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Acceleration of Renal Gluconeogenesis by Ketone Bodies and Fatty Acids

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1. Acetoacetate or short-chain fatty acids (acetate, butyrate, propionate, *n*-hexanoate, *n*-octanoate) accelerate the rate of glucose formation from lactate, fumarate and other precursors in slices of kidney cortex (rat, rabbit, sheep). The cause of this acceleration has been investigated. 2. There are two different mechanisms of acceleration. At low concentrations of glucogenic precursors the acceleration is mainly due to a 'sparing' action. The substances which accelerate are oxidizable and serve as fuel of respiration in place of the glucogenic precursor. This is indicated by the fact that the ratio lactate used/glucose formed falls in the presence of the accelerators and approaches the value 2. 3. At high concentrations of lactate the acceleration appears to be mainly due to the activation of pyruvate carboxylase by acetyl-coenzyme A. The evidence in support of this is summarized. The results indicate that the activation of pyruvate carboxylase by acyl-coenzyme A discovered by Utter & Keech (1963) in purified enzyme preparations also occurs in crude tissue homogenates and can play a part in the control of oxaloacetate synthesis and gluconeogenesis.

Krebs, Hems & Gascoyne (1963*a*) reported that acetoacetate, β-hydroxybutyrate and short-chain fatty acids, though themselves only slightly if at all gluconeogenic, can greatly increase the rate of glucose formation from lactate and certain other glucogenic precursors. To explain this effect it was suggested that the stimulating effect of acetoacetate and fatty acids may be due to a 'sparing' action: since acetoacetate and fatty acids are readily oxidized in kidney cortex they may spare potential glucose precursors which otherwise would serve as a fuel of respiration. Experiments are reported in this paper which indicate that apart from the sparing action there may be a second mechanism responsible for the increased gluconeogenesis in the presence of acetoacetate and related substances. This is the activation by acyl-coenzyme A of pyruvate carboxylase [pyruvate–CO_2 ligase (ADP); EC 6.4.1.1] discovered on purified enzyme preparations by Utter & Keech (1963). Some of the work described in the paper has already been briefly reported (Krebs, 1964).

EXPERIMENTAL

All experiments were carried out on washed slices of kidney cortex incubated at 40°. The general procedure was as described by Krebs, Bennett, de Gasquet, Gascoyne & Yoshida (1963*b*) except that the medium of incubation in most experiments was the phosphate-buffered saline of Krebs & de Gasquet (1964). This was used because it allowed the uptake of O_2 to be measured, with alkali but no filter paper in the centre wells of the manometer cups. The filter paper was omitted to maintain a somewhat higher steady-state pressure of CO_2, which is a reactant in gluconeogenesis.

Wistar rats, maintained (unless stated otherwise) on a low-carbohydrate diet, consisting of three parts of casein and one part of margarine, and supplemented with minerals and vitamins, were used when relatively small numbers of slices (30–50mg. wet wt. in 4ml. of medium) were needed. When larger quantities were required, sheep kidneys obtained from a slaughterhouse were used. The kidneys were removed from the animal as quickly as possible after death and transported to the laboratory in ice. To have sufficient material for various analyses about 800mg. (wet wt.) of tissue was suspended in 20ml. of medium in large conical Warburg manometer flasks of about 160ml. capacity. The gas space contained O_2. The temperature was 40°. Acidic substrates were added as solutions of sodium salts.

At the end of the incubation period the flasks were removed from the bath as quickly as possible and the tissue was taken out of the suspension, and when required for analysis it was lightly blotted and cooled to −70°. The frozen tissue was homogenized in 2ml. of 3% $HClO_4$ in a glass homogenizer. It was then centrifuged and the sediment was washed with small quantities of 3% $HClO_4$. The combined supernatant and washings were brought to pH 6·0 with 30% KOH. The total volume at this stage was about 4ml.

Glucose was determined by the glucose oxidase method as previously described. Lactate was determined according to Hohorst (1963), acetoacetate and D-β-hydroxybutyrate according to Williamson, Mellanby & Krebs (1962), and acetyl-CoA according to the procedure of Wieland & Weiss (1963), which is based on reaction systems first described by Ochoa (1955). Crotonate was determined spectrophotometrically at 235 mμ after extraction with ether and solution of the extract in 90% (w/v) H_2SO_4 (see Law & Slepecky, 1961).

Metabolic rates are expressed on either a fresh- or dry-weight basis. The dry weight was ascertained, as in previous work, by drying slices after incubation. In the course of the present work it became known that kidney slices may lose, during incubation, about 20% of their original dry weight.

RESULTS

Effects of acetate on gluconeogenesis from lactate in rat-kidney-cortex slices. Krebs, Hems & Gascoyne (1963) showed that acetoacetate, β-hydroxybutyrate, butyrate and hexanoate increased the formation of glucose in kidney cortex in the presence of lactate. Acetate, as shown in Table 1, has similar effects. When added alone, though increasing the oxygen uptake, it had no major effect on the glucose formation; slight increase occurred in some experiments. It had very large effects in the presence of lactate on both the oxygen consumption and the formation of glucose, especially at low lactate concentrations. Glucose formation was more than doubled under some conditions. Acetate also affected the ratio lactate used/extra glucose formed, lowering the values from between 3·5 and 7·6 to near 2·0. A value 2 indicates that almost all the lactate used served to form glucose. In the absence of acetate some of the lactate removed probably served as a substrate of respiration.

Effects of crotonate. The effects of crotonate were similar to those of acetate. It is especially noteworthy that the increase in the oxygen consumption was small when crotonate was added alone and in the absence of lactate there was no definite effect on the formation of glucose. With lactate there were marked increases in the oxygen consumption and glucose formation on addition of crotonate (Table 2).

Effects of hexanoate and octanoate. Again similar results were obtained when hexanoate was added in low concentrations (Table 3, Expts. 1 and 4). The stimulation of the oxygen uptake by both fatty acids was much greater in the presence of lactate, and with hexanoate this effect was again accompanied by an increased rate of glucose formation. At the higher concentrations of octanoate (5 mM) gluconeogenesis was inhibited, possibly because of inhibition of oxidative phosphorylation, and at 1 mM-octanoate the stimulation of gluconeogenesis was slight. It was marked at 0·5 mM-octanoate.

Table 1. *Effect of acetate on gluconeogenesis from* L-*lactate in rat kidney cortex*

For conditions of incubation see the text.

Expt. no.	Substrates added	Period of incubation (min.)	O_2 used (μmoles/g. dry wt.)	Glucose found (μmoles/g. dry wt.)	L-Lactate removed (μmoles/g. dry wt.)	Ratio: lactate removed / extra glucose found
1	None	90	1170	26	—	—
	Acetate (5 mM)		1920	24	—	—
	Lactate (1·25 mM)		1700	90	279	4·36
	Lactate (1·25 mM); acetate (5 mM)		2430	194	316	1·88
	Lactate (2·5 mM)		1880	115	439	4·9
	Lactate (2·5 mM); acetate (5 mM)		2560	269	479	1·97
	Lactate (5 mM)		2180	199	607	3·52
	Lactate (5 mM); acetate (5 mM)		2710	302	569	2·05
2	None	90	1060	24	—	—
	Acetate (5 mM)		1460	31	—	—
	Lactate (1·25 mM)		1610	74	225	4·50
	Lactate (1·25 mM); acetate (5 mM)		2720	224	320	1·60
3	None	60	640	17	—	—
	Acetate (5 mM)		890	20	—	—
	Lactate (10 mM)		1150	88	540	7·60
	Lactate (10 mM); acetate (5 mM)		1480	165	350	2·36

Table 2. *Effect of crotonate on gluconeogenesis from* L-*lactate in rat kidney cortex*

For conditions of incubation see the text.

Expt. no.	Substrates added	Period of incubation (min.)	O_2 used (μmoles/g. dry wt.)	Glucose found (μmoles/g. dry wt.)	L-Lactate removed (μmoles/g. dry wt.)
1	None	90	1210	22	—
	Crotonate (5 mM)		1335	26	—
	Lactate (1·25 mM)		2570	105	462
	Lactate (1·25 mM); crotonate (5 mM)		2875	185	413
2	None	60	920	24	—
	Crotonate (5 mM)		1000	21	—
	Lactate (10 mM)		1310	93	—
	Lactate (10 mM); crotonate (5 mM)		1680	166	—

Table 3. *Effects of hexanoate and octanoate on gluconeogenesis in the presence of* L-*lactate in rat kidney cortex*

For general conditions see the text. The period of incubation was 60 min.

Expt. no.	Substrates added	O_2 used (μmoles/g. dry wt.)	Glucose found (μmoles/g. dry wt.)
1	None	870	29
	n-Hexanoate (1 mM)	1130	30
	L-Lactate (2·5 mM)	1400	125
	L-Lactate (2·5 mM); *n*-hexanoate (1 mM)	1900	197
	L-Lactate (10 mM)	1710	200
	L-Lactate (10 mM); *n*-hexanoate (1 mM)	2185	281
2	None	878	17
	n-Octanoate (5 mM)	724	8
	L-Lactate (2·5 mM)	1455	118
	L-Lactate (2·5 mM); *n*-octanoate (5 mM)	1870	52
	L-Lactate (10 mM)	1780	190
	L-Lactate (10 mM); *n*-octanoate (5 mM)	2450	99
3	None	842	23
	n-Octanoate (1 mM)	1058	21
	L-Lactate (2·5 mM)	1500	121
	L-Lactate (2·5 mM); *n*-octanoate (1 mM)	2050	132
	L-Lactate (10 mM)	1760	165
	L-Lactate (10 mM); *n*-octanoate (1 mM)	2240	178
4	None	845	24
	n-Octanoate (0·5 mM)	1120	26
	L-Lactate (2·5 mM)	1450	116
	L-Lactate (2·5 mM); *n*-octanoate (0·5 mM)	2310	152

Effects of propionate. Propionate alone is glucogenic in kidney cortex but only at bicarbonate and carbon dioxide concentrations approaching the physiological range. Addition of propionate to rat-kidney-cortex slices suspended in phosphate-buffered saline increased glucose formation very little, in general by about 5 μmoles/g. dry wt./hr. In bicarbonate-buffered saline the corresponding values were found to be 30–40 μmoles. This effect of carbon dioxide is presumably due to its participation in propionate metabolism, being required as a reactant in the formation of methylmalonyl-CoA from propionyl-CoA. It may therefore be expected that some propionyl-CoA accumulates at low pressures of carbon dioxide. Addition of propionate in the presence of lactate in fact caused large increases in the yields of glucose (Table 4).

Effects of acetoacetate on the formation of glucose from fumarate, α-oxoglutarate and glutamate in rat kidney cortex. Krebs *et al.* (1963*a*) have already shown that at 2·5 mM-fumarate acetoacetate increased the rate of gluconeogenesis but that there was no effect at 10 mM-fumarate. This stimulating effect at low fumarate concentration is confirmed

Table 4. *Effect of propionate on gluconeogenesis from* L-*lactate in rat kidney cortex*

Rats were fed on the standard diet (see Krebs *et al.* 1963*b*). The period of incubation in phosphate-buffered saline was 120 min. For further details see the text.

Expt. no.	Substrates added	O_2 used (μmoles/g. dry wt.)	Glucose found (μmoles/g. dry wt.)	L-Lactate removed (μmoles/g. dry wt.)
1	None	1470	15	
	Propionate (1 mM)	1290	21	
	Lactate (10 mM)	3910	223	
	Lactate (10 mM); propionate (1 mM)	4320	379	
2	None	1720	25	
	Propionate (1 mM)	1290	40	
	Lactate (10 mM)	4120	299	
	Lactate (10 mM); propionate (1 mM)	4750	463	
3	Propionate (10 mM)	1365	16	
	Lactate (20 mM)	3840	275	995
	Lactate (20 mM); propionate (10 mM)	4395	350	1120

Table 5. *Effect of acetoacetate on gluconeogenesis from fumarate,* L-*malate, α-oxoglutarate and* L-*glutamate in rat kidney cortex*

For conditions of incubation see text.

Expt. no.	Substrates added	Period of incubation (min.)	O_2 used (μmoles/g. dry wt.)	Glucose found (μmoles/g. dry wt.)
1	None	90	1195	12
	Acetoacetate (5 mM)		1790	24
	Fumarate (1·25 mM)		1635	105
	Fumarate (1·25 mM); acetoacetate (5 mM)		1556	175
	Fumarate (2·5 mM)		1850	195
	Fumarate (2·5 mM); acetoacetate (5 mM)		2230	317
	Fumarate (5 mM)		2120	288
	Fumarate (5 mM); acetoacetate (5 mM)		2410	444
2	None	90	1220	19
	Acetoacetate (5 mM)		1550	20
	L-Malate (1·25 mM)		1960	204
	L-Malate (1·25 mM); acetoacetate (5 mM)		2810	349
3	None	90	1680	25
	Acetoacetate (5 mM)		1610	35
	L-Malate (2·5 mM)		2220	262
	L-Malate (2·5 mM); acetoacetate (5 mM)		2900	368
4	None	60	970	18·7
	Acetoacetate (5 mM)			18·8
	α-Oxoglutarate (2·5 mM)		1700	289
	α-Oxoglutarate (2·5 mM); acetoacetate (5 mM)		1950	243
	α-Oxoglutarate (10 mM)		1750	324
	α-Oxoglutarate (10 mM); acetoacetate (5 mM)		1870	186
5	None	60	825	24
	Acetoacetate (5 mM)		1010	21
	α-Oxoglutarate (2·5 mM)		1690	306
	α-Oxoglutarate (2·5 mM); acetoacetate (5 mM)		1870	287
	α-Oxoglutarate (10 mM)		1860	397
	α-Oxoglutarate (10 mM); acetoacetate (5 mM)		1810	284
6	None		863	30
	Acetoacetate (5 mM)			25
	L-Glutamate (2·5 mM)		1390	154
	L-Glutamate (2·5 mM); acetoacetate (5 mM)		1510	125
	L-Glutamate (10 mM)		1710	208
	L-Glutamate (10 mM); acetoacetate (5 mM)		1780	161

in Table 5 and is also demonstrated for malate. In contrast, glucose formation from α-oxoglutarate and glutamate is slightly inhibited by acetoacetate. The inhibition is presumably connected with the fact that gluconeogenesis from these precursors requires a substantial oxygen consumption to convert the substrates into oxaloacetate. The ready oxidation of acetoacetate in kidney cortex may compete with the oxidation of α-oxoglutarate and glutamate.

Effect of acetoacetate in sheep kidney. The 'acetoacetate effect' was also found in sheep kidney cortex (Table 6). Acetoacetate raised the level of glucose formation by about 80%, and caused a slight increase in the oxygen uptake, but it decreased the rate of lactate removal. When lactate was the sole added substrate the formation of glucose accounted for about two-thirds of the lactate removal. Thus some lactate was used for other reactions, probably as a fuel of respiration. When acetoacetate was present the ratio lactate removed/glucose found was about 1·8, i.e. under these conditions lactate was exclusively used as a precursor of glucose and about 10% of the glucose formed was derived from endogenous sources. The results show that acetoacetate replaces lactate as the fuel of respiration. These results, it should be emphasized, refer to experiments in which the concentration of lactate was relatively low (2·5 mM).

Concentrations of acetyl-coenzyme A in slices of sheep kidney cortex. If the stimulating effect on gluconeogenesis of various precursors of acetyl-CoA was due to an activation of pyruvate carboxylase by acetyl-CoA, the steady-state concentration of the latter in the tissue should increase in the presence of the precursors. To test this about 0·8 g. (wet wt.) of sheep-kidney slices was incubated under varying conditions (see Table 7). In all experiments both acetoacetate and acetate increased glucose formation in the presence of low concentrations of lactate, and as in rat kidney they caused a slight increase in glucose formation when

Table 6. *Time-course of glucose formation in slices of sheep kidney cortex and relationship between glucose formation,* L-*lactate removal and uptake of oxygen*

The concentration of added lactate was 2·5 mM and of added acetoacetate 5 mM. Slices were incubated in phosphate-buffered saline.

Substrates added	Period of incubation (min.)	O_2 used (μmoles/g. wet wt.)	Glucose formed (μmoles/g. wet wt.)	Lactate removed (μmoles/g. wet wt.)
L-Lactate	30	38	3·9	11·5
L-Lactate	60	90	6·5	20·0
L-Lactate	90	140	8·3	34
L-Lactate; acetoacetate	30	44	6·4	11·2
L-Lactate; acetoacetate	60	90	10·2	17·5
L-Lactate; acetoacetate	90	155	15·1	27

Table 7. *Effect of acetoacetate and acetate on glucose formation and acetyl-coenzyme A concentration in slices of sheep kidney cortex incubated in the presence of* L-*lactate*

About 800 mg. (wet wt.) of slices was incubated in 20 ml. of phosphate-buffered saline with O_2 in the gas space.

Expt. no.	Concn. of L-lactate (mM)	Other substrate (mM)	Period of incubation (min.)	Glucose formed (μmoles/g. wet wt.)	O_2 used (μmoles/g. wet wt.)	Acetyl-CoA found (μmoles/g. wet wt.)
1	5	None	90	8·6		0·024
	5	Acetoacetate (5 mM)		16·7		0·053
2	0	None		3·3	63	0·041
	0	Acetoacetate (5 mM)		4·3	80	0·110
	2·5	None	60	7·2	83	0·043
	2·5	Acetoacetate (5 mM)		12·9	95	0·081
3	5	None	45	7·8		0·032
	5	Acetate (5 mM)		9·7		0·048
4	0	None		3·1	62	0·057
	0	Acetate (5 mM)	60	3·5	89	0·073
	2·5	None		9·0	88	0·039
	2·5	Acetate (5 mM)		12·2	111	0·083

added as the sole substrate. The increased rates of gluconeogenesis were accompanied by a rise in the concentration of acetyl-CoA, which in some cases exceeded 100%. Without acetoacetate or acetate the acetyl-CoA concentration in the slices was between about 0·024 and 0·057 mM. Acetoacetate or acetate raised them to between about 0·053 and 0·11 mM. As the K_m for pyruvate carboxylase and acetyl-CoA is 0·019 mM (Keech & Utter, 1963), the increase in the concentrations of acetyl-CoA is within the range where the activating effect on the enzyme is to be expected. These findings are in agreement with the assumption (but do not prove it) that the carboxylation of pyruvate is one of the rate-limiting steps of glucose formation and that the rate of this reaction depends on the concentration of acetyl-CoA.

Effect of acetoacetate and concentrations of acetyl-coenzyme A in rabbit kidney cortex. Similar results were obtained with rabbit kidney cortex (Table 8). In this tissue the stimulating effect of acetoacetate on the formation of glucose was rather larger than in rat and sheep kidney (between 140 and 430%). These increases were accompanied by raised concentrations of acetyl-CoA in the tissues, especially in Expt. 2. The increase was slight in Expt. 3. In the absence of acetoacetate the rate of glucose synthesis was much lower than in the rat and sheep kidney. This may be connected with the circumstance that the need for gluconeogenesis in the rabbit, as a herbivore and non-ruminant, may be smaller than in the rat and the sheep.

Evidence on mechanism of action of ketone bodies and fatty acids. Many of the stimulating effects of ketone bodies and fatty acids on glucose formation from lactate can be explained either by a sparing action or by a stimulating effect on pyruvate carboxylase. Two further series of experiments were carried out to obtain evidence on the question to what extent the activation of pyruvate carboxylase may be responsible for the stimulating effect. The first series consisted of experiments at high lactate concentrations when the supply of lactate may have been taken to be in excess of possible needs and when therefore a sparing action was unlikely to be relevant. Increasing the concentration of lactate above 10 mM did not increase the rate of glucose formation in the absence of added acetoacetate (Table 9), but at the highest lactate concentrations (20 mM) the added acetoacetate still stimulated glucose formation, the absolute increase being about the same as at low lactate concentrations (Expt. 2). The same results were obtained with propionate (Table 4) and acetate and crotonate (Table 10). It is relevant to the interpretation of these observations that in similar experiments with pyruvate no acceleration occurred at high pyruvate concentrations.

The second series of experiments was based on the consideration that the sparing effect would show definable stoicheiometric relations between the amounts of lactate spared for glucose formation and the amounts of stimulating agent used: the amounts of lactate spared should be equivalent, in terms of oxygen needed for combustion, to the amounts of sparing material burnt. On the other hand if the stimulating effect is due to an activation of an enzyme, the stimulating agent should act catalytically, like the enzyme it activates, and the amount of the agent required should be expected to be smaller than the amounts of extra glucose formed. The volume of medium was kept low (2 ml.) to combine maximum concentrations with a minimum total amount of added substrates. The experiments were on the whole inconclusive and are therefore not reported in detail. In some experiments acetoacetate and crotonate gave catalytic effects when 10 mg. (dry wt.) rat kidney cortex was

Table 8. *Metabolism of acetoacetate and lactate in slices of rabbit kidney cortex*

About 750 mg. (wet wt.) of slices was incubated in 20 ml. of phosphate-buffered saline for 90 min. at 40°. Initial substrate concentrations were 5 mM. Values given are μmoles/g. wet wt.

Expt. no.	Substrates added	Glucose formed	Lactate used	Acetoacetate used	β-Hydroxybutyrate formed	Acetyl-CoA found
1	None	0·4	—	—	—	0·016
	Acetoacetate	0·7	—	38	8	0·036
	L-Lactate	1·9	24	—	—	0·016
	L-Lactate; acetoacetate	10·1	25	46	11	0·023
2	None	0·1	—	—	—	0·037
	Acetoacetate	0·2	—	20	11	0·032
	L-Lactate	1·2	27	—	—	0·013
	L-Lactate; acetoacetate	5·4	18	39	17	0·030
3	None	0·5	—	—	—	0·038
	Acetoacetate	0·4	—	12	10	0·044
	L-Lactate	3·3	23	—	—	0·027
	L-Lactate; acetoacetate	8·0	25	34	12	0·030

Table 9. *Effect of the concentration of* L-*lactate on the formation of glucose in the presence and absence of acetoacetate*

Rat-kidney-cortex slices were incubated for 1 hr. in Expts. 1 and 2, and for 90 min. in Expt. 3, in phosphate-buffered saline. The standard diet was given to the rats of Expts. 1 and 2 and the low-carbohydrate diet to the rat of Expt. 3.

Expt. no.	Substrate added	O_2 used (μmoles/g. dry wt.)	Glucose found (μmoles/g. dry wt.)	L-Lactate removed (μmoles/g. dry wt.)	Aceto-acetate removed (μmoles/g. dry wt.)	β-Hydroxy-butyrate found (μmoles/g. dry wt.)	Ratio: $\frac{\text{total } O_2 \text{ used}}{\text{lactate not used for glucose formation}}$	Ratio: $\frac{\text{extra } O_2 \text{ used}}{\text{lactate not used for glucose formation}}$	Ratio: $\frac{\text{total } O_2 \text{ used}}{\text{acetoacetate used}}$
1	None	820	17						
	Lactate (2·5 mM)	1670	113	510			5·9	3·0	
	Lactate (5 mM)	1690	140	690			4·1	2·1	
	Lactate (10 mM)	1970	166	800			4·2	2·5	
	Lactate (20 mM)	2200	173	840			4·5	2·6	
2	None	830	17	430					
	Acetoacetate (5 mM)	960	21		308				3·1
	Lactate (2·5 mM)	1500	90	430			6·0	2·7	
	Acetoacetate (5 mM); lactate (2·5 mM)	1870	142	380	390		19·5	10·8	4·8
	Lactate (5 mM)	1650	107	660			3·7	1·8	
	Acetoacetate (5 mM); lactate (5 mM)	1860	172	480	510		13·6	7·6	3·6
	Lactate (10 mM)	1710	126	860			28	1·4	
	Acetoacetate (5 mM); lactate (10 mM)	2020	198	670	490		7·4	4·3	4·1
	Lactate (20 mM)	2010	128						
	Acetoacetate (5 mM); lactate (20 mM)	2160	192		565				
3	Lactate (20 mM)	2320	216			10			
	Lactate (20 mM); acetoacetate (5 mM)	2600	325		345	65			
	Lactate (20 mM); acetoacetate (2·5 mM)	2640	348		390	65			
	Lactate (20 mM); acetoacetate (1·25 mM)	2600	323		300	30			

Table 10. *Acceleration of the formation of glucose by acetate and crotonate at high* L-*lactate concentrations*

Rat-kidney-cortex slices were incubated for the times specified. The animals had been kept on a low-carbohydrate diet.

Expt. no.	Incubation medium	Period of incubation (min.)	Substrate added	O_2 used (μmoles/g. dry wt.)	Glucose found (μmoles/g. dry wt.)
1	Bicarbonate-buffered saline	90	None		21
			Lactate (20 mM)		248
			Lactate (20 mM); acetate (20 mM)		372
2	Phosphate-buffered saline	60	Lactate (20 mM)	1790	212
			Lactate (20 mM); acetate (20 mM)	2240	313
3	Phosphate-buffered saline	60	Crotonate (5 mM)	1060	33
			Crotonate (10 mM)	1010	31
			Lactate (20 mM)	1780	245
			Lactate (20 mM); crotonate (5 mM)	2240	352
			Lactate (20 mM); crotonate (2.5 mM)	1940	340

incubated for 2 hr. in 2 ml. of phosphate-buffered medium with 40 μmoles of lactate and 0·4 μmole of acetoacetate or crotonate, but the effects were not regularly found. A catalytic effect would be expected if the formation of acyl-CoA and the combination of the latter with pyruvate carboxylase were the only reactions of acyl-CoA occurring in the test system. But this is not the case. As all acyl-CoA compounds are highly reactive, an increase in their tissue concentration is merely a reflection of a change in the steady state. It is therefore not altogether surprising that the test proved inconclusive. Catalytic quantities of substrate are used up too quickly to build up a raised steady-state concentration of acyl-CoA. To achieve this, an excess of substrate must be present.

DISCUSSION

Sparing action versus activation of pyruvate carboxylase. Two mechanisms could account for the acceleration of gluconeogenesis by ketone bodies and fatty acids, namely a 'sparing' effect in which the added fatty acid serves as a fuel of respiration and spares glucogenic substrates for gluconeogenesis or an activation of pyruvate carboxylase by acyl-CoA. The occurrence of the sparing effect is shown by the fact that less lactate is used and more glucose formed, and that the ratio lactate used/glucose formed is decreased and approaches the value 2 when substrates other than lactate are available as fuel of respiration.

There are, however, many observations which are difficult to reconcile with the view that a sparing effect is the only mechanism of acceleration and which support the assumption that the activation of pyruvate carboxylase is another major factor in stimulating gluconeogenesis:

(1) The effect occurs at high lactate concentrations when the excess of substrate, together with abundance of lactate dehydrogenase, should be sufficient to supply an excess of pyruvate for both gluconeogenesis and cell respiration; in other words the acceleration occurs in a situation when there is no case for a sparing action.

(2) The effect is absent at high concentrations of fumarate or other dicarboxylic acids where pyruvate carboxylase cannot be rate-limiting. It is also absent with pyruvate which alone can saturate the tissue with acetyl-CoA.

(3) Those substances (acetate, propionate, crotonate) the coenzyme derivatives of which activate the purified pyruvate carboxylase, according to Keech & Utter (1963), also stimulate gluconeogenesis from lactate. Propionate and crotonate are as effective as acetate although the rates of oxidation of propionate and crotonate are much lower than those of the other accelerating substrates.

(4) The postulated rise in the concentration of acetyl-CoA in the tissue has been demonstrated, and it is of the order of magnitude and in the range of concentrations where, according to the K_m, an activating effect is to be expected.

(5) In many experiments, far from sparing the total utilization of lactate, acetate increased the overall lactate utilization, expecially at low lactate concentrations (see Table 1). At the higher lactate concentrations (10 or 20 mM) the measurement of lactate removal is inaccurate because too small a proportion of the lactate present is used. The increased rate of gluconeogenesis from lactate and the diversion of lactate from combustion to gluco-

neogenesis indicate that acetate and related substances accelerate the carboxylation of pyruvate at the expense of the conversion of pyruvate into acetyl-CoA. A raised concentration of acetyl-CoA can account for both the acceleration of pyruvate carboxylase and the inhibition of pyruvate dehydrogenase (Garland & Randle, 1964).

The relevance of the results of the present work to the analysis of the control of gluconeogenesis has been discussed elsewhere (Krebs, 1964).

Oxygen consumption and gluconeogenesis. A stimulation of gluconeogenesis is expected to raise the rate of respiration on account of the ATP requirements of gluconeogenesis. Since the formation of 1 molecule of glucose requires 6 molecules of ATP the expected extra oxygen uptake is at least 1 molecule/molecule of glucose formed. In fact the increases caused by acetoacetate, acetate and related substrate were considerably greater, which suggests that the addition of these substances sets other energy-consuming reactions in motion. The nature of these remains to be explored.

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