Rates of Ketone-Body Formation in the Perfused Rat Liver

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1. The rates of formation of acetoacetate and β -hydroxybutyrate by the isolated perfused rat liver were measured under various conditions. 2. The rates found after addition of butyrate, octanoate, oleate and linoleate were about 100 μ moles/hr./g. wet wt. in the liver of starved rats. These rates are much higher than those found with rat liver slices. 3. The differences between the rates given by slices and by the perfused organ were much higher with the long-chain than with short-chain fatty acids. The increments caused by oleate and linoleate were 12 and 16 times as large in the perfused organ as in the slices, whereas the increments caused by butyrate and octanoate were about four times as large. 4. The rates of ketogenesis in the unsupplemented perfused liver of well-fed rats, and the increments caused by the addition of fatty acids, were about half of those in the liver from starved rats. 5. The value of the $[\beta\text{-hydroxybutyrate}]/[\text{acetoacetate}]$ ratio of the medium was raised by octanoate, oleate and linoleate. 6. Carnitine did not significantly accelerate ketogenesis from fatty acids. 7. Oleate formed up to 82% of the expected yield of ketone bodies. 8. In the liver of alloxan-diabetic rats the endogenous rates of ketogenesis were raised, in some cases as high as in the liver from starved rats, after addition of oleate. 9. On addition of either β -hydroxybutyrate or acetoacetate to the perfusion medium the liver gradually adjusted the $[\beta\text{-hydroxybutyrate}]/[\text{acetoacetate}]$ ratio towards the normal range. 10. The $[\beta\text{-hydroxybutyrate}]/[\text{acetoacetate}]$ ratio of the medium was about 0.4 when slices were incubated, but near the physiological value of 2 when the liver was perfused. 11. The experiments demonstrate that for the study of ketogenesis slices are in many ways grossly inferior to the perfused liver.

Earlier work on quantitative aspects of ketogenesis in isolated liver preparations had three major shortcomings. The absence of a specific and sensitive method for the determination of the ketone bodies, especially β -hydroxybutyrate, made it difficult to obtain reliable results when the quantities of the ketone bodies were small. Secondly, the ketone-body formation differed in at least one major way from that of the intact organism; the tissue preparations used, slices (Edson, 1935), homogenates or mitochondria (Lehninger, 1942, 1946), form predominantly acetoacetate, but liver and the body fluids *in vivo* contain predominantly β -hydroxybutyrate. This difference suggests that the isolated tissue preparations do not entirely reflect the situation *in vivo*. Thirdly, the formation of ketone bodies from long-chain fatty acids, the main precursors *in vivo*, in contrast with that from short-chain fatty acids up to C_{10} , was very slow in slices (Edson, 1935) and other tissue fragments.

Recent methodological progress has removed

these shortcomings. The analytical difficulties have been overcome by the spectrophotometric enzymic methods for the determination of acetoacetate and β -hydroxybutyrate (Williamson, Mellanby & Krebs, 1962). The other two failings, as shown in this paper (see also Löffler, Matschinsky & Wieland, 1965), do not apply to the isolated perfused rat liver. The perfused organ can form ketone bodies as rapidly from long-chain as from short-chain fatty acids, and under many conditions the proportion of β -hydroxybutyrate is the same as in the intact organism.

In view of the superior performance of the perfused liver, the rates of ketogenesis were systematically measured under a variety of conditions and compared with those in slices and homogenates. Rates from added precursors were much higher in the perfused organ, and the differences between the perfused organ and slices were much greater with the long-chain fatty acids (16-fold) than with short-chain fatty acids (fourfold).

METHODS AND MATERIALS

Liver perfusion. The procedure described by Hems, Ross, Berry & Krebs (1966), which is based on that of Miller, Bly, Watson & Bale (1951) and Schimassek (1963), was used. The bovine serum albumin, powder fraction V (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex), required in the preparation of the perfusion medium was dialysed at 4° against four changes of the saline medium (see below) over a period of 48 hr. The stock albumin solution in the saline medium contained 10% (w/v) and was diluted for use to 2.6%. Female albino rats of the Wistar strain, weighing about 200 g., were used.

Liver slices. These were cut with a dry razor blade by the method of Deutsch (1935) and weighed on a torsion balance. Each manometer cup contained about 100 mg. wet wt. (about 28 mg. dry wt.) of tissue. The slices were shaken in 4 ml. of the medium of Krebs & Henseleit (1932) supplemented with 2.6% (w/v) of dialysed bovine serum albumin and 1 mM-DL-carnitine. At the end of the incubation 1 ml. of 20% (w/v) perchloric acid was added and acetoacetate and β -hydroxybutyrate were determined after neutralization of the supernatant with KOH. For the determination of the initial ketone-body content a portion of the

slices was placed in 4% (w/v) perchloric acid immediately after slicing.

Solutions of oleate, linoleate and octanoate. Stock solutions (0.1 M) were freshly prepared by placing 1 m-mole of the fatty acid in a 10 ml. graduated test tube and adding water to a final volume of 8.5 ml. The tube was heated to about 60° and slightly more than 1 ml. of NaOH was added dropwise while the tube contents were agitated on a Vortex mixer. Complete solution of the fatty acid occurred after the addition of a small excess of alkali. The volume was then made up to 10 ml. The quantity of fatty acid solution to be added to the perfusion medium was placed in a test tube while still warm, and 3 ml. of dialysed 25% (w/v) bovine albumin solution (prepared in the same way as the 10% solution) was added with vigorous stirring. The pH of the mixture was adjusted to about 7.4 by the addition of dil. HCl. This stock solution was added to the perfusion medium after a preliminary perfusion period of 38 min., as described by Hems *et al.* (1966).

With palmitate and stearate the maximum concentration of substrate solution that could be prepared without risk of precipitation was 25 mM (before the addition of the albumin solution).

Alloxan-diabetes. To obtain diabetic rats, recrystallized

Table 1. Ketone-body formation from fatty acids in the perfused liver of starved rats

The rats were starved for 48 hr. After a preliminary perfusion period of 38 min. fatty acids and DL-carnitine were added to a final concentration of 2 mM. The rates of ketone-body formation were calculated from the linear portion of the time-course, which continued for 45–60 min. with the short-chain fatty acids and for 75–90 min. with the long-chain fatty acids. The results are given as means $\pm$ S.E.M. for four observations.

Fatty acid added	Total ketone bodies formed (μ moles/hr./g. wet wt.)	Total ketone bodies due to added substrate (μ moles/hr./g. wet wt.)	[β -Hydroxybutyrate]/[acetoacetate] ratio in medium			
			Before addition of substrate (at 38 min.)	After addition of substrate		
				30 min.	60 min.	90 min.
None	30.4 $\pm$ 3.3		1.44 $\pm$ 0.09 (20)	1.36 $\pm$ 0.15	1.05 $\pm$ 0.06	0.99 $\pm$ 0.12
Butyrate	94.4 $\pm$ 14.6	64		1.25 $\pm$ 0.08	0.95 $\pm$ 0.24	0.70 $\pm$ 0.29
Octanoate	128 $\pm$ 6.8	98		2.82 $\pm$ 0.54	2.08 $\pm$ 0.43	1.19 $\pm$ 0.47
Oleate	98 $\pm$ 4.7	68		3.41 $\pm$ 0.30	2.85 $\pm$ 0.39	2.69 $\pm$ 0.49
Linoleate	117 $\pm$ 6.3	87		2.86 $\pm$ 0.19	3.48 $\pm$ 0.42	3.04 $\pm$ 0.69

Table 2. Ketone-body formation from fatty acids in the perfused liver of well-fed rats

The experimental details were as described in Table 1. The results are expressed as means $\pm$ S.E.M. for the numbers of observations in parentheses.

Fatty acid added	Total ketone bodies formed (μ moles/hr./g. wet wt.)	Total ketone bodies due to added substrate (μ moles/hr./g. wet wt.)	[β -Hydroxybutyrate]/[acetoacetate] ratio in medium			
			Before addition of substrate (at 38 min.)	After addition of substrate		
				30 min.	60 min.	90 min.
None	4.9 $\pm$ 0.93 (3)		0.85 $\pm$ 0.10 (19)	0.64 $\pm$ 0.14	0.48 $\pm$ 0.16	0.36 $\pm$ 0.09
Butyrate	51.2 $\pm$ 5.7 (3)	46.3		1.98 $\pm$ 0.28	0.96 $\pm$ 0.05	0.68 $\pm$ 0.07
Butyrate (10 mM)	54.5 $\pm$ 3.07 (6)	49.6		1.26 $\pm$ 0.06	1.35 $\pm$ 0.09	1.26 $\pm$ 0.51
Octanoate	57.5 $\pm$ 5.08 (3)	52.6		3.38 $\pm$ 0.32	1.37 $\pm$ 0.34	0.65 $\pm$ 0.04
Oleate	33.0 $\pm$ 4.27 (4)	28.1		2.08 $\pm$ 0.35	1.18 $\pm$ 0.09	0.62 $\pm$ 0.06
Linoleate	42.9 $\pm$ 3.6 (5)	38.0		3.96 $\pm$ 0.91	1.57 $\pm$ 0.38	0.70 $\pm$ 0.06

alloxan monohydrate was injected into the right saphenous vein (8mg./100g. body wt.). After 48hr., the blood sugar concentration was about 55mM and the total blood ketone-body concentration was 8–10mM.

Analytical methods. β -Hydroxybutyrate and acetoacetate were determined enzymically by the method of Williamson *et al.* (1962).

RESULTS

Rate of ketogenesis in the perfused rat liver. In livers from rats starved for 48hr. (Table 1) high rates of ketone-body formation were found without addition of substrates, as expected, and the addition of fatty acids increased the rate three- to four-fold. The effects of butyrate, octanoate, oleate and linoleate were quantitatively about the same. The $[\beta\text{-hydroxybutyrate}]/[\text{acetoacetate}]$ ratio of the unsupplemented liver, as measured in the

medium, gradually decreased from 1.36 to 0.99, and butyrate had no effect on this ratio. Octanoate, oleate and linoleate greatly increased this ratio. In the presence of oleate and linoleate this ratio was maintained throughout the perfusion period at values within the physiological range (2–3). The rate of ketone-body formation in the unsupplemented liver from well-fed rats (Table 2) was about 15% of that of the liver of starved rats, and the increments caused by the addition of the fatty acids were only about half of those found in the liver of starved rats, except with butyrate, when they were about two-thirds. Thus the increased ketogenic capacity of the liver in starved rats seems to be due partly to the higher substrate concentration in the liver and partly to greater activity of the enzymes concerned. The $[\beta\text{-hydroxybutyrate}]/[\text{acetoacetate}]$ ratio was also raised in the liver of well-fed rats by the addition of the substrates. After 30min. the ratios, except that for butyrate, were within the physiological range, but subsequently the ratios fell more rapidly than in the liver of starved rats.

Effect of substrate concentration and carnitine on ketogenesis. The rates of ketone-body formation from oleate increased when the substrate concentration was raised from 0.5mM to 2.0mM (Table 3). Higher concentrations of oleate were not practicable because they tended to cause a blockage of the blood vessels. The addition of carnitine did not make a significant difference to the rate of ketogenesis. The $[\beta\text{-hydroxybutyrate}]/[\text{acetoacetate}]$ ratio was not affected by variations in the concentration of oleate or by carnitine.

Yield of ketone bodies from oleate in the perfused

Table 3. *Effect of substrate concentration and carnitine on the formation of ketone bodies in perfused rat liver*

The rats were starved for 48hr. The rates were calculated from the linear portions of the progress curves. The results are given as means $\pm$ S.E.M. for four observations. Further details are given in the Methods and Materials section.

Concn. of oleate (mM)	Total ketone bodies formed (μ moles/hr./g. wet wt.)	
	No carnitine added	2mM-DL-carnitine added
0	41.7 $\pm$ 5.8	30.4 $\pm$ 3.3
0.5	67.3 $\pm$ 8.2	75.1 $\pm$ 6.1
1.0	82.8 $\pm$ 5.0	88.1 $\pm$ 6.7
2.0	98.0 $\pm$ 2.4	98.4 $\pm$ 4.7

Table 4. *Endogenous formation of ketone bodies in perfused rat livers in acute alloxan-diabetes*

Experimental details are given in the Methods and Materials section. The rates of ketone-body formation were obtained from the approximately linear time-course during the first 60min. after the start of the perfusion. As the severity of the diabetes varied (see blood-sugar concentrations) no statistical treatment is attempted. The rats had free access to food, but the food intake of the diseased rats was erratic and in some cases was probably much decreased.

Expt. no.	Time after alloxan injection (hr.)	Blood glucose concn. at death (mM)	Rates of ketone-body formation (μ moles/hr./g. wet wt.)			$[\beta\text{-Hydroxybutyrate}]/$ $[\text{acetoacetate}]$ ratio in perfusion medium		
			β -Hydroxybutyrate	Acetoacetate	Total ketones	30 min.	60 min.	90 min.
1	24	34.9	7.22	16.1	23.3	0.28	0.46	0.23
2	24	35.6	9.10	6.63	15.7	0.96	1.40	0.87
3	48	33.3	42.5	25.4	67.9	3.10	1.72	1.61
4	48	50.1	49.5	22.7	72.2	2.80	2.20	1.30
5	48	67.8	41.1	11.8	52.9	2.33	3.18	1.65
6	48	53.8	34.7	18.2	52.9	1.71	1.91	1.49
7	72	36.4	10.0	7.1	17.1	1.04	1.40	0.61
8	72	63.1	61.1	26.5	87.6	2.86	2.33	3.29
9	72	71.0	50.1	16.5	66.6	2.49	3.04	1.98
10	72	65.0	14.0	11.6	25.5	2.40	1.22	0.62

Table 5. *Formation of ketone bodies in the perfused liver of rats with acute alloxan-diabetes after addition of fatty acids*

Alloxan was injected 24–36 hr. before perfusion. Unless the urine contained at least 0.5% glucose, the animals were rejected. The rats had free access to food. Experimental details were as described in Table 4. The results are given as means $\pm$ s.e.m. for the numbers of observations in parentheses.

Substrate added	Ketone bodies formed (μ moles/hr./g. wet wt.)	[β -Hydroxybutyrate]/[acetoacetate] ratio		
		30 min.	60 min.	90 min.
Butyrate (10mM)	61.7 $\pm$ 1.5 (3)	1.68 $\pm$ 0.44	1.60 $\pm$ 0.22	2.04 $\pm$ 0.49
Oleate (2mM)	50.0 $\pm$ 9.3 (15)	3.52 $\pm$ 0.58	3.15 $\pm$ 1.1	2.21 $\pm$ 1.03
Octanoate (2mM)	80.0 $\pm$ 8.8 (4)	3.85 $\pm$ 0.78	3.02 $\pm$ 0.40	2.17 $\pm$ 0.05
Linoleate (2mM)	96.8 $\pm$ 22.0 (3)	4.34 $\pm$ 0.52	4.47 $\pm$ 0.47	4.14 $\pm$ 1.56

Table 6. *Formation of ketone bodies in the liver of chronic alloxan-diabetic rats perfused with 2mM-oleate*

The alloxan-treated rats were given 2 i.u. of zinc-protamine-insulin subcutaneously each day for 3 weeks. The insulin was then withheld, and 24–36 hr. later, when the rats were very weak and excreted glucose and ketone bodies, the liver was perfused. The results are expressed as means $\pm$ s.e.m. for six observations.

Ketone bodies formed (μ moles/min./g. wet wt.)	[β -Hydroxybutyrate]/[acetoacetate] ratio		
	30 min.	60 min.	90 min.
65.4 $\pm$ 12.3	4.69 $\pm$ 0.81	4.36 $\pm$ 0.95	3.60 $\pm$ 0.86

liver. When a small amount of oleate (0.6mM) was added to the perfusion medium, the rate of ketone-body formation increased for a limited period, and then returned to the rate observed before the addition of oleate. The extra ketone-body formation over a 90min. period, in four experiments in which 75 μ moles of oleate had been added, was 275 $\pm$ 21.2 μ moles of total ketone bodies. This value is 82% of that expected for the quantitative conversion of the added oleate into ketone bodies (337.5 μ moles).

Ketone-body formation by the liver in alloxan-diabetes. The ketone-body formation by the liver of alloxan-diabetic rats perfused without addition of substrates is shown in Table 4. There were considerable individual variations in the metabolic behaviour of the liver after alloxan treatment. Thus in Expts. 8 and 10, where the increases in the blood sugar concentration at death were about equal, the rates of ketone-body formation were very different, being much lower in Expt. 10. In all cases the rates of total ketone-body production were increased, compared with the rate of 4.9 μ moles/hr./g. observed for the liver of well-fed rats (Table 2). The rates were not as high 24hr. after alloxan administration as they were after 48

or 72hr. In some cases the rates were almost as high as in oleate-supplemented liver of starved rats, and much higher than in liver of well-fed rats after addition of long-chain fatty acids. The [β -hydroxybutyrate]/[acetoacetate] ratio was relatively low 24hr. after injection, when it was similar to the value found in well-fed normal rats. After 72hr. the ratio was high (2–3) and of similar values to those in the starved liver after addition of oleate (Table 1). Addition of fatty acids (Table 5) did not cause a significant further rise of the rate of ketone-body production, nor did it affect the [β -hydroxybutyrate]/[acetoacetate] ratio. Thus the liver of alloxan-diabetic rats behaved like that of starved rats, supplemented with fatty acids. Livers of rats with chronic alloxan-diabetes, maintained with insulin for 3 weeks, gave, 24–36 hr. after the withdrawal of insulin, rates of ketogenesis and [β -hydroxybutyrate]/[acetoacetate] ratios similar to those of rats with acute alloxan-diabetes (Table 6).

Adjustment of the [β -hydroxybutyrate]/[acetoacetate] ratio by the perfused liver. When the ratio of the ketone-body concentrations was disturbed by the addition of either 2mM-DL- β -hydroxybutyrate, i.e. 1mM-D- β -hydroxybutyrate, or 1mM-acetoacetate at 38min., raising it to 3.88 on addition of β -hydroxybutyrate and decreasing it to 0.56 on addition of acetoacetate, this was followed by a gradual approach to the original value (Table 7). The respective values of the ratios were 1.42 and 1.01 1hr. after the additions and they were 0.99 and 0.79 after 90min. Thus equal ratios were not obtained, but this is not surprising because the amounts of ketone bodies added were relatively large, the volume of the perfusion medium being about 20 times that of the liver tissue. The amounts of ketone bodies added were about equal to the amounts formed during a 45min. period of perfusion in the starved livers. Table 7 demonstrates that the liver possesses the capacity to adjust the redox state of the ketone bodies to the normal range when it has been artificially upset.

Table 7. Time-course of changes in the $[\beta\text{-hydroxybutyrate}]/[\text{acetoacetate}]$ ratio in the perfusion medium

The liver of a rat starved for 48 hr. was perfused under standard conditions. After 38 min. either DL- β -hydroxybutyrate or acetoacetate was added, to a final concentration of 1 mM-D- β -hydroxybutyrate or 1 mM-acetoacetate. The results are given as means $\pm$ S.E.M. for four observations. Other details are given in the Methods and Materials Section.

	Time (min.)	...	10	38	40	55	70	85	100	115	130
β-Hydroxybutyrate added											
Total ketone bodies (μ moles/ml.)			0.93 $\pm$ 0.13	1.37 $\pm$ 0.15	2.32 $\pm$ 0.08	2.78 $\pm$ 0.14	3.14 $\pm$ 0.19	3.29 $\pm$ 0.33	3.60 $\pm$ 0.38	3.85 $\pm$ 0.49	4.35 $\pm$ 0.36
$[\beta\text{-Hydroxybutyrate}]/[\text{acetoacetate}]$ ratio			1.37 $\pm$ 0.06	1.80 $\pm$ 0.32	3.88 $\pm$ 0.28	2.19 $\pm$ 0.23	1.76 $\pm$ 0.29	1.84 $\pm$ 0.36	1.42 $\pm$ 0.17	1.31 $\pm$ 0.23	0.99 $\pm$ 0.25
Acetoacetate added											
Total ketone bodies (μ moles/ml.)			0.51 $\pm$ 0.11	—	1.96 $\pm$ 0.14	2.37 $\pm$ 0.09	2.76 $\pm$ 0.23	2.82 $\pm$ 0.23	3.23 $\pm$ 0.32	3.36 $\pm$ 0.25	3.65 $\pm$ 0.35
$[\beta\text{-Hydroxybutyrate}]/[\text{acetoacetate}]$ ratio			0.91 $\pm$ 0.29	—	0.56 $\pm$ 0.04	0.93 $\pm$ 0.06	1.01 $\pm$ 0.09	1.10 $\pm$ 0.18	1.01 $\pm$ 0.22	0.88 $\pm$ 0.22	0.79 $\pm$ 0.24

Ketone-body formation in rat liver slices. A comparison of Tables 1 and 8 shows that the ketone-body formation from added substrates in rat liver slices was far below that found in the perfused organ. The endogenous ketone-body production was only slightly less in slices, but the rates of total ketone-body production from butyrate and octanoate were only one-quarter, that from oleate about 8% and that from linoleate less than 6% of that in the perfused organ. Thus most of the capacity for converting long-chain fatty acids into ketone bodies was lost on slicing. At the same time the value of the $[\beta\text{-hydroxybutyrate}]/[\text{acetoacetate}]$ ratio was very much lower in the slices than in the liver *in situ* or on perfusion. The time-curve of the ketone-body formation in slices (Table 9) was near-linear over a period of 90 min. from both endogenous sources and from added butyrate, and the $[\beta\text{-hydroxybutyrate}]/[\text{acetoacetate}]$ ratio was fairly constant near 0.4 over the 90 min. period. Since this ratio reflects the intramitochondrial redox state of the NAD couple (Williamson, Lund & Krebs, 1967) it follows that, with respect to this couple, the mitochondria in slices are more highly oxidized than *in vivo*, as they are in homogenates (Krebs & Gascoyne, 1968).

DISCUSSION

The experiments demonstrate the great superiority of the perfused rat liver over slices in the study of ketogenesis. Much of the ketogenic capacity, at least 90% in the case of ketogenesis from added long-chain fatty acids, was lost on slicing, and slices no longer maintained the $[\beta\text{-hydroxybutyrate}]/[\text{acetoacetate}]$ ratio within the normal range of 2–3. It is noteworthy that the capacity for oxidizing long-chain acids is lost to a much greater extent than that for oxidizing short-chain acids. The cause of this loss on slicing, and of the differences between the long-chain and short-chain acids, remains to be clarified. Such differences are not unexpected because the oxidation of long-chain and short-chain fatty acids is known to involve different enzymes (Leloir & Muñoz, 1939; Green, Mii, Mahler & Bock, 1954; Crane, Mii, Hauge, Green & Beinert, 1956; Beinert & Page, 1957).

As slices retain a mere fraction of the original ketogenic capacity of the liver, their use in the study of ketogenesis is evidently limited.

The increased ketogenesis in starvation can in part be explained by the higher concentration of free fatty acids in the blood plasma and presumably in the tissue. Since, however, addition of free fatty acids to the liver of well-fed animals does not raise the rate of ketogenesis to the same value as in the liver of starved rats, factors other than the concentration of free fatty acids must determine the

Table 8. *Ketone-body formation from fatty acids by rat liver slices*

Female rats were starved for 48 hr. Liver slices were incubated for 1 hr. as described in the text. The results are given as means $\pm$ S.E.M. for the numbers of observations in parentheses. Initially the slices contained 4.09 ± 0.15 (10) μ moles of total ketone bodies/g. wet wt. and the $[\beta$ -hydroxybutyrate]/[acetoacetate] ratio was 1.79 ± 0.089 (10). The concentrations of added substrates were 2 mM. The medium contained 1 mM-DL-carnitine.

Substrate added	Total ketone-body formation (μ moles/hr./g. wet wt.)		$[\beta$ -Hydroxybutyrate] [acetoacetate] ratio
	Observed	Increment caused by addition of substrate	
None	24.5 ± 1.0 (10)		0.35 ± 0.01 (10)
Butyrate	40.9 ± 2.43 (10)	16.4	0.41 ± 0.02 (10)
Hexanoate	50.8 ± 2.45 (8)	26.3	0.60 ± 0.03 (8)
Octanoate	47.9 ± 3.54 (8)	23.4	0.70 ± 0.04 (8)
Palmitate	28.0 ± 1.89 (8)	3.5	0.47 ± 0.01 (8)
Oleate	30.0 ± 1.87 (8)	5.5	0.59 ± 0.02 (8)
Linoleate	29.3 ± 2.29 (8)	4.8	0.54 ± 0.02 (8)

Table 9. *Time-course of ketone-body formation from butyrate and endogenous precursors by rat liver slices*

The conditions were as described in the Methods and Materials section. The results are given as means $\pm$ S.E.M. (where appropriate) for eight observations. Initially the slices contained 3.27 ± 0.17 μ mole (3) of total ketone bodies/g. wet wt. and the $[\beta$ -hydroxybutyrate]/[acetoacetate] ratio was 2.84 ± 0.13 (8).

	Time (min.)	30	60	90
Endogenous ketogenesis				
Total ketone bodies found (μ moles/g. wet wt.)		11.8 ± 0.23	18.0 ± 0.72	23.7 ± 1.02
Total ketone bodies, increment over initial (μ moles/g. wet wt.)		8.5	14.7	20.4
$[\beta$ -Hydroxybutyrate]/[acetoacetate] ratio		0.42 ± 0.02	0.42 ± 0.02	0.39 ± 0.02
Ketogenesis with 2 mM-butyrate				
Total ketone bodies found (μ moles/g. wet wt.)		17.1 ± 0.90	29.3 ± 1.15	44.0 ± 1.79
Total ketone bodies, increment over initial (μ moles/g. wet wt.)		12.8	26.0	40.7
$[\beta$ -Hydroxybutyrate]/[acetoacetate] ratio		0.34 ± 0.02	0.38 ± 0.02	0.39 ± 0.01

rate of ketogenesis. One of these may be the availability of non-fat substrates. Assays of the enzymes concerned with ketogenesis in the liver (Williamson, Bates & Krebs, 1968) indicate that the enzyme activities do not increase on starvation.

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