¹⁴C]-glucose. 2. Added acetoacetate (5 mM) or butyrate (10 mM) provided up to 80%, and added oleate (2 mM) up to 50% of the fuel of respiration. The oxidation of endogenous substrates was suppressed correspondingly. 3. More [U-¹⁴C]oleate was removed by the tissue than could be oxidized by the amount of oxygen taken up; less than 25% of the oleate removed was converted into respiratory carbon dioxide and about two-thirds was incorporated into the tissue lipids. The rate of oleate incorporation into the neutral-lipid fraction was calculated to be equivalent to the rate of oxidation of endogenous fat, which provided the chief remaining fuel. 4. The contribution of endogenous substrates to the respiration (50%) in the presence of added oleate is taken to reflect either a high turnover rate of the endogenous neutral lipids (approx. half-life 2.5 hr.) or a raised rate of lipolysis caused by the experimental conditions *in vitro*. 5. Added L- α -glycerophosphate (2.5 mM) increased oleate incorporation into the neutral-lipid fraction by up to 40% (i.e. caused a net synthesis of triglyceride). 6. Lactate (2.5 mM) added as sole substrate supplied 30% of the respiratory fuel, but with added oleate (2 mM) lactate was converted quantitatively into glucose. Oleate stimulated the rate of gluconeogenesis from lactate by 45%. 7. The oxidation of both long-chain and short-chain even-numbered fatty acids was accompanied by ketone-body formation. Ketone-body synthesis from oleate, but not from butyrate, increased six- to seven-fold after 48 hr. of starvation. The maximum rates of renal ketogenesis (80 μ moles/hr./g. dry wt., with butyrate) were about 20% of the maximum rates observed in the liver (on a weight-for-weight basis) and accounted for, at most, 35% of the fatty acid removed. 8. DL-Carnitine (1.0 mM) had no effect on the rates of uptake of acetate, butyrate or oleate or on the rate of radioactive carbon dioxide formation from [U-¹⁴C]oleate, but increased ketone-body formation from oleate by more than 100%. Ketone-body formation from butyrate was not increased. 9. There is evidence supporting the assumption that there are cells in which gluconeogenesis and ketogenesis occur together, characterized by equal labelling of [U-¹⁴C]oleate and the ketone bodies formed, and other cells that oxidize fat and do not form ketone bodies. 10. Inhibitory effects of unlabelled acetoacetate on the oxidation of [1-¹⁴C]butyrate and of unlabelled butyrate on [4-¹⁴C]acetoacetate oxidation show that fatty acids and ketone bodies compete as fuels on the basis of their relative concentrations. 11. The pathway of ketogenesis in renal cortex must differ from that of the liver, as β -hydroxy- β -methylglutaryl-CoA synthetase is virtually absent from the kidney. In contrast with the liver the kidney possesses 3-oxo acid CoA-transferase (EC 2.8.3.5), and the ready reversibility of this reaction and that of thiolase (EC 2.3.1.9) provide a mechanism for ketone-body formation from acetyl-CoA. This mechanism may apply to extrahepatic tissues generally, with the possible exception of the epithelium of the rumen and intestines.

The low respiratory quotient of kidney-cortex slices (about 0.75; Dickens & Šimer, 1930) has been

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taken to indicate that fat is the principal physiological fuel of this tissue. Direct evidence in support of this was provided by experiments with [¹⁴C]palmitate on rabbit kidney-cortex slices (Lee,

Vance & Cahill, 1962) and on dog kidneys receiving infusions of labelled fatty acid through the renal artery *in vivo* (Gold & Spitzer, 1964). The oxidation of long-chain fatty acids has been taken to contribute 60–80% of the fuel of respiration of the kidney (Hohenleitner & Spitzer, 1961; Lee *et al.* 1962; Gold & Spitzer, 1964; Nieth & Schollmeyer, 1966). Kidney cortex can also oxidize ketone bodies and the intermediates of the tricarboxylic acid cycle and of glycolysis (see Krebs & Johnson, 1948; Krebs, 1961).

The experiments reported in the present paper were designed to measure the contributions that can be made by glucose, lactate, acetoacetate, butyrate, oleate and endogenous substrates to the fuel of respiration in rat kidney-cortex slices, and to supply information on the factors that regulate the choice of fuel in this tissue.

EXPERIMENTAL

Incubation procedure. Washed kidney-cortex slices (approx. 50 mg. wet wt.) from male rats (300–400 g.) were incubated at 40° in 4.0 ml. of the phosphate-buffered saline of Krebs & de Gasquet (1964) as described by Weidemann & Krebs (1969). Where glucose uptake was measured, the volume of the incubation medium used was 2.0 ml., and approx. 70–75 mg. wet wt. of tissue was added/cup. The respiratory CO₂ was collected and estimated as described by Krebs, Hems, Weidemann & Speake (1966). The value obtained was corrected for the CO₂ content of all reagents used, measured in a control cup without slices. Kidney perfusions were carried out as described by Nishiitsutsuji-Uwo, Ross & Krebs (1967).

Reagents. Commercial L- α -glycerophosphate (Calbiochem, Los Angeles, Calif., U.S.A.) was found to contain 84.6% of the L-isomer by the enzymic assay of Hohorst (1963). DL-Carnitine hydrochloride and oleic acid of high purity were obtained from Fluka A.-G., Buchs, Switzerland.

Analytical methods. Glucose, L-lactate, pyruvate, α -glycerophosphate, acetoacetate and β -hydroxybutyrate were determined by enzymic methods as described by Weidemann & Krebs (1969).

Acetate and butyrate were estimated by gas-liquid chromatography as described by Baumgardt (1964) with valeric acid as the internal standard. Oleate uptake was estimated by two different methods: (1) by disappearance of isotope from the medium when [U-¹⁴C]oleate was added; (2) by the colorimetric method of Itaya & Ui (1965). Oleate was extracted into heptane from neutral samples (0.5 ml.) of the medium before and after incubation (Dole & Meinertz, 1960) and samples were taken for liquid-scintillation counting. Measured samples of the top layer were transferred to chloroform-washed stoppered tubes, evaporated to dryness in a stream of N₂ and made up to 6.0 ml. with redistilled chloroform. The colorimetric estimation was carried out on these solutions. Chloroform washing of the glassware was necessary to remove traces of soaps and detergents.

Preparation of bovine serum albumin-oleate complex. Bovine serum albumin (fraction 5; Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex) was freed of contaminating

long-chain fatty acids and bound to sodium oleate as described by Garland, Newsholme & Randle (1964). Where [U-¹⁴C]oleate was used, the labelled and unlabelled materials were mixed together before addition to the albumin. The resulting solution was dialysed overnight at 0° against several changes of phosphate-buffered saline to equilibrate the inorganic ions.

Radiochemical methods. [U-¹⁴C]Oleate, [1-¹⁴C]butyrate, [U-¹⁴C]glucose and ethyl [4-¹⁴C]acetoacetate were obtained from The Radiochemical Centre, Amersham, Bucks. [4-¹⁴C]Acetoacetate was prepared from its ethyl ester and checked for radiochemical purity as described by Krebs *et al.* (1966).

The radioactivity in glucose and other metabolic products not decomposing on paper was separated by two-dimensional paper chromatography and radioautography as described by Gevers & Krebs (1966) and Crowley, Moses & Ullrich (1963). [1-¹⁴C]Butyric acid was recovered after incubation by steam-distillation and [U-¹⁴C]oleate by the method described above (see under 'Analytical methods'). Where [4-¹⁴C]acetoacetate was the labelled substrate the radioactivity in acetoacetate, acetone and β -hydroxybutyrate at the beginning and end of the incubation was separated by the method of Mayes & Felts (1967). This method was found to be unsuitable for the separation of labelled ketone bodies formed from [1-¹⁴C]butyrate, as a substantial proportion of the substrate in addition to [¹⁴C]acetone was collected in the hydrazine-lactic acid trap. In experiments with this substrate the method of Mayes & Felts (1967) was modified. The general principle of separating radioactivity in the three ketone bodies was followed, but the radioactivity in the acetone moiety of acetoacetate was determined by precipitation of the acetone with Denigès's reagent as an acetone-HgSO₄ complex (Van Slyke, 1917; Edson, 1935). The precipitate was washed twice with water (sufficient to remove 100% of the radioactivity added as [1-¹⁴C]butyrate), dissolved in 0.5 ml. of 2M-HCl and neutralized, and a sample was added to 14.0 ml. of the scintillation medium previously described (Weidemann & Krebs, 1969). For each experiment the percentage recovery of radioactivity from a standard [4-¹⁴C]acetoacetate solution was determined (usually 83%) and used to correct the values obtained.

To determine the radioactivity in the respiratory CO₂, samples (0.5 ml.) of the NaOH solution from the centre wells (diluted 1:10) containing labelled bicarbonate were added directly to the scintillation medium.

Incorporation of [U-¹⁴C]oleate into tissue lipids. Slices were removed from the incubation medium before addition of HClO₄ and drained of adhering medium. After washing to remove contaminating radioactivity (12 × 4.0 ml. changes of 2% HClO₄) the slices were homogenized in water (2.0 ml.). Samples (1.0 ml.) of the homogenate were added to 20 ml. of chloroform-methanol (2:1, v/v) and, after being shaken, the mixture was kept overnight at room temperature. The lipids were extracted by the method of Folch, Lees & Sloane-Stanley (1957). After removal of the top layer, the lower phase was made up to 20 ml. and the solution was divided into two 10 ml. portions. Both were evaporated to dryness in a stream of N₂. One portion was redissolved in heptane (3.0 ml.) and duplicate samples (1.0 ml.) were taken for liquid-scintillation counting. This gave the total radioactivity in the tissue lipids. The second portion was redissolved in a small quantity of chloroform

and transferred quantitatively to columns containing silicic acid (100 mesh)-Celite (2:1, w/w). The neutral lipids plus free fatty acids, and the phospholipids, were eluted serially with chloroform and methanol as described by Bezman-Tarcher, Otway & Robinson (1965). The methanol fraction was evaporated to dryness and redissolved in heptane (3.0 ml.), and duplicate samples (1.0 ml.) were taken for liquid-scintillation counting. This gave the total radioactivity in the tissue phospholipids. The free fatty acids and neutral lipids were then separated on Florisil columns by the method of Enser, Kunz, Borensztajn, Opie & Robinson (1967) and prepared for counting as described for the phospholipids. Approx. 95–98% of the radioactivity in the lipid fraction was recovered by this procedure.

Incorporation of oleate carbon into water-soluble compounds. The radioactivity in water-soluble compounds other than ketone bodies released into the incubation medium was determined by fractionating samples (0.5 ml.) of deproteinized neutral medium by the method of Dole & Meinertz (1960). To remove traces of labelled oleate the top layer was removed and the bottom layer extracted a second time with heptane. Samples of the bottom layer (1.0 ml.) were taken for estimation of radioactivity and the values obtained were corrected for radioactivity in the three ketone bodies. In the experiments with $[U-^{14}C]$ oleate further identification of the compounds bearing radioactivity in this fraction was not attempted.

Counting procedure. Radioactivity in the separated products was determined by liquid-scintillation counting as described by Weidemann & Krebs (1969). The counting efficiency was 82.5% and was corrected to 100% by the use of a standard $[^{14}C]$ toluene solution.

RESULTS

Contribution of glucose to the respiratory fuel. Experiments in which the uptake of glucose, the

formation of lactate and pyruvate and the gas exchange were measured on addition of 5 mM- $[U-^{14}C]$ glucose are shown in Table 1. Kidney-cortex slices of rats from three different dietary regimes were used. There was no significant effect of the high-carbohydrate diet on glucose uptake, but starvation for 48 hr., in accordance with the findings of Underwood & Newsholme (1967), decreased the rate of glucose uptake by more than 50%. The rates of gas exchange were not significantly affected by the diets, although the respiratory quotient was slightly lower after starvation.

On the assumption that the glucose not recovered as lactate or pyruvate was oxidized, the contribution made by glucose to the respiratory fuel was 30.3% on the standard diet and 27.6% on the high-carbohydrate diet, against 11.3% after starvation. Measurements of the specific radioactivity of the respiratory carbon dioxide formed indicate that in fact less glucose was oxidized by slices from the well-fed rats than the calculations based on the above assumptions indicate. With tissue from the starved animals the agreement between the specific-radioactivity measurements and the results obtained on the above assumptions was good. It follows that in the well-fed animal some glucose must have been converted into metabolic products other than lactate, pyruvate and carbon dioxide. The data in Table 2 show that some radioactivity (approx. 5%) was present in the water-insoluble material of the tissue. Of this the lipid-soluble fraction accounted for only 20%. Thus fatty acid synthesis was a minor process under the experimental conditions (Chernick, Masoro & Chaikoff, 1950;

Table 1. Contribution of $[U-^{14}C]$ glucose to the fuel of respiration of rat-kidney cortex slices

Kidney-cortex slices (14–15 mg. dry wt.) were incubated for 60 min. in phosphate-buffered saline (2.0 ml.). For full experimental details see the text. The specific radioactivity of $[U-^{14}C]$ glucose was 19.6×10^4 c.p.m./ μ g. atom of C and the total radioactivity added/cup was 1294×10^4 c.p.m. The formation of metabolites is indicated by a + sign and disappearance by a – sign. The high-carbohydrate diet consisted of 70% sucrose, 20% casein, 5% arachis oil and 5% minerals and was given for 3 days. The results are given as means $\pm$ s.d. for the numbers of experiments in parentheses.

Metabolic changes (μ moles/hr./g. dry wt. of tissue)						
Substrate added ...	None			Glucose (5.0 mM)		
	Balanced diet (3)	High-carbo-hydrate diet (6)	Starved (48 hr.) (3)	Balanced diet (3)	High-carbo-hydrate diet (6)	Starved (48 hr.) (3)
Nutritional state ...						
O ₂	–970 $\pm$ 26	–950 $\pm$ 74	–1030 $\pm$ 57	–1070 $\pm$ 100	–1030 $\pm$ 69	–1070 $\pm$ 48
CO ₂	+820 $\pm$ 19	+820 $\pm$ 44	+820 $\pm$ 61	+890 $\pm$ 86	+880 $\pm$ 58	+880 $\pm$ 32
Respiratory quotient	0.85 $\pm$ 0.03	0.86 $\pm$ 0.04	0.80 $\pm$ 0.02	0.83 $\pm$ 0.03	0.86 $\pm$ 0.02	0.82 $\pm$ 0.05
Glucose	+7.9 $\pm$ 1.3	+9.5 $\pm$ 2.4	+18.1 $\pm$ 3.2	–60 $\pm$ 7.9	–53 $\pm$ 11.5	–27 $\pm$ 12.4
Lactate	+2.0 $\pm$ 0.4	+1.8 $\pm$ 0.5	+3.1 $\pm$ 2.5	+9.5 $\pm$ 2.2	+10.6 $\pm$ 3.7	+10.7 $\pm$ 3.8
Pyruvate	+1.6 $\pm$ 0.2	+1.0 $\pm$ 0.5	+1.5 $\pm$ 0.4	+2.0 $\pm$ 0.4	+1.7 $\pm$ 0.8	+1.7 $\pm$ 0.6
% contribution of glucose oxidation to O ₂ consumption				30.3 $\pm$ 6.3	27.6 $\pm$ 7.2	11.3 $\pm$ 6.5
Sp. radioactivity of $^{14}CO_2$				25.0	23.8	11.8
Sp. radioactivity of $[U-^{14}C]$ glucose $\times 100$						

Table 2. Isotope distribution on addition of $[U-^{14}C]$ glucose to rat kidney-cortex slices at the end of incubation for the experiment recorded in Table 1

The radioactivity recovered in the respiratory CO_2 , incubation medium and water-insoluble material of the slice is given. The total radioactivity other than glucose recovered from the incubation medium included the following substances: lactate, pyruvate, hexose phosphates, 2- and 3-phosphoglyceric acid, α -glycerophosphate, citrate, α -oxoglutarate, succinate, fumarate, malate, glutamate, glutamine, glycine, serine, alanine, protein and glycogen. For these substances no significant differences in the radioactivity recovered were found between the three nutritional states.

Nutritional state ...	Balanced diet			High-carbohydrate diet			Starved (48 hr.)		
	$10^{-2} \times$ Radio-activity recovered (c.p.m.)	% of recovered (not including glucose)	$10^{-4} \times$ Sp. radioactivity (μg .atom of C)	$10^{-2} \times$ Radio-activity recovered (c.p.m.)	% of recovered (not including glucose)	$10^{-4} \times$ Sp. radioactivity (μg .atom of C)	$10^{-2} \times$ Radio-activity recovered (c.p.m.)	% of recovered (not including glucose)	$10^{-4} \times$ Sp. radioactivity (μg .atom of C)
Radioactivity recovered in									
Glucose	110140		19.19	111025		18.89	110983		18.25
CO_2	6662	48.1	4.82	7123	48.3	4.56	3530	31.5	2.24
Water-soluble substances in medium (other than glucose)	6560	47.3		6880	46.6		7066	62.9	
Slice									
Non-lipid fraction	530	3.8		625	4.2		534	4.8	
Lipid fraction	109	0.8		135	0.9		94	0.8	
Total radioactivity recovered	124001			125788			122207		

Lee *et al.* 1962; Spencer & Lowenstein, 1967). Even a high-carbohydrate diet, which stimulates fat synthesis in the liver, did not increase fat synthesis in kidney-cortex slices.

It is noteworthy that the total radioactivity recovered in glycolytic and tricarboxylic acid-cycle intermediates and amino acids in the incubation medium was at least as great as the amount recovered in the respiratory carbon dioxide. In the 48 hr.-starved animals nearly two-thirds of the recovered radioactivity was found in this fraction. The retention of radioactivity in the intermediates may be in part connected with the fact that the label entering the tricarboxylic acid cycle from $[U-^{14}C]$ glucose is retained for several turns and in part by the relatively high concentration of some of the labelled intermediates formed (e.g. glutamate, glutamine and alanine, which between them contained 36–44% of the radioactivity in substances other than glucose recovered from the medium).

It is known that in cardiac and skeletal muscle the addition of insulin increases the glucose uptake. In preliminary experiments no major effect of insulin was found, although small effects (25% stimulation of glucose uptake) cannot be excluded.

Contribution of long-chain fatty acids to the respiratory fuel. It is already known that the utilization of glucose by kidney-cortex slices is decreased on addition of palmitate or butyrate to the medium (Underwood & Newsholme, 1967), and that glucose, in contrast, has no effect on the formation of $^{14}CO_2$ from labelled palmitate (Allen, Friedmann & Weinhouse, 1955). These observations indicate that fatty acids are a preferred fuel of respiration. The following experiments were designed to obtain more detailed information on the fate of a labelled long-chain fatty acid in kidney-cortex slices.

Oleate (2.0 mm) added to the medium was taken up by slices at the rate of 89.0 ± 7.9 and 70.3 ± 3.4 μ moles/hr./g. dry wt. in the well-fed and 48 hr.-starved states respectively (four observations). In both nutritional conditions more oleate was removed than could be oxidized by the measured amount of oxygen taken up. The fate of $[U-^{14}C]$ -oleate was therefore investigated (Table 3). The addition of oleate increased the oxygen consumption by 10%, lowered the respiratory quotient from 0.82 to 0.75 and caused a striking increase in the formation of ketone bodies. DL-Carnitine (1.0 mm) did not affect the rate of oxygen uptake or the rate of removal of oleate, but it more than doubled the rate of ketone-body formation. L- α -Glycerophosphate (2.5 mm), which was added to test whether it promoted the esterification of oleate, increased the rate of oleate uptake by 20% and, as expected, increased the formation of glucose.

The distribution of radioactivity at the end of

Table 3. *Effects of carnitine and α -glycerophosphate on the fate of oleate in rat kidney-cortex slices (net changes)*

Kidney-cortex slices from 48 hr.-starved rats were incubated for 60 min. at 40° in phosphate-buffered saline. All cups contained, in addition to substrates, 2% (w/v) of bovine serum albumin. For full experimental details see the text. The specific radioactivity of [U-¹⁴C]oleate was 20.73×10^3 c.p.m./ μ g. atom of C and the total radioactivity added/cup 3×10^6 c.p.m. Each cup contained 12–13 mg. dry wt. of tissue. The formation of metabolites is indicated by a + sign and the disappearance by a – sign.

Substrate(s) added ...	Metabolic changes (μ moles/hr./g. dry wt. of tissue)							
	None	DL-Carnitine (1.0 mm)	[U- ¹⁴ C]-Oleate (2.0 mm)	[U- ¹⁴ C]Oleate (2.0 mm) + DL-carnitine (1.0 mm)	L- α -Glycerophosphate (2.5 mm) +			
					No further addition	DL-Carnitine (1.0 mm)	[U- ¹⁴ C]-Oleate (2.0 mm)	[U- ¹⁴ C]Oleate (2.0 mm) + DL-carnitine (1.0 mm)
O ₂	–1137	–1085	–1217	–1260	–1168	–1192	–1462	–1490
CO ₂	+929	+830	+912	+923	+925	+1033	+1090	+1108
Respiratory quotient	0.82	0.77	0.75	0.74	0.79	0.87	0.75	0.74
Oleate			–73.5	–72.5			–91.6	–91.9
Acetoacetate	+1.8	+1.6	+16.3	+40.6	+1.2	+1.7	+13.2	+30.1
β -Hydroxybutyrate	+1.1	+2.0	+5.0	+9.0	+0.2	+1.8	+4.5	+4.9
L- α -Glycerophosphate					–108.9	–109.4	–88.3	–81.8
Glucose	+17.7	+19.9	+18.4	+19.4	+31.6	+35.0	+39.9	+39.0
L- α -Glycerophosphate used					3.45	3.13	2.21	2.10
Glucose formed								

60 min. of incubation is given in Table 4. Under all conditions tested about 70% of the radioactivity removed from the medium was recovered in the lipid-soluble material of the tissue, and this fraction contained 47% neutral lipid, 13% phospholipid and 40% unesterified fatty acid. Addition of α -glycerophosphate increased oleate incorporation into the neutral-lipid fraction by nearly 40%, but had no effect on the tissue concentration of free oleate. The average concentration of free oleate in the tissue reached 4.5 mm after 60 min. of incubation. At 0° the tissue accumulated oleate up to 1.4 mm in 15 min. whereas less than 4% of the label was found in the phospholipid and triglyceride fractions at this temperature. The uptake of oleate by the tissue was thus rapid and little affected by the temperature, which suggests that renal protein, like plasma albumin, combines with long-chain fatty acids. An alternative explanation is a high solubility in a lipid cell phase such as fat droplets.

In all cups less than 25% of the label in the oleate taken up was recovered as ¹⁴CO₂. Added DL-carnitine, in contrast with its effect on ketogenesis, had no stimulating effect on the incorporation of the label into carbon dioxide. There was a small, but consistent, inhibitory effect of carnitine on the incorporation of label into neutral lipid. The low specific radioactivity of the respiratory carbon dioxide (36% of that of the labelled oleate) indicates that the greater part of the carbon dioxide formed was derived from endogenous substrates. This fact

and the finding that the specific radioactivity of the ketone-body carbon was about the same as that of the oleate carbon leads to the conclusion that the carbon dioxide and ketone bodies were formed from separate pools of acetyl-CoA.

α -Glycerophosphate increased the formation of ¹⁴CO₂ from oleate by 25%. The increased oxygen consumption in the presence of both oleate and α -glycerophosphate was presumably due to the increased energy requirements for fatty acid esterification and gluconeogenesis.

The uptake of oleate depends on the concentration of the substrate (Table 5). Decreasing the concentration from 2.0 mm to 0.5 mm decreased the incorporation of oleate into the lipid fraction of the tissue tenfold. The formation of ¹⁴CO₂ fell only threefold and nearly 50% of the oleate taken up at 0.5 mm could be accounted for by oxidation. The contribution of oleate to the fuel of respiration at 0.5 mm was small (approx. 16%) and ketone-body formation was also much decreased (Fig. 1). The stimulating effect of carnitine on ketogenesis occurred at all concentrations tested (0.25–4.0 mm oleate).

A further analysis of the fate of added oleate (Table 6) and the calculation of the contribution of oleate to the fuel of respiration is based on the following assumptions:

(1) The complete oxidation of 1 mole of oleate requires 25.5 moles of oxygen and yields 18 moles of carbon dioxide.

Table 4. *Isotope distribution on addition of [U-¹⁴C]oleate to rat kidney cortex slices at the end of incubation for the experiment recorded in Table 3*

Substrate(s) added ...	[U- ¹⁴ C]Oleate (2.0 mm) +						[U- ¹⁴ C]Oleate (2.0 mm) + L- α -glycerophosphate (2.5 mm) +					
	No further addition			DL-Carnitine (1.0 mm)			No further addition			DL-Carnitine (1.0 mm)		
	10 ⁻¹ × Radio-activity recovered (c.p.m.)	% of recovered radio-activity (not including oleate)	10 ⁻³ × Sp. radio-activity (c.p.m./ μ g. atom of C)	10 ⁻¹ × Radio-activity recovered (c.p.m.)	% of recovered radio-activity (not including oleate)	10 ⁻³ × Sp. radio-activity (c.p.m./ μ g. atom of C)	10 ⁻¹ × Radio-activity recovered (c.p.m.)	% of recovered radio-activity (not including oleate)	10 ⁻³ × Sp. radio-activity (c.p.m./ μ g. atom of C)	10 ⁻¹ × Radio-activity recovered (c.p.m.)	% of recovered radio-activity (not including oleate)	10 ⁻³ × Sp. radio-activity (c.p.m./ μ g. atom of C)
Radioactivity recovered in												
Oleate	258990		20.73	260025		20.73	249140		20.73	249140		20.73
CO ₂	10140	23.0	7.45	9810	21.3	7.11	12755	24.0	7.82	12230	22.3	7.40
Ketone bodies	2645	6.0	20.76	5940	12.9	20.03	2285	4.3	21.60	4390	8.0	21.06
Lipid fraction of slice												
Free fatty acid	11810	26.8		11885	25.8		12120	22.8		12770	23.1	
Neutral lipid	13575	30.8		12440	27.0		18655	35.1		17660	32.2	
Phospholipid	3660	8.3		3180	6.9		4090	7.7		3950	7.2	
Water-soluble substances in medium (other than ketone bodies)	2250	5.1		2810	6.1		3240	6.1		3950	7.2	
Total radioactivity recovered	303070			306090			302285			304090		
% recovered	101.0			102.1			100.9			101.6		

Table 5. *Effect of [U-¹⁴C]oleate concentration on distribution of radioactivity at the end of incubation*

Kidney-cortex slices from 48 hr.-starved rats were incubated for 60 min. as described in the text. In the cups containing 0.5 mm-[U-¹⁴C]oleate, 7.5 × 10⁵ c.p.m. was added of specific radioactivity 19.74 × 10³ c.p.m./μg. atom of C; the cups with 2.0 mm-[U-¹⁴C]oleate contained 3 × 10⁶ c.p.m. of identical specific radioactivity.

Substrate(s) added ...	[U- ¹⁴ C]Oleate +			
	No further addition		DL-Carnitine (1.0 mm)	
	10 ⁻¹ × Radioactivity recovered (c.p.m.)	% of recovered radioactivity	10 ⁻¹ × Radioactivity recovered (c.p.m.)	% of recovered radioactivity
Expt. 1: 0.5 mm-[U- ¹⁴ C]oleate				
CO ₂	3040	46.1	2960	46.3
Ketone bodies	165	2.5	476	7.5
Lipid fraction of slices				
Free fatty acid	1085	16.5	1000	15.6
Neutral lipid	1975	30.0	1680	26.3
Phospholipid	322	4.9	278	4.3
Expt. 2: 2.0 mm-[U- ¹⁴ C]oleate				
CO ₂	9445	24.0	9275	25.8
Ketone bodies	890	2.3	1745	4.9
Lipid fraction of slice				
Free fatty acid	10280	26.2	9600	26.6
Neutral lipid	17285	44.0	14030	38.9
Phospholipid	1390	3.5	1375	3.8

Fig. 1. Effect of oleate concentration and DL-carnitine on ketone-body formation by kidney-cortex slices. Slices from 48 hr.-starved rats were incubated for 60 min. in phosphate-buffered saline. O, No added carnitine; ●, DL-carnitine (1.0 mm) added.

(2) The calculation of the oxygen required for the formation of ketone bodies is based on the equation:

This assumption ignores the formation of β-hydroxybutyrate, which was no more than 20% of the total ketone bodies formed.

(3) The small amount of radioactivity in substances other than ketone bodies recovered in the water-soluble fraction of the medium (e.g. glucose) was taken to be incorporated by exchange reactions as described by Krebs *et al.* (1966) and was therefore considered to be equivalent to an amount of oleate oxidized to carbon dioxide.

On the basis of these assumptions only about 46% of the oxygen uptake can be accounted for by the complete oxidation of the added oleate and about 3% by the formation of ketone bodies. This is in agreement with the measurements of the specific radioactivity of the carbon dioxide formed, which was about 36% of that of the added oleate. Thus endogenous substrates contributed about 50% of the fuel of respiration. The value of the respiratory quotient (0.75) and the absence of other oxidizable substrates imply that it was endogenous fat that provided this fraction of the respiratory fuel. The amount of endogenous fat used can be calculated from the oxygen consumption and was found to be about equivalent to the amount of oleate incorporated into neutral fat in the tissue. Whether this equivalence is accidental is an open question.

Table 6. *Contribution of [U-¹⁴C]oleate oxidation to oxygen consumption of kidney-cortex slices*

The calculations are based on the experiments recorded in Tables 3 and 4. The O₂ equivalent to the complete combustion of 1 mole of oleate is 25.5 moles; the production of acetoacetate from 1 mole of oleate requires 7.5 moles of O₂. The [U-¹⁴C]oleate used and O₂ equivalents are expressed as μmoles/hr./g. dry wt. of tissue. The experimentally observed O₂ consumption was converted into 'O₂ equivalents' as described by Williamson & Krebs (1961).

Substrate(s) added ...	[U- ¹⁴ C]Oleate (2.0mm) +				[U- ¹⁴ C]Oleate (2.0mm) + L-α-glycerophosphate (2.5mm) +					
	No further addition		DL-Carnitine (1.0mm)		No further addition		DL-Carnitine (1.0mm)			
	[U- ¹⁴ C]- Oleate used	O ₂ equiv- alents	% of total O ₂ equiv- alents	[U- ¹⁴ C]- Oleate used	[U- ¹⁴ C]- Oleate used	O ₂ equiv- alents	% of total O ₂ equiv- alents	[U- ¹⁴ C]- Oleate used	O ₂ equiv- alents	% of total O ₂ equiv- alents
Radioactivity recovered in										
CO ₂	18.2	464	37.5	17.5	22.9	584	36.0	22.0	561	34.8
Intermediates (by metabolic incorporation)	4.0	103	8.3	5.0	5.9	151	9.3	7.1	181	11.2
Ketone bodies	4.8	36	2.9	10.6	4.2	32	2.0	7.9	59	3.7
Neutral lipid	24.4	622	50.3	22.1	33.5	854	52.7	31.8	811	50.3
Total	51.4	1235	100	55.4	66.5	1621	100	68.8	1612	100
Experimental observations (oleate uptake and O ₂ consumption in μmoles/hr./g. dry wt. of tissue)	73.5	1220		72.5	91.6	1465		91.9	1493	
Calc. O ₂ consumption										
Observed O ₂ consumption										
		101.2				110.8			108.0	

Table 7. *Effect of starvation on ketone-body formation from oleate by rat kidney-cortex slices*

Kidney-cortex slices were incubated for 60 min. in phosphate-buffered saline containing oleate (2.0 mM) and bovine serum albumin (2%, w/v). For full experimental details see the text. The results are given as means $\pm$ S.D.

Duration of starvation (hr.)	No. of animals	Addition ...	Total ketone-body formation (μ moles/hr./g. dry wt. of tissue)		[β -Hydroxybutyrate]/[acetoacetate] ratio	
			No added carnitine	DL-Carnitine (1.0 mM)	No added carnitine	DL-Carnitine (1.0 mM)
0	4		3.1 $\pm$ 0.6	6.5 $\pm$ 1.2	0.70 $\pm$ 0.21	0.56 $\pm$ 0.12
24	3		3.1 $\pm$ 0.2	12.2 $\pm$ 2.6	0.26 $\pm$ 0.04	0.20 $\pm$ 0.03
48	8		10.8 $\pm$ 4.5	30.8 $\pm$ 8.1	0.21 $\pm$ 0.05	0.21 $\pm$ 0.03
72	5		19.1 $\pm$ 7.2	38.1 $\pm$ 9.2	0.28 $\pm$ 0.04	0.26 $\pm$ 0.03

Effect of starvation on oleate oxidation. Ketone-body formation from oleate was stimulated six- to seven-fold by starvation (Table 7). Irrespective of the nutritional state DL-carnitine (1.0 mM) increased ketone-body formation, but the percentage increase was less after prolonged starvation. The total uptake of oleate did not increase on starvation. Apart from minor changes due to the increased ketogenesis, the pattern of isotope distribution and the percentage contribution of oleate (2.0 mM) to the fuel of respiration in tissue from well-fed and 48 hr.-starved rats, as calculated above, were similar.

The stimulation of [^{14}C]oleate incorporation into the neutral lipids of the tissue by α -glycerophosphate ceased when the starvation period was prolonged for 72 hr.

Contribution of lactate to the respiratory fuel. Previous experiments (Krebs, Hems & Gascoyne, 1963; Krebs, Speake & Hems, 1965; Krebs *et al.* 1966; Krebs, Gascoyne & Notton, 1967) have shown that in the kidney cortex of the well-fed rat lactate could contribute up to 48% to the fuel of respiration when added at 10 mM or 2.5 mM as sole substrate. When acetoacetate or short-chain fatty acids were also added, lactate no longer served as fuel, but was virtually quantitatively converted into glucose. Table 8 shows that in kidney-cortex slices from starved rats addition of oleate (2.0 mM) plus DL-carnitine (1.0 mM) also abolished the utilization of lactate as fuel of respiration: under these conditions all the lactate used was recovered as glucose or pyruvate, the formation of pyruvate being a very minor process contributing less than 0.8% to the oxygen consumption. When carnitine was absent, about 10% of the respiration could still be attributed to the oxidation of lactate. Like short-chain fatty acids, oleate, with and without carnitine, increased the rate of glucose formation from lactate (up to 45%) and it is noteworthy that this increase was accompanied by a rise in the rate of ketogenesis. Both increases can be accounted for by an increased steady-state concentration of

mitochondrial acetyl-CoA in those cells that convert lactate into glucose; acetyl-CoA activates pyruvate carboxylase and also serves as a precursor of acetoacetate.

Contribution of acetoacetate to the respiratory fuel. Earlier work (Krebs, 1961; Krebs *et al.* 1966) showed that 5 mM- or 10 mM-acetoacetate suppresses the endogenous respiration of kidney cortex and can supply 60–75% of the respiratory fuel. As shown in Table 9, this effect depends on the concentration of acetoacetate, but even at 1.0 mM acetoacetate still contributes 40% to the fuel and at 0.5 mM it contributes 25%. Since acetoacetate caused an increase in the rate of oxygen consumption the inhibition of endogenous respiration was somewhat smaller than the contribution to the fuel. As expected when the rate of gluconeogenesis by slices is insignificant the ratio:

$$\frac{\text{Specific radioactivity of } ^{14}\text{CO}_2}{\text{Specific radioactivity of } [^{14}\text{C}]\text{acetoacetate}} \times 100$$

was almost identical with the percentage contribution of acetoacetate to the fuel. Starvation for 48 hr. (Table 10) diminished the contribution to the fuel at low concentrations of acetoacetate (0.25–1.0 mM), but had little effect when the acetoacetate concentration was higher than 5 mM. Thus in the competition between acetoacetate and endogenous substrates the latter obtain a greater share of the fuel after a period of starvation.

The addition of oleate (1.0 mM) plus carnitine decreased the contribution of acetoacetate oxidation to the oxygen consumption from 63% to 45% in slices from well-fed rats (Table 11). After 48 hr. of starvation oleate plus carnitine lowered the rate of acetoacetate oxidation by 40% and under these conditions the net contribution of acetoacetate to the fuel of respiration was decreased to 24%.

Ketogenesis from short-chain fatty acids. The rate of ketone-body formation from short-chain fatty acids depends on the fatty acid concentration. The optimum concentrations were established in

Table 8. *Contribution of lactate (2.5mm) to the fuel of respiration of rat kidney-cortex slices in the presence and absence of oleate ± carnitine*

Kidney-cortex slices from 48 hr.-starved rats were incubated under standard conditions. All cups contained bovine serum albumin (2%, w/v). The results are given as means ± s.d. for three experiments.

Substrate(s) added ...	Metabolic changes (μmoles/hr./g. dry wt. of tissue)									
	Oleate (2.0mm) +			L-Lactate (2.5mm) +			Oleate (2.0mm) + DL-carnitine (1.0mm)			ratio
	DL-Carnitine (1.0mm)	No further addition	DL-Carnitine (1.0mm)	DL-Carnitine (1.0mm)	No further addition	DL-Carnitine (1.0mm)	DL-Carnitine (1.0mm)	No further addition	DL-Carnitine (1.0mm)	
O ₂	None	—	—	—	—	—	—	—	—	
Glucose	1030 ± 82	—	1290 ± 120	1315 ± 81	—	1360 ± 31	1430 ± 52	—	1520 ± 44	
Lactate	+19 ± 2.1	+23 ± 3.5	+17 ± 1.9	+21 ± 0.1	+107 ± 5	+114 ± 9	+139 ± 9	+155 ± 3	—	
Pyruvate	+7 ± 1.9	+6 ± 0.9	+8 ± 3.9	+8 ± 3.1	—	—	—	—	—	
Total ketone bodies	+1.2	+1.3	+1.0	+1.1	+21	+22	+20	+20	+20	
[β-Hydroxybutyrate]	+6.6	+7.8	+16.6	+39.8	+11.2	+12.3	+15.6	+24.0	+24.0	
[Acetoacetate]	0.22	0.36	0.27	0.26	0.24	0.24	0.27	0.25	0.25	
Lactate used — pyruvate formed					3.2 ± 0.1	3.1 ± 0.1	2.3 ± 0.2	2.0 ± 0.1	2.0 ± 0.1	
% contribution of lactate oxidation to O ₂ consumption					27 ± 2.5	29 ± 3.2	8 ± 5.4	0 ± 1.5	0 ± 1.5	

preliminary tests and these are given, together with the corresponding rates of ketogenesis, in Table 12. When no carnitine was added butyrate (10mm) gave the highest rate among the fatty acids tested, and the rate was not increased by carnitine. The stimulating effect of carnitine increased with the chain length of the fatty acids and was by far the greatest with oleate. It should be emphasized that the absence of a carnitine effect under the test conditions does not prove that carnitine is not involved, as kidney cortex is known to contain about 0.25mm-L-carnitine (Broekhuysen, Rozenblum, Ghislain & Deltour, 1965).

Added DL-carnitine had no effect on the rates of removal of acetate and butyrate from the phosphate-buffered medium under conditions where propionate removal is increased by 100% (Weidemann & Krebs, 1969). At 2.5mm acetate was removed at 300 μmoles/hr./g. dry wt. of tissue and butyrate at 230 μmoles/hr./g. dry wt. of tissue. Ketogenesis is therefore small in relation to the total uptake of fatty acids by the slices. Most of the acetate or butyrate taken up is oxidized to completion (see the next section).

Competition between acetoacetate and butyrate. Since acetoacetate can be readily oxidized by kidney cortex, its accumulation on addition of fatty acids suggests that the utilization of acetoacetate is inhibited by fatty acids, presumably competitively. To study the competition, various concentrations of acetoacetate and butyrate were added to slices and the net changes in the concentrations of ketone bodies were measured. Low butyrate concentrations (less than 1mm) inhibited the net removal of acetoacetate (initial concentration 2.5mm) by 20%. At higher concentrations (10mm) butyrate completely suppressed the utilization of 2.5mm-acetoacetate and even caused a net production of ketone bodies. This is illustrated in Figs. 2(a) and 2(b), which show at which concentrations of butyrate and acetoacetate no net changes in the concentrations of total ketone bodies occurred.

A detailed analysis of the fate of butyrate and acetoacetate was carried out with the help of ¹⁴C-labelled substrates (Tables 13, 14 and 15). The net changes in the medium (Table 13) indicate that at 2.5mm acetoacetate suppressed the uptake of butyrate (10mm) by 50%. Acetoacetate uptake was suppressed by 87% on addition of butyrate. With both acetoacetate (2.5mm) and butyrate (10mm) present, acetoacetate made no net contribution to the fuel of respiration, as β-hydroxybutyrate formation exceeded the rate of acetoacetate uptake.

Isotope distribution. In the first type of experiment the effect of unlabelled acetoacetate on the oxidation of [1-¹⁴C]butyrate was studied. With [1-¹⁴C]butyrate as sole substrate 10% of the added radioactivity was removed and, of the radioactivity

Table 9. *Effect of [4-¹⁴C]acetoacetate concentration on acetoacetate oxidation by rat kidney-cortex slices*

Kidney-cortex slices from well-fed rats were incubated for 60 min. in phosphate-buffered saline. [4-¹⁴C]Acetoacetate of specific radioactivity 11.3×10^4 c.p.m./ μ g. atom of C was added/cup. For full experimental details see the text. The percentage contribution of acetoacetate to the fuel of respiration was calculated as given in Williamson & Krebs (1961).

Substrate added ...	Metabolic changes (μ moles/hr./g. dry wt. of tissue)						
	None	[4- ¹⁴ C]Acetoacetate					
		(0.1mm)	(0.5mm)	(1.0mm)	(2.0mm)	(5.0mm)	(10.0mm)
O ₂	-834	-908	-1060	-1030	-1118	-1169	-1023
CO ₂	+611	+632	+795	+810	+882	+944	+938
Acetoacetate	—	-16.6	-73.0	-119	-159	-243	-245
β -Hydroxybutyrate	—	0	+5.0	+14.6	+31.3	+44.4	+49.6
Acetoacetate 'oxidized'	—	-16.6	-68.0	-104	-128	-199	-195
% contribution of acetoacetate oxidation to O ₂ consumption		7.3	25.6	40.1	45.2	66.8	74.5
% suppression of endogenous respiration		0	5.2	25.6	25.4	52.7	68.0
$10^{-2} \times ^{14}\text{CO}_2$ formed (c.p.m.)		864	3474	5260	7130	7150	10880
$10^{-4} \times$ Sp. radioactivity (c.p.m./ μ g. atom of C)		0.91	3.02	4.32	5.10	5.87	7.91
Sp. radioactivity of CO ₂							
Sp. radioactivity of acetoacetate $\times 100$		8.1	26.7	38.3	45.1	52.0	70.0

Table 10. *Effect of starvation for 48 hr. on the contribution of acetoacetate to the fuel of respiration of rat kidney-cortex slices*

Kidney-cortex slices from well-fed and 48 hr.-starved rats were incubated for 120 min. in phosphate-buffered saline. For full experimental details see the text. The results are given as means $\pm$ S.D. for three experiments.

Nutritional state ... Concn. of added acetoacetate (mm)	% contribution of acetoacetate oxidation to O ₂ consumption	
	Well-fed	Starved (48 hr.)
0.25	30 $\pm$ 2	14 $\pm$ 6
1.0	45 $\pm$ 4	27 $\pm$ 8
5.0	63 $\pm$ 8	47 $\pm$ 12
10.0	83 $\pm$ 12	73 $\pm$ 19

recovered, 58% was found in carbon dioxide and 34% in the three ketone bodies (Table 14). Minor amounts were incorporated into intermediates and other metabolic products. Addition of acetoacetate (2.5mm) depressed the uptake of [1-¹⁴C]butyrate by 53% and caused a greater proportion (55%) of the radioactivity of the butyrate taken up to appear in the ketone bodies. The radioactivity of the respiratory carbon dioxide was depressed by 52% (i.e. to the same extent as butyrate uptake) and accounted for only 39% of the butyrate removed. At 0.25mm, on the other hand, acetoacetate depressed ¹⁴CO₂ production from [1-¹⁴C]-butyrate by less than 3%.

Table 11. *Effect of added oleate (1.0mm) on the rate of acetoacetate oxidation by rat-cortex kidney slices*

Kidney-cortex slices from well-fed and 48 hr.-starved rats were incubated for 120 min. in phosphate-buffered saline. The initial substrate concentrations were: acetoacetate, 5.0mm; oleate, 1.0mm; DL-carnitine, 1.0mm. The results are given as means $\pm$ S.D. for four experiments.

Substrate(s) added	Acetoacetate used - β -hydroxybutyrate formed (μ moles/2hr./g. dry wt. of tissue)	% contribution of acetoacetate oxidation to O ₂ consumption
Expt. 1: well-fed rats		
Acetoacetate	253 $\pm$ 36	63 $\pm$ 8
Acetoacetate + oleate + DL-carnitine	194 $\pm$ 14	45 $\pm$ 5
Expt. 2: 48 hr.-starved rats		
Acetoacetate	170 $\pm$ 24	47 $\pm$ 8
Acetoacetate + oleate + DL-carnitine	106 $\pm$ 22	24 $\pm$ 4

In the absence of added acetoacetate the specific radioactivities of the carbon atoms of carbon dioxide, acetoacetate and D(-)- β -hydroxybutyrate were identical, indicating that all arise from a common pool of acetyl-CoA. This is in contrast with the finding with oleate (Table 4). The dilution of label confirms that about 30% of the carbon was derived from endogenous sources. As expected, added unlabelled acetoacetate diluted the specific

radioactivity of the [^{14}C]acetoacetate formed from [$1\text{-}^{14}\text{C}$]butyrate, but neither the $^{14}\text{CO}_2$ nor the $\text{D}(-)\text{-}\beta\text{-hydroxy}[^{14}\text{C}]\text{butyrate}$ formed was diluted to the same extent. The dilution of label in carbon dioxide was greater than that expected on the

assumption that the butyrate not accounted for by net ketone-body formation was completely oxidized. Thus more of the carbon dioxide was derived from added unlabelled acetoacetate than could be predicted from the net changes. The specific radioactivity of the $\text{D}(-)\text{-}\beta\text{-hydroxybutyrate}$ carbon suggests that acetoacetate was not in exchange equilibrium with $\text{D}(-)\text{-}\beta\text{-hydroxybutyrate}$ in the cup.

Table 12. Ketone-body formation from fatty acids by rat kidney-cortex slices

The optimum concentrations were established by incubating kidney-cortex slices from 48 hr.-starved rats in phosphate-buffered saline containing fatty acids in the concentration range 0.5–20 mM. Rates of ketogenesis with carnitine are given at the optimum concentration established in its absence. For full experimental details see the text.

Substrate	Optimum concn. of fatty acid (mM)	Rate of ketogenesis ($\mu\text{moles/hr./g. dry wt. of tissue}$)	
		No carnitine added	DL-Carnitine (1.0 mM)
None		2	4
Acetate	20	25	
Crotonate	10	40	
Butyrate	10	81	76
Hexanoate	1	46	56
Octanoate	0.5	60	81
Oleate (+2% serum albumin)	2	11	31

The effect of unlabelled butyrate on [$4\text{-}^{14}\text{C}$]acetoacetate oxidation is shown in Table 15. In the absence of butyrate 56–77% of the acetoacetate removed (not converted into $\beta\text{-hydroxybutyrate}$ and acetone) was completely oxidized. A higher percentage of the radioactivity was recovered in glucose, tricarboxylic acid-cycle intermediates and amino acids than with [$1\text{-}^{14}\text{C}$]butyrate. This is as expected, because of the longer retention of label from [$2\text{-}^{14}\text{C}$] than from [$1\text{-}^{14}\text{C}$]acetyl-CoA in the oxaloacetate formed after one turn of the cycle (Krebs *et al.* 1966). Butyrate (10 mM) decreased the uptake of [$4\text{-}^{14}\text{C}$]acetoacetate by 57% and 46% at 0.25 mM and 2.5 mM respectively. This was accompanied by an inhibition of $^{14}\text{CO}_2$ formation of 50% at 0.25 mM and of 28% at 2.5 mM, and a fall in the specific radioactivity of carbon dioxide due to dilution by carbon dioxide from butyrate. At 2.5 mM the fall in $^{14}\text{CO}_2$ production from [$4\text{-}^{14}\text{C}$]acetoacetate was much smaller than that predicted from the net changes (Table 13).

Fig. 2. Effect of (a) acetoacetate concentration and (b) butyrate concentration on the net oxidation or production of ketone bodies when both substrates are added together. Kidney-cortex slices from 48 hr.-starved rats were incubated for 60 min. in phosphate-buffered saline. (a) Changes in total ketone bodies with: $\circ$, no butyrate added; $\bullet$, butyrate (10 mM) added. (b) Changes in total ketone bodies with: $\circ$, no acetoacetate added; Δ , acetoacetate (2.5 mM) added; $\blacktriangle$, acetoacetate (5.0 mM) added.

Table 13. *Net changes on addition of butyrate (10.0mM) and acetoacetate (0.25mM and 2.5mM) to rat kidney-cortex slices*

Kidney-cortex slices from 48hr.-starved rats were incubated for 60min. in phosphate-buffered saline. For other experimental details see the text. The results are given as means $\pm$ S.D. for three experiments, except those for changes in butyrate, which are given as means of two experiments only.

Substrate(s) added ...	Metabolic changes (μ moles/hr./g. dry wt. of tissue)					
	None	Acetoacetate (0.25mM)	Acetoacetate (2.5mM)	Butyrate (10mM) +		
				No further addition	Acetoacetate (0.25mM)	Acetoacetate (2.5mM)
O ₂	-992 $\pm$ 32	-1069 $\pm$ 43	-1026 $\pm$ 39	-1045 $\pm$ 13	-1066 $\pm$ 67	-1019 $\pm$ 34
CO ₂	+889 $\pm$ 24	+1039 $\pm$ 44	+1061 $\pm$ 53	+912 $\pm$ 62	+924 $\pm$ 41	+920 $\pm$ 57
Acetoacetate	+5 $\pm$ 1	-49 $\pm$ 7	-177 $\pm$ 18	+78 $\pm$ 13	+64 $\pm$ 18	-24 $\pm$ 23
β -Hydroxybutyrate	+3 $\pm$ 2	+7 $\pm$ 3	+65 $\pm$ 3	+14 $\pm$ 1	+19 $\pm$ 1	+45 $\pm$ 2
Net change in total ketone bodies	+8 $\pm$ 3	-42 $\pm$ 9	-112 $\pm$ 16	+92 $\pm$ 14	+83 $\pm$ 19	+21 $\pm$ 25
Butyrate				-245	-191	-114
% contribution of acetoacetate oxidation to O ₂ consumption		16	43			
% contribution of complete oxidation of butyrate not converted into ketone bodies to O ₂ consumption				73	50	44

The fall in the specific radioactivity of [4-¹⁴C]-acetoacetate, especially at 0.25mM, shows that ketone bodies were formed from endogenous fatty acids during the oxidation of acetoacetate. Added butyrate caused a much larger dilution of label than endogenous fuels.

The general inference from these competition studies is that the rate of ketone-body formation from a fatty acid depends, not only on the concentration of the fatty acid, but also on that of the ketone bodies in the incubation medium.

Distribution of carboxyl carbon of butyrate in acetoacetate. The distribution of radioactivity derived from [1-¹⁴C]butyrate (Table 16) shows that both C-1 and C-3 of the ketone bodies were labelled. The labelling patterns in acetoacetate and β -hydroxybutyrate were virtually the same and C-1 was labelled preferentially, with about 60% of the label in this position. These findings are similar to those reported for rat liver, sheep rumen epithelium and sheep omasum by Hird & Symons (1961, 1962).

Rates of ketone-body synthesis in the perfused and sliced rat kidney. The isolated perfused rat kidney formed ketone bodies from butyrate (5.0mM) at about the same rate as in kidney-cortex slices (Table 17). This is in contrast with the rat liver, where the perfused organ gave much higher rates than slices (Krebs, 1967). It is noteworthy, however, that the [β -hydroxybutyrate]/[acetoacetate] ratio (measured in the perfusate) was much lower (0.2) with the kidney preparation than with the perfused liver, where it was 1-3 (H. A. Krebs, P. G. Wallace & R. Hems, unpublished work). As

shown by Nishiitsutsuji-Uwo *et al.* (1967) the oxygen uptake by the perfused kidney was consistently higher than in slices.

Whereas renal ketogenesis from oleate was increased by starvation (Table 7) the nutritional state made no difference with butyrate.

DISCUSSION

Preferred fuel of respiration. It is now firmly established that fatty acids and acetoacetate, when available in concentrations that may be regarded as physiological, are the main exogenous fuels of respiration in rat kidney cortex. Glucose and lactate make practically no contribution to the fuel when fatty acids, or ketone bodies, or both, are available (Table 8; Krebs *et al.* 1966; Underwood & Newsholme, 1967). Lactate can serve as fuel when present in excess and when fatty acids or ketone bodies are absent. Fatty acids and ketone bodies compete as fuels and the preference for one over the other depends on their relative concentrations. The contribution made by amino acids to the fuel of respiration, although not negligible, is likely to be very small, as the carbon skeletons of many amino acids are converted quantitatively into glucose.

Under the test conditions (i.e. in slices) only fatty acids and ketone bodies suppressed the oxidation of endogenous substrates, but the suppression was not complete. With butyrate (10mM) it was 80%. With oleate (2mM) the endogenous substrates still contributed approx. 50% of the fuel. The main endogenous fuel must have been triglyceride as, in

Table 14. Isotope distribution on addition of [1-¹⁴C]butyrate (10 mm) to rat kidney-cortex slices oxidizing acetoacetate (0.25 mm and 2.5 mm)

The specific radioactivity of [1-¹⁴C]butyrate was 7.85×10^4 c.p.m./ μ g. atom of C and the total radioactivity added/cup was 125640×10^3 c.p.m. Radioactivity in water-soluble substances other than those given was recovered in: hexose phosphates, citrate, α -oxoglutarate, malate, alanine, aspartate, glutamate, glutamine, glycine, serine, tryptophan, glycogen and protein.

Substrate(s) added ...	[1- ¹⁴ C]Butyrate (10 mm)	[1- ¹⁴ C]Butyrate (10 mm) + acetoacetate (0.25 mm)			[1- ¹⁴ C]Butyrate (10 mm) + acetoacetate (2.5 mm)		
		10 ⁻³ x Radioactivity (c.p.m.)	% of recovered radioactivity (not including butyrate)	10 ⁻⁴ x Sp. radioactivity (c.p.m./ μ g. atom of C)	10 ⁻² x Radioactivity (c.p.m.)	% of recovered radioactivity (not including butyrate)	10 ⁻⁴ x Sp. radioactivity (c.p.m./ μ g. atom of C)
Radioactivity recovered in							
Butyrate	113130	7.85		7.85	118950		7.85
Acetoacetate	2980	5.22	28.6	3.76	3500	47.7	0.92
Acetone	107		1.0		115	1.6	
β -Hydroxybutyrate	500	5.78	4.8	5.52	405	5.5	1.64
CO ₂	6000	5.28	57.6	4.25	2860	39.0	2.07
Glucose	103	0.57	1.0	0.74	97	1.3	0.80
Lactate + pyruvate	185		1.8		107	1.5	
Other water-soluble substances	530	5.2	5.2		254	3.4	
in medium							
Total radioactivity recovered	123535				126288		

addition to the low respiratory quotient, both the total amount of stored carbohydrate and the rates of production of ammonia by the tissue were low. Remarkably, the incorporation of oleate into neutral fat was equivalent to the amount of endogenous triglyceride oxidized. This indicates a high turnover rate of the neutral-lipid fraction.

The data in Tables 3, 4 and 6 allow calculations to be made of the turnover rates of triglycerides and phospholipids under the test conditions. According to Williams *et al.* (1945) kidneys from 70-day-old male rats contain 3.16% of the dry weight as neutral fat and 15.19% as phospholipid. On the assumption that 100% of the respiration was due to fat oxidation, approx. 24 μ moles of the total pool size of 117 μ moles of triglyceride fatty acids/g. dry wt. of tissue were replaced in 1 hr. when slices were incubated with 2 mm-oleate (Table 6). This gives a half-life of only 2.5 hr. for the endogenous neutral fat. A similar calculation for the half-life of the tissue phospholipids gives a value of 43 hr. Analogous results have been obtained by Olson & Hoeschen (1967) and Denton & Randle (1967) for the isolated perfused rat heart.

The close proximity of neutral-lipid droplets to the mitochondria of the cells of the proximal-tubule epithelium of the dog kidney (Carlson, Liljedahl & Wirsén, 1965; Maunsbach & Wirsén, 1966) is of interest in this context. If other endogenous substrates (e.g. amino acids) contributed significantly to the fuel of respiration, the rate of turnover of the triglyceride pool would be correspondingly lower, but would still be relatively high.

It must be emphasized, however, that the high turnover rate may be an experimental artifact. It is possible that the endogenous fat is normally tapped only when the tissue is not supplied with sufficient fuel from the circulation. This would imply that tissues contain regulatory mechanisms that control the supply of fuel from the endogenous triglycerides (e.g. an intracellular lipase). The activity of the tissue lipase may be abnormally high in the sliced cortex owing to loss of control mechanisms on slicing.

Acetyl-CoA pools. The conclusion (Table 4) that the respiratory carbon dioxide and the ketone bodies formed in the presence of [U-¹⁴C]oleate come from different acetyl-CoA pools does not imply that the two postulated pools are in the same cell. Kidney cortex comprises many different types of cells, and it is feasible that the formation of ketone bodies occurs only in certain cells, which readily oxidize added oleate, and that other cells, which under the test conditions mainly oxidize endogenous fat, degrade this to completion and do not form ketone bodies. This is supported by the observation that the endogenous net ketogenesis of the kidney is very low, even after starvation and with added carnitine

Table 15. *Isotope distribution on addition of butyrate (10 mm) to rat kidney-cortex slices oxidizing [4-¹⁴C]acetoacetate (0.25 mm and 2.5 mm)*

The specific radioactivity of [4-¹⁴C]acetoacetate was 26.10×10^4 c.p.m./ μ g. atom of C and the total radioactivity added/cup was 10300×10^2 c.p.m. at 0.25 mm and 102980×10^2 c.p.m. at 2.5 mm. Radioactivity in water-soluble substances other than those given was recovered in: hexose phosphates, citrate, α -oxoglutarate, succinate, fumarate, malate, alanine, aspartate, glutamate, glutamine, glycine, serine, tryptophan, glycogen and protein.

Substrate(s) added ...	[4- ¹⁴ C]Acetoacetate (0.25 mm) +						[4- ¹⁴ C]Acetoacetate (2.5 mm) +					
	No further addition			Butyrate (10 mm)			No further addition			Butyrate (10 mm)		
	10 ⁻² × Radio-activity (c.p.m.)	% of recovered radio-activity (not including ketone bodies)	10 ⁻⁴ × Sp. radio-activity (c.p.m./ μ g. atom of C)	10 ⁻² × Radio-activity (c.p.m.)	% of recovered radio-activity (not including ketone bodies)	10 ⁻⁴ × Sp. radio-activity (c.p.m./ μ g. atom of C)	10 ⁻² × Radio-activity (c.p.m.)	% of recovered radio-activity (not including ketone bodies)	10 ⁻⁴ × Sp. radio-activity (c.p.m./ μ g. atom of C)	10 ⁻² × Radio-activity (c.p.m.)	% of recovered radio-activity (not including ketone bodies)	10 ⁻⁴ × Sp. radio-activity (c.p.m./ μ g. atom of C)
Radioactivity recovered in												
Acetoacetate	1720		18.98	6640		11.32	64620		23.44	82265		22.55
Acetone	205			370			3350			1370		
β -Hydroxybutyrate	445		18.62	0		0	7060		22.14	2750		10.06
CO ₂	6295	76.0	5.51	2975	63.5	2.36	16900	64.0	12.20	12200	51.3	8.95
Glucose	820	9.9	5.19	346	7.4	3.56	2450	9.4	16.83	1130	4.8	11.64
Lactate + pyruvate	66	0.8		86	1.8		370	1.4		360	1.5	
Other water-soluble substances in medium	1100	13.3		1290	27.3		6700	25.2		10110	42.4	
Total radioactivity recovered	10650			11710			101450			110190		

Table 16. *Incorporation of isotope from [1-¹⁴C]-butyrate into C-3 of acetoacetate and β -hydroxybutyrate formed by rat kidney-cortex slices*

The radioactivity is that recovered in the ketone bodies formed from [1-¹⁴C]butyrate at the end of incubation for the experiment recorded in Table 14. Radioactivities were determined separately in ¹⁴CO₂ and [¹⁴C]acetone after decarboxylation of [¹⁴C]acetoacetate by the method described in the text. β -Hydroxy[¹⁴C]butyrate was first converted enzymically into acetoacetate, as described by Mayes & Felts (1967).

	Ketone body formed	Radio-activity (c.p.m.)	% distribution of radioactivity	
			C-1	C-3
Cup 1	Acetoacetate	298 000	58.2	41.8
	β -Hydroxybutyrate	49 700	60.0	40.0
Cup 2	Acetoacetate	335 600	60.6	39.4
	β -Hydroxybutyrate	71 250	60.7	39.3
Cup 3	Acetoacetate	349 500	68.4	31.6
	β -Hydroxybutyrate	40 500	64.0	36.0

(Table 3), when the bulk of the endogenous respiration is an oxidation of fat. However, the possibility of different mitochondrial populations within one cell cannot be excluded (Brdiczka, Pette & Sippell, 1965), but it is unlikely that there are two pools of acetyl-CoA derived from fatty acids in one and the same mitochondrion.

An extension of this interpretation is the assumption that gluconeogenesis also occurs only in certain cells, and that these cells are the same as those that convert oleate into ketone bodies and oxidize acetoacetate. Table 8 shows that a stimulation of gluconeogenesis from lactate by oleate and a stimulation of ketone-body formation from oleate occurred together. It is known that both ketogenesis and gluconeogenesis from lactate have a regulatory factor in common, i.e. the concentration of acetyl-CoA. This is a limiting factor of ketogenesis and it regulates the activity of pyruvate carboxylase (Utter & Keech, 1963), one of the key steps in gluconeogenesis from lactate. It would appear that the stimulation of gluconeogenesis by long-chain fatty acids in the starved state is inseparable from an increased ketogenesis when, as is the case, thiolase and 3-oxo acid CoA-transferase are present.

Mechanism of renal ketogenesis. There is a major difference between the mechanisms of ketogenesis in the liver and in renal cortex. In the liver the β -hydroxy- β -methylglutaryl-CoA pathway (Lynen, Henning, Bublitz, Sörbo & Kröplin-Rueff, 1958) is the mechanism of ketone-body formation (Hird & Symons, 1962; Sauer & Erfle, 1966; Williamson, Bates & Krebs, 1968). In the kidney a key enzyme

Table 17. *Ketone-body formation from butyrate by perfused rat kidney and rat kidney-cortex slices*

The initial butyrate concentration was 5.0 mM; that of DL-carnitine was 1.0 mM. Other experimental details are given in the text. Single determinations of the O₂ consumption are given for each treatment. The ketone-body values are given as means $\pm$ s.d. for three experiments.

Substrate(s)	Physiological state	Perfused kidney				Kidney-cortex slices			
		Total ketone bodies formed		[β -Hydroxybutyrate] [acetoacetate] ratio		Total ketone bodies formed		[β -Hydroxybutyrate] [acetoacetate] ratio	
		O ₂ consumed (μ moles/hr./g. dry wt. of tissue)	O ₂ consumed (μ moles/hr./g. dry wt. of tissue)	O ₂ consumed (μ moles/hr./g. dry wt. of tissue)	O ₂ consumed (μ moles/hr./g. dry wt. of tissue)	O ₂ consumed (μ moles/hr./g. dry wt. of tissue)	O ₂ consumed (μ moles/hr./g. dry wt. of tissue)	O ₂ consumed (μ moles/hr./g. dry wt. of tissue)	O ₂ consumed (μ moles/hr./g. dry wt. of tissue)
Butyrate	Well-fed	2120	81 $\pm$ 3	0.25 $\pm$ 0.15	1440	70 $\pm$ 10	0.27 $\pm$ 0.01	0.27 $\pm$ 0.01	0.27 $\pm$ 0.01
Butyrate + DL-carnitine	Well-fed	1820	84 $\pm$ 4	0.30 $\pm$ 0.15	1654	74 $\pm$ 8	0.28 $\pm$ 0.01	0.28 $\pm$ 0.01	0.28 $\pm$ 0.01
Butyrate	Starved (48 hr.)	1900	85 $\pm$ 8	0.21 $\pm$ 0.06	1130	77 $\pm$ 10	0.21 $\pm$ 0.02	0.21 $\pm$ 0.02	0.21 $\pm$ 0.02
Butyrate + DL-carnitine	Starved (48 hr.)	1810	86 $\pm$ 8	0.14 $\pm$ 0.04	1230	84 $\pm$ 10	0.21 $\pm$ 0.04	0.21 $\pm$ 0.04	0.21 $\pm$ 0.04

Fatty acids

Scheme 1.

of this pathway, β -hydroxy- β -methylglutaryl-CoA synthetase, is virtually absent (Williamson, 1967). On the other hand the kidney possesses 3-oxo acid CoA-transferase, an enzyme absent from the liver. Since the acetoacetyl-CoA deacylase is very weak in kidney (Williamson, 1967) the most likely mechanism of renal ketogenesis from long-chain fatty acids is that shown in Scheme 1.

The activities of thiolase (EC 2.3.1.9) and 3-oxo acid CoA-transferase (EC 2.8.3.5) are high enough to account for the observed rates of ketogenesis (Wieland, Reinwein & Lynen, 1956; Williamson, 1967). This mechanism has already been discussed for heart muscle by Beinert & Stansly (1953), Stern (1955) and Hartmann & Lynen (1961). In the absence of the β -hydroxy- β -methylglutaryl-CoA pathway, the high activity of thiolase must be a major factor in renal ketogenesis from long-chain fatty acids. Whenever acetyl-CoA accumulates, a proportion is converted into acetoacetyl-CoA and made available for the transferase reaction.

Ketone-body formation from butyrate. When butyrate is the substrate acetoacetate can be formed via acetoacetyl-CoA without the participation of acetyl-CoA, as indicated in Scheme 1. The equilibrium position of the thiolase reaction ($K = 6 \times 10^{-5}$; Goldman, 1954) is highly in favour of the formation of acetyl-CoA. This fact, together with the relatively high activity of renal thiolase, explains why a proportion of the acetoacetyl-CoA derived from butyrate passes through the acetyl-CoA pool. In the absence of the thiolase reaction the specific radioactivities of C-1 of butyrate and acetoacetate would be identical. The reversibility of the thiolase reaction causes a fall in the specific radioactivity of C-1 and a rise in the specific radioactivity of C-3 of acetoacetate, and measurements of the specific radioactivities of C-1 and C-3 of acetoacetate (Table 16) form a basis for calculating the relative extent of the thiolase reaction. The fact that C-3 of the ketone bodies formed from [^{14}C]-butyrate contained 30–40% of the label indicates that 60–80% of the acetoacetyl-CoA entered the thiolase reaction before the transferase reaction.

Competition between acetoacetate and butyrate. The reactions shown in Scheme 1 also illustrate the basis of the competition between butyrate and aceto-

acetate described in Tables 13, 14 and 15. Although 10mm-butyrate caused a net production of ketone bodies in the presence of 2.5mm-acetoacetate and apparently contributed a major part of the respiratory fuel compared with no net contribution by acetoacetate, approx. 34% of the carbon dioxide carbon was formed from acetoacetate and only 26% came from butyrate. This is possible if acetoacetate and butyrate both contribute carbon to the same acetyl-CoA pool via acetoacetyl-CoA, as mixing of carbon atoms from both sources would occur in a single intracellular compartment (i.e. the mitochondrion). The ready reversibility of the transferase reaction would ensure that carbon atoms of acetoacetate exchanged with the acetyl-CoA pool (and consequently entered the tricarboxylic acid cycle) even if the net direction of the reaction:

favoured acetoacetate synthesis.

Whether there is a net oxidation of either fatty acids or ketone bodies would depend on the relative rates at which each generates acetyl-CoA. This may be regulated by a competition for the available CoA by the corresponding activating reactions. Because of the equilibrium conditions discussed above a high value for the $[\text{succinyl-CoA}]/[\text{acetoacetyl-CoA}]$ ratio would favour ketone-body activation and a low value ketone-body synthesis.

General comments on extrahepatic ketogenesis. It has been known for almost 30 years that many animal tissues can form small quantities of ketone bodies from pyruvate (Krebs & Johnson, 1937) or from short-chain fatty acids (Jowett & Quastel, 1935; Weil-Malherbe, 1936; Geyer & Cunningham, 1950; Geyer, Cunningham & Pendergast, 1950; Pennington, 1952). The earlier methods were unspecific with reference to β -hydroxybutyrate, and as they were altogether not absolutely specific some doubts remained as to the reality of extrahepatic ketogenesis. The use of the enzymic method employing β -hydroxybutyrate dehydrogenase has removed these doubts, and assays of enzymes related to ketogenesis (Williamson, 1967) have supplied essential additional information on possible pathways of extrahepatic ketogenesis. The absence of β -hydroxy- β -methylglutaryl-CoA synthetase from tissues other than liver (Lynen *et al.* 1958; Williamson, 1967) signifies fundamental differences between hepatic and extrahepatic ketone-body formation. However, according to Hird & Symons (1961) the mechanism of ketogenesis by the epithelium of the rumen and omasum of the sheep is the same as that in liver.

It is evident that the ketogenic capacity of extrahepatic tissues is an inseparable aspect of their ability to utilize acetoacetate as a fuel of respiration.

Once the enzymes of acetoacetate utilization (i.e. 3-oxo acid CoA-transferase and thiolase) are present in sufficiently high activity the tissues are bound to have a ketogenic capacity that shows itself whenever the concentration of acetyl-CoA (derived either from pyruvate or from fatty acids) is relatively high. In this situation the same reactions that are responsible for the utilization of acetoacetate, in the reverse direction, cause the formation of acetoacetate. However, it is unlikely that in cardiac muscle and in renal cortex conditions favouring net ketone-body production often obtain *in vivo*.

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