Fatty Acid Metabolism in the Perfused Rat Liver

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1. The formation of acetoacetate, β-hydroxybutyrate and glucose was measured in the isolated perfused rat liver after addition of fatty acids. 2. The rates of ketone-body formation from ten fatty acids were approximately equal and independent of chain length (90–132 μmol/h per g), with the exception of pentanoate, which reacted at one-third of this rate. The [β-hydroxybutyrate]/[acetoacetate] ratio in the perfusion medium was increased by long-chain fatty acids. 3. Glucose was formed from all odd-numbered fatty acids tested. 4. The rate of ketone-body formation in the livers of rats kept on a high-fat diet was up to 50% higher than in the livers of rats starved for 48h. In the livers of fat-fed rats almost all the O_2 consumed was accounted for by the formation of ketone bodies. 5. The ketone-body concentration in the blood of fat-fed rats rose to 4–5mM and the [β-hydroxybutyrate]/[acetoacetate] ratio rose to 11.5. 6. When the activity of the microsomal mixed-function oxidase system, which can bring about ω-oxidation of fatty acids, was induced by treatment of the rat with phenobarbitone, there was no change in the ketone-body production from fatty acids, nor was there a production of glucose from even-numbered fatty acids. The latter would be expected if ω-oxidation occurred. Thus ω-oxidation did not play a significant role in the metabolism of fatty acids. 7. Arachidonate was almost quantitatively converted into ketone bodies and yielded no glucose, demonstrating that gluconeogenesis from poly-unsaturated fatty acids with an even number of carbon atoms does not occur. 8. The rates of ketogenesis from unsaturated fatty acids (sorbate, undecylenate, crotonate, vinylacetate) were similar to those from the corresponding saturated fatty acids. 9. Addition of oleate together with shorter-chain fatty acids gave only a slightly higher rate of ketone-body formation than oleate alone. 10. Glucose, lactate, fructose, glycerol and other known antiketogenic substances strongly inhibited endogenous ketogenesis but had no effects on the rate of ketone-body formation in the presence of 2mM-oleate. Thus the concentrations of free fatty acids and of other oxidizable substances in the liver are key factors determining the rate of ketogenesis.

The isolated perfused rat liver offers the possibility of studying many quantitative aspects of liver metabolism with much greater accuracy than is practical in the whole organism or any other tissue preparations. The perfused organ has proved to be superior to slices, homogenates and other tissue preparations because much of the normal metabolic capacity as well as the control mechanisms are lost on disturbing the structure of the organ (Krebs, Wallace, Hems & Freedland, 1969).

The present work continues previous experiments on the perfused rat liver (Krebs *et al.* 1969) and is concerned with a systematic investigation of the rates of ketone-body formation from fatty acids of various chain lengths, of the rate of glucose forma-

tion from fatty acids with uneven numbers of carbon atoms, the question of the occurrence of ω-oxidation, and of the effects of high-fat diets and other factors on the capacity of the liver to degrade fatty acids and to convert them into ketone bodies.

MATERIALS AND METHODS

The liver perfusions were carried out as described by Krebs *et al.* (1969). Substrates were added 38min after the start of the perfusion and continued for a total of 130min. 'Zero' time was 40min after the start. The fatty acids used were the purest commercially available. Oleic acid ('puriss') was obtained from Fluka A.G. Chemische Fabrik, Buchs, Switzerland, as a colourless fluid stated to be at least 99% pure. Yellow-coloured samples of oleic acid from other sources gave lower

metabolic rates. Arachidonic acid was kindly supplied by Dr J. M. Osbond of Roche Products Ltd., Welwyn Garden City, Herts., U.K. It was obtained from the ethyl ester by mild alkaline hydrolysis. The ester had been prepared by the method of Osbond (1966). The preparation of long chain fatty acid stock solutions was as described by Krebs et al. (1969); it was not necessary to add bovine albumin to the stock solution when the chain length was below eight carbon atoms. Female albino rats of the Wistar strain, supplied by Scientific Products Farm, Ash Research Centre, Canterbury, Kent, U.K., weighing about 200 g were used.

Analytical methods were the same as previously described (Krebs et al. 1969) except that the Van Slyke apparatus instead of the Natelson apparatus was used for the determination of oxygen in the perfusion fluid. The oxygen determinations were carried out on 3 ml samples. The term 'total ketone bodies' refers to the sum of β-hydroxybutyrate and acetoacetate. There was no evidence to indicate a significant formation of acetone under test conditions. When acetoacetate or β-hydroxybutyrate was added to the perfused liver the amount of 'total ketone bodies' recovered was always greater than the initial amount added.

High-fat diets consisted of either 90 parts of margarine and 10 parts of casein or 95 parts of margarine and 5 parts of casein, with a supplement of salts and vitamins.

To increase the activity of the hepatic microsomal oxidases, taken to be responsible for ω-oxidation of fatty acids (see Das, Orrenius & Ernster, 1968), phenobarbitone, dissolved in 0.5 ml of 0.9% NaCl, was given to rats intraperitoneally on five consecutive days. The injections (100 mg/kg body wt. per day) were given in the morning so that during the night the rats were active and taking in food (Remmer, 1959; Remmer, Greim, Schenkman & Estabrook, 1967). On the fourth and fifth days the rats were starved to be used on the sixth day.

RESULTS

Rates of formation of ketone bodies and of glucose in the perfused rat liver in the presence of fatty acids. Table 1 expands information given previously, which covered four fatty acids with an even number of carbon atoms (Krebs et al. 1969). The rates of total ketone-body formation in the presence of optimal concentrations of ten fatty acids in the livers from starved rats were about the same and independent of the length of the carbon chain (between 90 and 132 μmol/h per g), with the exception of *n*-valerate (pentanoate) which reacted at one-third of the rate of the other acids, and propionate, which is glucogenic and antiketogenic. The rates of total ketone-body production were fairly constant throughout the perfusion time of 90 min after addition of the substrate, but, as reported previously (Table 1 in Krebs et al. 1969), the [β-hydroxybutyrate]/[acetoacetate] ratio tended to decrease during the perfusion, the decrease being similar for all substrates. The ratio depended on the length of the carbon chain and on the rate of oxidation. Long-chain substrates gave

Table 1. *Rates of formation of ketone bodies and of glucose in the presence of fatty acids in the perfused rat liver*

The rats were starved for 48 h. The rates are the initial rates, which were usually constant for 60 min after the addition of substrate. The rates are means ±S.E.M. for four observations in each case. The weights are fresh weights. The values for [β-hydroxybutyrate]/[acetoacetate] are the ratios of means of the concentrations in the medium at the time stated.

Substrate added	Initial concn. of substrate (mM)	β-Hydroxybutyrate formed (μmol/h per g)	Acetoacetate formed (μmol/h per g)	Total ketone bodies formed (μmol/h per g)	[β-Hydroxybutyrate]/[acetoacetate] ratio in medium after addition of substrate			Glucose formed (μmol/h per g)
					30 min	60 min	90 min	
None	—	19.6± 1.9	10.8±0.9	30.4± 3.3 (4)	1.36±0.15	1.05±0.06	0.99±0.12	8.4±1.6
Propionate	10	4.7± 1.4	11.4±2.4	15.7± 1.6 (3)	0.39±0.12	0.20±0.03	0.18±0.04	28.8±4.5
Butyrate	2	46.8± 8.1	47.5±8.0	94.4±14.6 (4)	1.25±0.08	0.95±0.24	0.70±0.29	
Pentanoate	4	12.0± 1.7	22.2±3.7	34.5± 5.1 (4)	0.64±0.04	0.59±0.07	0.42±0.10	22.4±1.7
Hexanoate	5	56.1± 3.9	68.5±7.3	125 ± 8.0 (4)	0.86±0.13	0.94±0.14	0.74±0.09	
Heptanoate	4	40.2± 6.6	49.8±6.6	90 ±12 (4)	0.88±0.11	0.66±0.09	0.62±0.11	25.8±1.8
Octanoate	5	72.3± 6.1	55.6±8.5	128 ± 6.8 (4)	2.82±0.54	2.08±0.43	1.19±0.47	
Nonanoate	5	47.3± 7.6	32.8±4.2	103 ±12 (4)	2.06±0.57	1.64±0.37	1.31±0.15	16.8±1.8
Decanoate	5	80.3±16	51.7±9	132 ± 9.0 (4)	1.41±0.09	1.69±0.16	1.87±0.27	
Dodecanoate	2.5	86.6± 5.5	35.4±3.4	122 ± 4.9 (4)	3.35±0.42	2.26±0.27	1.36±0.20	
Oleate	2	75.7± 3.1	22.3±3.1	98.4± 4.7 (4)	3.41±0.30	2.85±0.39	2.69±0.49	

Table 2. *Yield of glucose and ketone bodies on perfusing rat livers with fatty acids with an odd number of carbon atoms*

Livers of starved (48h) rats were perfused for 90min after addition of the fatty acids. The experiments were those for which the initial rates were given in Table 1. The expected ratio of ketone bodies formed/glucose formed was calculated on the assumption that per molecule of fatty acid 0.5molecule of glucose was formed and that the remaining carbon atoms formed ketone bodies.

Fatty acid added	Glucose formed after addition of substrate (μmol)	Ketone bodies formed after addition of substrate (μmol)	Ketone bodies formed / Glucose formed	
			Found	Expected
Pentanoate	242± 22 (4)	370± 57 (4)	1.5	1.0
Heptanoate	306± 18.5 (4)	888±165 (4)	2.9	2.0
Nonanoate	267±134 (2)	899± 92 (2)	3.35	3.0

Table 3. *Rates of formation of ketone bodies from fatty acids in the perfused liver of rats fed on a diet rich in fat*

The general conditions were as described in Table 1. The rats were kept for at least 8 days on a diet containing 90% margarine. The remainder was casein, vitamins and salts. The values are means ±S.E.M. for four observations. The concentrations of fatty acids used were those which, as established in preliminary tests, give maximal rates.

Substrate added	Initial concentration of substrate (mM)	β-Hydroxy-butyrate formed (μmol/h per g)	Acetoacetate formed (μmol/h per g)	Total ketone bodies formed (μmol/h per g)	$\dfrac{[\beta\text{-Hydroxybutyrate}]}{[\text{Acetoacetate}]}$
None		9.09± 0.76	35.1± 4.4	44.3± 3.8	0.26
Hexanoate	5	62.9±11.1	79.3± 7.9	142 ±18.9	0.79
Octanoate	5	91.8± 6.8	53.4± 3.9	145 ± 7.7	1.72
Dodecanoate	2.5	115 ±12.1	43.7±11.9	159 ±12.8	2.63
Oleate	2	113 ±28.6	33.1± 7.6	146 ± 7.6	3.41

high ratios, medium-length fatty acids raised the ratio above that of the unsupplemented control, but pentanoate, which was oxidized slowly, caused a decrease, and propionate also gave very low ratios. In general, fatty acids of approximately the same molecular weight and having either an even or odd number of carbon atoms gave about the same ratios. Glucose was formed, as expected, from all fatty acids with an odd number of carbon atoms tested. None of the fatty acids with an even number of carbon atoms gave significant increases of the glucose output over the controls without added substrate (not recorded in Table 1).

The total yield of glucose formed from the fatty acids with an odd number of carbon atoms was in each case less than expected on the basis of the formation of ketone bodies (Table 2). The deficit was small in the case of nonanoate where the yield of glucose was 89% of the theoretical yield. With pentanoate and heptanoate the yields were only 65 and 69% respectively. The fate of the three carbon atoms not recovered as glucose is uncertain. Glycogen synthesis was negligible under the test conditions.

Adaptation to high-fat diets. Experience of prolonged starvation indicates that the mammalian body can adapt its energy metabolism to the utilization of fat (Cahill, Owen & Morgan, 1968). To test whether such adaptation occurs in liver, rats were given the diet containing 90% margarine and 10% casein. The animals were kept on this diet for 7–18 days. As shown in Table 3 the rates of ketone-body formation were substantially higher than in rats starved for 48h (Table 1), although the fat-fed rats were not starved. After two or three days of under-eating and some weight loss they ate enough food to maintain their body weight after the initial loss. When oleate was added as substrate the increase in the rate of ketone-body formation was about 50%. It was between 10 and 20% with the other fatty acids tested. The endogenous rate was increased by about 50%. The [β-hydroxybutyrate]/[acetoacetate] ratio tended to be higher with the long-chain fatty acids, but was much lower when no substrate had been added.

The comparison of the rate of ketone-body formation and oxygen consumption by the livers from rats on the high-fat diet (Tables 4 and 5)

Table 4. *Ketone-body formation and oxygen uptake in perfused liver of rats fed on a high-fat diet*

The diet consisted of 90% margarine and 10% casein with salt and vitamin supplements. The animals were kept on this diet for 7–17 days (mean 11.5 days). There were slight losses of weight during this period. Oleate (2 mM) was added to the medium 38 min after the start of the perfusion. The values of the rates are means ±S.E.M. for four experiments. The oxygen uptake before the addition of oleate was 2.24±0.21 (4) μmol/min per g. The O_2 uptake required for the formation of ketone bodies was calculated on the assumption that the added oleate was the only source of ketone bodies (see Table 5). This assumption implies that the added oleate is oxidized in preference to endogenous fatty acids. It is very probable that this is the case, but even if stearate or palmitate were also substrates this will not affect the calculations significantly, as shown in Table 5.

Period of perfusion (min)	Acetoacetate formed (μmol/min per g)	β-Hydroxy-butyrate formed (μmol/min per g)	[β-Hydroxybutyrate] [Acetoacetate] ratio	Total ketone bodies formed (μmol/min per g)	Oxygen uptake (μmol/min per g)	Oxygen uptake calculated for formation of ketone bodies (μmol/min per g)	Oxygen taken up/Ketone bodies formed Found	Oxygen taken up/Ketone bodies formed Calculated
0–45	0.50±0.20	2.29±0.29	4.58	2.74±0.10	3.67±0.34	3.50±0.06	1.34	1.28
45–90	0.70±0.18	1.42±0.25	2.03	2.17±0.19	3.48±0.38	2.82±0.17	1.60	1.30

indicates that almost all the oxygen consumption was used in the formation of ketone bodies from the added oleate (95% during the first 45 min and 81% during the second 45 min). On the assumption that oleate was the only substrate oxidized, 75% of the added oleate had disappeared after 90 min. As the rate of oxidation of oleate is concentration-dependent (see Krebs *et al.* 1969) the decrease in the ratio O_2 used/ketone bodies formed was probably due to a decreased concentration of oleate. Thus when fatty acids are available in saturating concentrations, i.e. those giving maximal rates (as is likely *in vivo* in the liver of fat-fed rats), almost the whole of the respiration of livers from rats kept on a high-fat diet serves to supply the body with ketone bodies. The needs of the liver for ATP synthesis are presumably met by the energy released in the dehydrogenations involved in the formation of ketone bodies.

The rate of ketone-body formation in the livers of well-fed rats (see Krebs *et al.* 1969) in the presence of saturating concentrations of oleate was no more than one-quarter of the rates found with fat-fed livers.

There was a loss of body weight of approx. 35 g per rat of 240 g weight during the initial week on the high-fat diet, because of a decreased food intake (Table 6). After the first week the body weights remained fairly constant during the period of observation (18 days).

Blood ketone-body concentrations in fat-fed rats. When fat-fed rats were used for the perfusion experiments blood samples were taken for the determination of ketone bodies (Table 6). The total ketone-body concentration rose to between 4 and 5 mM and the [β-hydroxybutyrate]/[acetoacetate] ratio also increased, to a mean value of 7.2 after 7–12 days and 11.5 after 17–18 days. No definite trends were discernible between days 7 and 18 on the high-fat diet. Variations were considerable, as indicated by the values for the S.E.M.; the variations were erratically distributed over the periods of observation.

Effect of diet on liver weight. The wet weight of the liver per 100 g body weight remained unchanged (Table 7). Starvation on the other hand caused a loss of fresh weight of 12.8% and of dry weight of 19.8% (Table 6). The greater loss of dry weight can be explained by the assumption that the loss concerned mainly storage materials, e.g. glycogen. The fresh-weight gain on the high-fat diet must have been mainly water since it was not accompanied by a parallel gain in dry weight.

Question of ω-oxidation of fatty acids. The occurrence of ω-oxidation of fatty acids was discovered by Verkade & van der Lee (1934) (see also Verkade, van der Lee & van Alphen, 1938); but experiments by Bernhard (1941) suggested that it is a minor

Table 5. *Calculated quantitative relations between O_2 uptake and ketone-body formation from long-chain fatty acids*

Fatty acid	Overall reaction	O_2 used / Acetoacetate formed	O_2 used / β-Hydroxybutyrate formed
Oleate	$C_{18}H_{34}O_2 + 7.5\ O_2 \rightarrow 4.5$ acetoacetate	1.66	
Oleate	$C_{18}H_{34}O_2 + 5.25\ O_2 \rightarrow 4.5\ \beta$-hydroxybutyrate		1.17
Stearate	$C_{18}H_{36}O_2 + 8\ O_2 \rightarrow 4.5$ acetoacetate	1.78	
Stearate	$C_{18}H_{36}O_2 + 5.75\ O_2 \rightarrow 4.5\ \beta$-hydroxybutyrate		1.28
Palmitate	$C_{16}H_{32}O_2 + 7\ O_2 \rightarrow 4$ acetoacetate	1.75	
Palmitate	$C_{16}H_{32}O_2 + 5\ O_2 \rightarrow 4\ \beta$-hydroxybutyrate		1.25

Table 6. *Effect of high-fat diet on body weight and ketone-body concentrations in whole blood*

The rats were maintained on a high-fat diet containing 90% margarine (for details see the Materials and Methods section). The values are means $\pm$s.e.m. and, in parentheses, number of observations.

Initial body weight (g)	Days on high-fat diet	Final body weight (g)	Mean loss of body weight		Ketone bodies in whole blood	
			Absolute (g)	%	Total (mM)	β-Hydroxybutyrate / Acetoacetate
244$\pm$ 8 (8)	7–12	209$\pm$9 (8)	-35	14.4	4.32$\pm$0.87 (10)	7.2$\pm$1.8 (10)
245$\pm$10 (3)	17–18	208$\pm$8 (3)	-37	15.1	5.10$\pm$0.44 (3)	11.5$\pm$3.9 (3)

side reaction of no physiological importance. Recently it has been established that ω-oxidation is an extramitochondrial process brought about by the microsomal cytochrome P-450 system. Like the hydroxylation of drugs it involves a coupled oxidation with NADPH (Omuro & Sato, 1964a,b; Orrenius, 1965). The activity of this enzyme system greatly increases on treatment of the animal with various drugs, phenobarbitone being particularly effective (Remmer, 1959).

The ω-oxidation of fatty acids is notable because it could play a role in the formation of carbohydrate from fatty acids, especially during prolonged starvation or on a diet low in carbohydrate and protein. ω-Oxidation of fatty acids with an even number of carbon atoms would lead to the formation of succinyl-CoA, a glucogenic precursor. Thus tri-stearine would yield two molecules of glucose jointly from the glycerol moiety and three terminal carbon atoms of each chain. Without ω-oxidation tri-stearine would give only 0.5 mol of glucose.

Rats were therefore treated with phenobarbitone to induce a maximum capacity of the microsomal oxidizing system. However, the treatment did not affect the rate of glucose formation, and, as shown in Table 8, the rate of ketone-body formation was the same as without phenobarbitone treatment. Thus even under optimum conditions the fraction of fatty acids undergoing ω-oxidation is negligible.

Question of gluconeogenesis from polyunsaturated fatty acids. Linoleic acid (9,12-octadecadienoic acid), linolenic acid (9,12,15-octadecatrienoic acid) and arachidonic acid (5,8,11,14-eicosatetraenoic acid) contain C_3 units between double bonds and it has been suggested (Tidwell, Pope & Gifford, 1966) that these may yield glucose via propionate. Propionate or propionyl-CoA would arise if the degradation of the chain started at the double bonds and if the chain were broken at the site of the double bonds. If this was true arachidonic acid could provide nine out of its 20 carbon atoms for the formation of glucose. The experiment shows that arachidonic acid forms ketone bodies as rapidly as oleate; that the yield of ketone bodies is almost quantitative (Table 9) and that no extra glucose is formed. These results disprove gluconeogenesis from polyunsaturated fatty acids and are in accordance with the conclusions of Stoffel & Caesar (1965) that the β-oxidation of polyunsaturated fatty acids yields Δ^2-*cis*-enoyl-CoA esters which are hydrated by Δ^2-enoyl-CoA hydratase to D($-$)-3-hydroxyacyl-CoA esters which then epimerize to the L($+$)-isomeride through the action of 3-hydroxyacyl-CoA epimerase. These reactions channel the whole carbon chain into the pathway of β-oxidation.

Ketogenesis from other unsaturated and branched-chain fatty acids. Values for the rates of ketogenesis from linoleic acid and oleic acid in the perfused rat liver were reported by Krebs *et al.* (1969). Values

Table 7. *Weight of perfused livers from well-fed, starved (48h), fat-fed and phenobarbitone-treated rats*

The weights refer to livers that had been perfused for 130min. The wet weight was obtained by immediate weighing of the organ after severing blood vessels. The whole liver was then placed in a drying oven at 110°C for 3 days, the time required to attain constant weight. The high-fat diet contained 90% margarine (see the Materials and Methods section).

Nutritional state	Liver wet wt./100g body weight	Change from wet wt. of well-fed liver		Liver dry wt./100g body weight	Change from dry wt. of well-fed liver		Wet wt./Dry wt.
		Absolute change (g)	% change		Absolute change (g)	% change	
Well-fed	4.06±0.10 (20)			1.143±0.02 (20)			3.55
Starved	3.54±0.06 (20)	−0.52	−12.8	0.917±0.02 (20)	−0.226	−19.8	3.86
High-fat diet	4.43±0.07 (20)	+0.37	+ 9.1	1.16 ±0.02 (20)	+0.017	+ 1.5	3.82
Phenobarbitone-treated, starved	4.47±0.09 (17)	+0.41	+10.1	1.14 ±0.03 (17)	0	0	3.92

for the rates from sorbate (hexa-2,4-dienoate), undecylenate (hendecenoate), crotonate (but-2-10-enoate), vinylacetate (but-3-enoate) and isovalerate (3-methylbutanoate) are given in Table 10. The rates are very similar to those found with the corresponding saturated fatty acids.

Ketone-body formation from substrate combinations. Since short- and long-chain fatty acids are converted into their CoA esters by different enzymes (Leloir & Muñoz, 1939; Lang, 1939, 1940a,b; Beinert, 1963; Rossi & Gibson, 1964; Smith & Osborne-White, 1965; Smith & Russell, 1967), and since the thiolase reaction as well as the hydrogenation of saturated acyl-CoA esters may also involve different enzymes for short and long chains (see Lynen, 1965), it may be expected that the rates of degradation, as measured by ketone-body formation, are additive when a short- and a long-chain fatty acid are added together. The results in Table 11 show that several combinations of oleate with shorter-chain fatty acids gave only slightly higher rates than with oleate alone. The non-additive behaviour indicates that some enzymes are shared by long- and short-chain fatty acids. According to Lynen (1965) this may be the β-hydroxyacyl-CoA dehydrogenase and the enoyl-CoA hydratase (see also Decker, 1959).

Antiketogenic effects. Substances known to be antiketogenic *in vivo* (glucose, lactate, fructose, xylitol, glycerol, dihydroxyacetone, proline) strongly inhibited endogenous ketogenesis in the starved liver (Table 12), but there were no significant effects when 2mM-oleate was present in the perfusion medium. These results confirm the view that the relative concentrations of ketogenic and antiketogenic substrates are important factors determining the rate of formation of ketone bodies (Edson, 1936).

Endogenous sources of ketone bodies. The concentration of free fatty acids in the starved rat liver is about 1.3mM (Foster, 1967; Falkhoff & Kipnis, 1966). This amount can supply at most 6μmol of ketone bodies/g fresh weight, as most of the free fatty acids are C_{16} and C_{18} acids (Muto & Gibson, 1970). The rate of endogenous ketogenesis observed in starved-rat liver was 60μmol/g for the 2h period of observation. At this rate the store of free fatty acids would have been exhausted after 5min. The perfusion medium, made up with albumin as purchased, was found to contain 0.4mM free fatty acids as determined by the method of Itaya & Ui (1965). After defatting of the albumin with charcoal as described by Chen (1967) the free fatty acid content of the medium was 0.04mM. Defatting did not decrease the rate of 'endogenous' ketogenesis. When this medium was used 7g of liver still formed about 420μmol of ketone bodies in 2h. To this the free fatty acids of the medium could have contri-

Table 8. *Effect of phenobarbitone on the rate of ketone-body formation in the perfused rat liver*

The conditions were as described in Table 1, except that the rats had been treated with phenobarbitone as described in the Materials and Methods section. No extra glucose was formed on addition of the fatty acids.

Substrate added	β-Hydroxy-butyrate formed (μmol/h per g)	Acetoacetate formed (μmol/h per g)	Total ketone bodies formed (μmol/h per g)	$\dfrac{[\beta\text{-Hydroxybutyrate}]}{[\text{Acetoacetate}]}$
None	6.2± 1.2	20.4± 1.21	26.6± 1.75	0.31
Hexanoate (4 mM)	43.1± 3.6	95.4± 8.2	138.5± 5.2	0.45
Octanoate (5 mM)	92 ±28	66 ±14.9	158 ±43.0	1.39
Oleate (2 mM)	49.4± 5.1	51.8± 8.5	101 ±12.9	0.95

Table 9. *Formation of ketone bodies and of glucose in perfused rat liver on addition of arachidonate*

The livers were perfused for 90 min after the addition of arachidonate. The rate of total ketone-body production was constant when the initial substrate concentration was 1 or 2 mM. It decreased with 0.5 mM substrate. The rate given in the latter case is the initial rate. The [β-hydroxybutyrate]/[acetoacetate] ratio was about 1 initially and fell to 0.5. The liver weights were between 5.1 and 7.4 g. The rate of glucose formation in controls without added substrate was up to 70 μmol/90 min per liver.

Treatment of rat	Arachidonate added	Rate of ketone-body formation (μmol/h per g)	Total ketone bodies formed (μmol)	Theoretical ketone-body yield from added substrate (μmol)	Rate of glucose formation (μmol/h per g)	Total glucose formed (μmol)
Starved (48 h)	2 mM (300 μmol)	73.6	565	1500	4.4	34
Starved (48 h)	2 mM (300 μmol)	71.6	649	1500	2.7	25
Starved (48 h)	1 mM (150 μmol)	79.5	705	750	5.7	59
Starved (48 h)	1 mM (150 μmol)	99.6	765	750	4.0	35
Starved (48 h)	0.5 mM (75 μmol)	63.1	420	375	5.3	58
Starved (48 h)	0.5 mM (75 μmol)	43.0	382	375	8.0	71
High-fat diet	2 mM (300 μmol)	71.8	761	1500	4.8	43
High-fat diet	2 mM (300 μmol)	86.7	668	1500	2.1	23

Table 10. *Ketone-body formation from unsaturated and branched-chain fatty acids*

The experimental conditions were as described in Table 1. The values are means ±S.E.M., with numbers of observations in parentheses.

Substrate	Total ketone bodies formed (μmol/h per g)	[β-Hydroxybutyrate]/[acetoacetate] ratio in medium after addition of substrate		
		30 min	60 min	90 min
Crotonate (but-2-enoate) (4 mM)	70.0±10 (3)	0.22±0.6	0.14±0.04	0.11±0.03
Vinylacetate (but-3-enoate) (4 mM)	76.0±7.5 (3)	0.44±0.04	0.32±0.02	0.24±0.01
Sorbate (hexa-2,4-dienoate) (2 mM)	75.5±5.5 (3)	0.42±0.12	0.28±0.11	0.21±0.04
Undecylenate (hendec-10-enoate) (2 mM)	70.6±7.9 (3)	1.51±0.66	1.00±0.32	0.69±0.30
Isovalerate (3-methylbutanoate) (10 mM)	97.5±2.5 (3)	0.28±0.07	0.30±0.16	0.29±0.11

buted at most 27 μmol. It follows that the pre-formed fatty acids of the perfusion medium were not major precursors of the endogenous ketone bodies. The main precursors must have been the fatty acids set free by hepatic lipolysis. In the intact organism the main precursors are the fatty acids reaching the liver through the circulation.

DISCUSSION

Rates of ketogenesis from fatty acids. Qualitatively ketogenesis from the precursors tested in this work was already known (for review see Deuel, 1957), but quantitative information on the ketogenic capacity was not available before the use of the

isolated perfused rat liver. Compared with slices the rates of ketogenesis in the perfused organ are very high. With long-chain fatty acids the rate is more than ten times as fast in the intact organ at the same substrate concentration (Krebs, 1967).

One of the most drastic changes that occurs on slicing, and occurs with great rapidity, is the decrease in the ATP concentration and the loss of total adenine nucleotides (see Krebs, 1970). It is likely that this is one of the factors responsible for the different behaviour of slices and perfused organs. It might be expected that all energy-requiring reactions are equally affected by the loss of ATP, but this is not the case. It appears that the rate of urea synthesis can be high even if much ATP has been lost.

Effect of diet on the rate of ketone-body formation. Maximal rates of hepatic ketogenesis were obtained on rats given a diet containing 90% (w/w) fat. With 2mM-oleate in the perfusion medium the rates

were about 30% higher than those of starved rats, and 300% higher than those of rats fed on a standard diet. The endogenous rate of ketogenesis was also raised by about 30% (Table 3). One of the factors responsible for the high rate of ketogenesis on fat feeding is probably the increased capacity of hydroxymethylglutaryl-CoA synthase, the rate-limiting enzyme of ketogenesis. The capacity of this enzyme is known to increase by 140% on fat feeding (Williamson, Bates & Krebs, 1968). Stadie (1940) and Stadie, Zapp & Lukens (1940, 1941) found that in severe diabetic ketosis most of the O_2 consumption of rat or cat liver was used for the conversion of fatty acids into ketone bodies, as indicated by a very low respiratory quotient (0.32). This change of liver metabolism towards the generation of ketone bodies is even more pronounced in the livers of fat-fed rats. The significance of hepatic ketogenesis lies in the supply of a fuel of respiration to peripheral tissues (Krebs, 1961; Cahill *et al.* 1968; Owen *et al.* 1967) and it follows from the results given in Table 4 that virtually the whole of the respiration is concerned with this function when fat is the predominant fuel of respiration of the organism. The high rates of ketogenesis, it is true, occurred on addition of oleate to the perfusion medium, but the concentrations used (2mM initially) are near the physiological range in animals depending on fat as energy source. At a rate of ketone-body production of 2.7 μmol/min per g (Table 4) the daily ketone-body production would be about 3g for a rat weighing 200g. A rat of this size consumes, under normal conditions, about 14g (dry weight) of rat cubes daily. Thus maximally about 20% of the calorie needs could be provided by the ketone bodies generated in the liver.

Mechanism of antiketogenesis. The fact that the

Table 11. *Rates of ketone-body formation on addition of two substrates*

The experimental conditions were as described in Table 1. For the values of the rates with sorbate alone see Table 10, and of the rates for the other substances see Table 1.

Substrates added	Rate of total ketone-body formation (μmol/h per g)
Oleate (2mM)	98.4±4.7 (4)
Oleate (2mM)+butyrate (10mM)	110 ±7.5 (4)
Oleate (2mM)+heptanoate (2mM, added twice)	100 ±9.5 (2)
Oleate (2mM)+dodecanoate (2.5mM)	122 ±1.0 (2)
Oleate (2mM)+sorbate (2mM)	111 ±7.5 (4)

Table 12. *Antiketogenic effects in the perfused rat liver*

The rats were starved for 48h. The initial concentration of the antiketogenic agent was 10mM. The values are means ±S.E.M. with numbers of observations in parentheses.

Antiketogenic substrate	Rate of endogenous total ketone-body formation (μmol/h per g)	Inhibition of endogenous ketogenesis (%)	Rate of total ketone-body formation in the presence of 2mM-oleate (μmol/h per g)	Inhibition of ketogenesis from oleate (%)
None	30.4±3.3 (4)		98± 4.7 (4)	
L-Lactate	6.5±0.29 (3)	79	85± 5.4 (6)	13
Glucose	11.5±2.9 (3)	62		
Fructose	9.7±0.9 (4)	68		
Xylitol	12.2±3.3 (4)	60	101±12.4 (3)	0
Glycerol	10.5±1.6 (4)	65	90± 8.8 (4)	8
Dihydroxyacetone	15.0±3.0 (3)	51		
Proline	13.3±3.8 (3)	56		

rate of ketogenesis from fatty acids increases with the substrate concentration (see Heimberg, Weinstein & Kohout, 1969; Van Harken, Dixon & Heimberg, 1969; Krebs et al. 1969) and that the antiketogenic effects of carbohydrates, carbohydrate derivatives and amino acids (Table 12) depend on the concentration of ketogenic precursors implies that the regulation of ketogenesis is related to the regulation of the tissue concentration of free fatty acids, the main ketogenic precursors. This concentration in turn depends mainly on the rate at which fatty acids reach the liver from the triglyceride stores of adipose tissue and from the intestine.

Effect of long-chain fatty acids on the [β-hydroxybutyrate]/[acetoacetate] ratio. A striking effect of long-chain fatty acids is a rise of the [β-hydroxybutyrate]/[acetoacetate] ratio, or the maintenance of a high ratio, in the perfusion medium (Tables 1 and 3; see also Williamson, Browning & Scholz, 1969). The ratios found after addition of long-chain fatty acids to the medium are those found *in vivo* in liver and in blood plasma, and it appears that long-chain fatty acids are the only physiological substrates that maintain a high ratio in the perfused organ. As the ratio is indicative of the mitochondrial redox state it follows that the long-chain fatty acids play a role in the control of the mitochondrial redox state in the liver.

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