CCCXXII. KETOGENESIS-ANTIKETOGENESIS.
V. METABOLISM OF KETONE BODIES.

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ACETOACETIC and β-hydroxybutyric acids are known to undergo two chief metabolic changes, interconversion and oxidative breakdown. The reversibility of the reaction, acetoacetic acid $\rightleftharpoons$ β-hydroxybutyric acid, was demonstrated in liver brei by Dakin & Wakeman [1910, 1, 2] and in perfused liver by Friedmann & Maase [1910]. Furthermore, the interconversion of these keto- and hydroxy-acids, both in liver and in kidney, was clearly illustrated by the perfusion experiments of Snapper & Grünbaum [1927, 1, 2, 3]. Using slices, Jowett & Quastel [1935] have shown that β-hydroxybutyric acid is oxidized to acetoacetic acid in tissues other than liver and kidney.

Apart from the interconversion Snapper & Grünbaum found a true destruction of "ketone bodies", which was large in kidney but very small in liver. β-Hydroxybutyric acid was also destroyed in the extremities of the dog and by the tongue muscles of the calf [Snapper & Grünbaum, 1928].

Quastel & Wheatley [1935] studied the breakdown of acetoacetic acid in kidney slices. Acetoacetic acid disappeared under both aerobic and anaerobic conditions; in the former instance only one-quarter to one-third of the change was due to reduction to β-hydroxybutyric acid, whereas in the latter reduction was almost quantitative. The distinct nature of the two processes was demonstrated by the action of inhibitors, e.g. malonate.

Methods.

Various tissues of rats, guinea-pigs and pigeons were used in this work. Slices of lung, pancreas, submaxillary gland and skeletal muscle were cut by the method of Deutsch [1936]. Lung slices float on cold Ringer solution but sink into the medium as soon as the containing vessel is transferred to a thermostat at 37·5°. The quantities of tissue employed in manometric experiments were: kidney and brain, 8–10 mg. (dry weight), other tissues 10–20 mg. (dry weight). Slices of striped muscle were suspended in a "kochsaft" made from pigeon's breast muscle and buffered to *p*H 7·4 with phosphate according to the method of Krebs (unpublished).

Aerobic experiments. Tissue respiration was measured in Warburg manometric vessels of the conical type, the medium being 2–3 ml. phosphate saline [Krebs, 1933]. The vessels were filled with O_2 and shaken in a thermostat at 37·5°. At the end of 2 hours the slices were removed and the change in ketone bodies, either formation or disappearance, was determined by methods already described [Edson, 1935].

Determination of ketone bodies. Acetoacetic acid was usually determined manometrically by the aniline citrate method, but if β-hydroxybutyric acid was to be estimated simultaneously it was more convenient to apply the modified Van Slyke procedure. The method of Ostern [1933] was employed

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when mesoxalic acid was present, since the aniline citrate method is inaccurate under such conditions. β-Hydroxybutyric acid was estimated by the modified Van Slyke method.

We have found that certain substances interfere with the Van Slyke determinations.

1. Pyruvate gives a large quantity of insoluble mercury compound during the first boiling (30 min.) with Denigès' reagent. In presence of pyruvate it is impossible to determine acetoacetic acid as mercury-acetone compound, but since the whole of the pyruvate reacts during the first boiling, it is possible to remove the precipitate and proceed to a determination of β-hydroxybutyric acid. Control experiments with pyruvate have shown that there is no further precipitation on heating for 90 min. after addition of dichromate, and that β-hydroxybutyric acid can be determined satisfactorily in solutions which originally contained pyruvate. If simultaneous determinations of both ketone bodies in presence of pyruvate are required, acetoacetic acid must be estimated manometrically in a separate sample.

2. Crotonic acid reacts with Denigès' reagent during both steps of the Van Slyke method. If the precipitate is considered to be wholly acetone-Hg compound, 1 mol. crotonic acid gives 0·33 mol. acetone.

3. 2 ml. malonic acid solution, 0·02 *M*, react to a slight extent with Denigès' reagent during the first boiling. A satisfactory blank correction is readily made. 2 ml. malonic acid solution, 0·01 *M*, or 2 ml. hydroxymalonic acid solution, 0·01 and 0·02 *M*, give no mercury precipitates under the conditions of the determination.

Anaerobic experiments. In examining the anaerobic disappearance of ketone bodies we used the bicarbonate-Ringer solution of Krebs & Henseleit [1932] in equilibrium with 5 % CO_2 and 95 % N_2. Anaerobic acid production was measured during the course of 2-hour experiments at 37·5°.

Larger scale experiments. In some experiments it was necessary to use greater amounts of tissue (60 mg. dry weight) and of Ringer solution (10 ml.). The shaking was then performed in the large vessels described by Krebs [1933]. 2 ml. samples were extracted for duplicate determinations of ketone bodies.

Units. The rate of metabolism is expressed in the usual gas notation by the following quotients:

Q_{O_2} = μl. O_2 (N.T.P.) consumed per mg. dry weight of tissue per hour.

Q_{Acac} = μl. CO_2 (N.T.P.) β-ketonic acid formed per mg. dry weight of tissue per hour. 1 millimol β-ketonic acid = 1 millimol CO_2.

$Q_{\beta\text{-Hydroxy}}$ = μl. CO_2 (N.T.P.) β-hydroxybutyric acid formed per mg. dry weight of tissue per hour. 1 millimol β-hydroxybutyric acid = 1 millimol CO_2.

Experimental.

Anaerobic disappearance of acetoacetic acid in animal tissues.

Quastel & Wheatley [1935] have shown that kidney slices reduce large amounts of acetoacetic acid to β-hydroxybutyric acid when the conditions are virtually anaerobic (respiration poisoned with HCN).

The experiments of Table I were performed with the object of comparing the rates of anaerobic disappearance of acetoacetic acid in different tissues and in presence of substrates which might be expected to affect the process. Preliminary work led us to attach particular importance to pyruvate and fructose.

Table I. *Anaerobic disappearance of acetoacetic acid in presence of tissue slices.*

2–3 ml. Bicarbonate-Ringer solution, *p*H 7·4. 2 hours at 37·5°. Gas: 5% CO_2 and 95% N_2.

Exp.	Animal	Substrate	$Q_{CO_2}^{N_2}$	Q_{Acac}
		M initial concentration		
		Liver.		
1	Rat	Acetoacetic acid, 0·0033	3·14	−1·68
		,, +glucose, 0·01	2·92	−1·83
		,, +glycerol, 0·01	2·73	−2·08
		,, +sorbitol, 0·01	2·86	−2·00
		,, +lactate, 0·01	3·57	−2·38
		,, +pyruvate, 0·01	6·40	−2·66
		,, +glyceraldehyde, 0·01	5·86	—
		,, +alanine, 0·01	3·39	−2·12
2	Rat	Pyruvate, 0·01	3·16	0·00
		,, 0·02	3·22	0·00
		Glyceraldehyde, 0·01	4·68	0·00
		Acetoacetic acid, 0·0033	2·31	−2·31
		,, +pyruvate, 0·01	5·36	−3·78
		,, +pyruvate, 0·02	5·20	−3·03
		,, +glyceraldehyde, 0·01	5·57	—
		,, +glucose, 0·01	2·25	−1·81
3	Rat	Acetoacetic acid, 0·0033	1·99	−1·06
		,, +glucose, 0·01	1·57	−0·98
		,, +galactose, 0·01	1·42	−1·07
		,, +mannose, 0·01	1·72	−1·35
		,, +fructose, 0·01	5·46	−2·49
		,, +pyruvate, 0·01	5·98	−2·33
		,, +lactate, 0·01	2·25	−1·26
		,, +pyruvate, 0·01 +glucose, 0·01	7·75	−3·05
4	Rat	Acetoacetic acid, 0·0023	1·88	−1·04
		,, +pyruvate, 0·01	5·22	−2·18
		,, +fructose, 0·01	3·42	−2·42
		,, +fructose, 0·01 +pyruvate, 0·01	5·85	−2·56
5	Rat	Acetoacetic acid, 0·0033	1·93	−1·27
		,, +hexosediphosphate, 0·01	2·77	−1·53
		,, +β-phosphoglycerate, 0·01	1·62	−1·14
		,, +α-phosphoglycerate, 0·01	2·15	−1·41
		,, +α-glycerophosphate, 0·01	2·24	−1·99
		,, +butyrate, 0·01	1·61	−1·08
		,, +acetate, 0·01	2·08	−1·67
		,, +*n*-valerate, 0·01	2·10	−1·41
6	Rat	Acetoacetic acid, 0·0033	1·49	−1·92
		,, +methylglyoxal, 0·005	4·40	—
		,, +methylglyoxal, 0·0025	3·44	—
		,, +acetaldehyde, 0·01	4·02	−2·40
		,, +acetaldehyde, 0·005	2·80	−2·57
		,, +1 mg. glutathione	1·20	−1·93
		,, +malonate, 0·01	1·01	−1·76
7	Rat, starved 24 hours	Nil	1·40	+0·14
		Fructose, 0·01	1·80	+0·05
		Acetoacetic acid, 0·0023	1·57	−0·71
		,, +fructose, 0·01	2·34	−1·61
		,, +glucose, 0·01	1·70	−0·88
		,, +pyruvate, 0·01	3·95	−2·33
		,, +pyruvate, 0·001	2·09	−1·28
		,, +glucose, 0·01 +pyruvate, 0·01	4·38	−1·96
		,, +glucose, 0·01 +pyruvate, 0·001	4·46	−1·69

Table I (*cont.*).

Exp.	Animal	Substrate	$Q_{CO_2}^{N_2}$	Q_{Acac}
		M initial concentration		
		Kidney cortex.		
8	Rat	Acetoacetic acid, 0·0033	1·90	−1·50
		,, +glucose, 0·01	5·03	−1·78
		,, +glycerol, 0·01	1·67	−1·55
		,, +pyruvate, 0·01	4·25	−1·85
		,, +alanine, 0·01	1·88	−2·62
		,, +lactate, 0·01	2·32	−1·55
		,, +cysteine, 0·01	1·75	−1·23
9	Rat	Acetoacetic acid, 0·0033	2·00	−1·39
		,, +pyruvate, 0·02	3·92	−1·44
		,, +hexosediphosphate, 0·01	2·41	−1·35
		,, +α-phosphoglycerate, 0·01	2·43	−1·79
		Pyruvate, 0·02	3·06	+0·01
10	Rat	Acetoacetic acid, 0·0033	1·74	−1·63
		,, +α-glycerophosphate, 0·01	1·80	−1·90
11	Rat	Acetoacetic acid, 0·0033	1·65	−1·34
		,, +fructose, 0·01	2·07	−1·33
		Fructose, 0·01	1·86	0·00
12	Rat	Acetoacetic acid, 0·0033	1·58	−1·56
		,, +sorbitol, 0·01	1·37	−1·56
		,, +glyceraldehyde, 0·01	4·10	—
		,, +succinate, 0·01	1·07	−1·55
		,, +glutarate, 0·01	1·73	−1·41
		,, +malonate, 0·01	1·61	−1·35
		,, +propionate, 0·01	1·70	−1·21
13	Guinea-pig	Acetoacetic acid, 0·0033	1·69	−1·26
		,, +fructose, 0·01	2·70	−1·80
		,, +pyruvate, 0·01	3·24	−1·47
		,, +alanine, 0·01	1·54	−1·79
		,, +1 mg. glutathione	1·72	−1·52
14	Pigeon	Acetoacetic acid, 0·0033	1·41	−1·69
		,, +pyruvate, 0·01	4·00	−2·32
15	Pigeon	Acetoacetic acid, 0·0033	1·43	−1·04
		,, +pyruvate, 0·01	4·02	−2·25
		Testis.		
16	Rat	Acetoacetic acid, 0·0033	1·76	−1·33
		,, +pyruvate, 0·01	3·78	−1·18
		,, +glyceraldehyde, 0·01	3·08	—
		,, +glucose, 0·01	5·06	−1·57
		,, +alanine, 0·01	1·45	−1·20
17	Rat	Acetoacetic acid, 0·0033	2·61	−1·24
		,, +fructose, 0·01	3·92	−1·12
		,, +α-glycerophosphate, 0·01	2·44	−0·83
		Brain cortex.		
18	Rat	Acetoacetic acid, 0·0033	0·92	−1·19
		,, +pyruvate, 0·01	3·05	−0·94
		,, +fructose, 0·01	0·94	−0·66
19	Guinea-pig	Acetoacetic acid, 0·0033	1·07	−0·71
		,, +pyruvate, 0·01	2·34	−0·87
		,, +fructose, 0·01	2·14	−1·01
		,, +glucose, 0·01	21·2	−1·15
		Glucose, 0·01	20·3	0·00
20	Guinea-pig	Acetoacetic acid, 0·0033	1·08	−0·86
		+pyruvate, 0·01	2·68	−0·51
		,, +fructose, 0·01	1·95	−0·42

Table I (*cont.*).

Exp.	Animal	Substrate	$Q^{N_2}_{CO_2}$	Q_{Acac}
		M initial concentration		
		Brain cortex (*cont.*).		
		(1 hour only)		
21	Guinea-pig	Acetoacetic acid, 0·0023	2·53	−2·07
		,, +glucose, 0·01	22·1	2·28
		,, +glyceraldehyde, 0·01	2·96	—
		,, +α-glycerophosphate, 0·01	1·80	−4·75
22	Pigeon	Acetoacetic acid, 0·0033	1·73	−1·97
		,, +pyruvate, 0·01	2·66	−1·73
		,, +fructose, 0·01	1·51	−1·63
		Spleen.		
23	Rat	Acetoacetic acid, 0·0033	1·52	−1·04
		,, +pyruvate, 0·01	2·23	−1·04
		Intestine.		
24	Rat	Acetoacetic acid, 0·0033	4·60	−1·04
		,, +pyruvate, 0·01	5·50	−0·84
		,, +fructose, 0·01	4·98	−0·85
		Pancreas.		
25	Rat	Acetoacetic acid, 0·0023	1·99	−0·52
		,, +pyruvate, 0·01	2·81	−0·35
		,, +fructose, 0·01	2·47	−0·34
26	Pigeon	Acetoacetic acid, 0·0023	1·88	−1·05
		,, +pyruvate, 0·01	2·64	−1·25
		Submaxillary gland.		
27	Guinea-pig	Acetoacetic acid, 0·0033	0·85	−0·27
		,, +pyruvate, 0·01	1·58	−0·43
		Diaphragm.		
28	Rat	Acetoacetic acid, 0·0033	1·08	−0·48
		,, +hexosediphosphate, 0·01	1·55	−0·45
		,, +pyruvate, 0·01	1·74	−0·63

Sodium acetoacetate solutions were prepared according to Ljunggren [1924]. In all experiments the initial concentration of acetoacetate was determined by setting up a control vessel without tissue; this was treated in exactly the same way as the vessels to which tissue slices were added.

The experiments show that added acetoacetic acid disappears anaerobically in all the tissues which have been examined. The rates of disappearance are slightly higher in kidney, liver and pigeon brain than in other tissues, but in testis, rat and guinea-pig brains, spleen, intestine and pigeon pancreas there is a fairly uniform rate, the values of Q_{Acac} being about -1. In rat pancreas, submaxillary gland and diaphragm the rates are distinctly slower. There is acid production in every case.

When substrates are added in addition to acetoacetic acid the following changes occur:

1. In presence of pyruvate there is a marked increase of CO_2 production in all tissues, but acceleration of acetoacetic acid disappearance is constantly observed only in rat liver and in pigeon brain and kidney. In the other tissues examined there is no significant effect on acetoacetic acid reduction.

2. In liver there is a large acid formation in presence of fructose [Oppenheimer, 1912; Dickens & Greville, 1932; Rosenthal, 1930; 1931], and we have found that this is accompanied by a more rapid disappearance of acetoacetic acid. In kidney, where fructolysis is low, the rate of acetoacetic acid dis-

appearance is not altered significantly; likewise there is no effect in testis, brain or other tissues.

3. When *dl*-glyceraldehyde is added to slices of liver, kidney and testis there is acid formation [see Rosenthal, 1930; 1931] and acceleration of acetoacetic acid disappearance equal to that observed with pyruvate. In kidney there is a similar acid formation in presence of methylglyoxal and acetaldehyde, but a smaller effect on disappearance of ketonic acid.

Since glyceraldehyde and methylglyoxal react with acetoacetic acid *in vitro* in absence of tissue, and since the presence of tissue slices causes little or no acceleration of this effect, it is difficult to assess the action of these substances in surviving tissue. We have therefore omitted the $-Q_{Acac}$ values from Table I.

4. Glucose, galactose, mannose, hexosediphosphate, phosphoglycerate, α-glycerophosphate, glycerol, sorbitol, lactate, acetate, propionate, butyrate, cysteine, succinate and glutathione in the concentrations employed have no significant influence on anaerobic disappearance of acetoacetic acid in the tissues to which they have been added. Alanine, however, was effective in both liver and kidney, whilst α-glycerophosphate increased the rate of disappearance in guinea-pig brain during a short experiment.

Anaerobic disappearance of acetoacetic acid is somewhat slower in the liver of a starved rat but is increased by pyruvate and fructose just as in the well-nourished organ (Exp. 7).

Table II.

(*a*) *Anaerobic acid formation and acetoacetic acid disappearance in rat liver slices.*

Substrate		$Q_{CO_2}^{N_2}$	Q_{Acac}
1. During the first hour:			
Acetoacetic acid, 0·0033 *M*		2·97	−2·16
,,	+pyruvate, 0·01 *M*	13·5	−4·05
,,	+fructose, 0·01 *M*	11·4	−6·11
2. During the second hour:			
Acetoacetic acid, 0·0033 *M*		2·42	−1·33
,,	+pyruvate, 0·01 *M*	6·59	−2·65
,,	+fructose, 0·01 *M*	5·54	−2·91

(*b*) *Effects of fluoride and iodoacetate on the anaerobic disappearance of acetoacetic acid in liver slices (rat).*

2 hours at 37·5°.

Fluoride. Concentration of NaF = 0·01 *M*. Ca-free medium.			
Acetoacetic acid, 0·003 *M*		1·70	−1·25
,,	+fluoride	0·98	−1·17
,,	+pyruvate, 0·01 *M*	6·62	−3·88
,,	+pyruvate, 0·01 *M* +fluoride	5·70	−3·70
,,	+fructose, 0·01 *M*	2·56	−2·06
,,	+fructose, 0·01 *M* +fluoride	1·37	−1·58
Concentration of sodium fluoride = 0·02 *M* Ca-free medium.			
Acetoacetic acid, 0·003 *M*		1·93	−1·18
,,	+fluoride	1·00	−1·21
,,	+pyruvate, 0·01	9·82	−3·16
,,	+pyruvate, 0·01 +fluoride	4·98	−2·55
,,	+fructose, 0·01	3·95	−2·86
,,	+fructose, 0·01 +fluoride	1·39	−1·29
Iodoacetate. Concentration of sodium iodoacetate = 0·00067 *M*.			
Acetoacetic acid, 0·003 *M*		2·05	−0·75
,,	+iodoacetate	1·93	−0·82
,,	+pyruvate, 0·01 *M*	7·02	−2·36
,,	+pyruvate, 0·01 *M* +iodoacetate	4·09	−1·57
,,	+fructose, 0·01 *M*	5·10	−2·27
,,	+fructose, 0·01 *M* +iodoacetate	2·48	−1·51

The relationship between anaerobic glycolysis and disappearance of acetoacetic acid.

In view of the well-known effects of pyruvate and fructose on anaerobic acid production in liver we performed a number of experiments bearing on the relationship between glycolysis and the reduction of acetoacetic acid (Table II).

Table II (*a*) shows that the rate of acetoacetic acid disappearance diminishes with time in proportion to the fall in fructolysis or in CO_2 production caused by pyruvate. The inhibitors of glycolysis, fluoride and iodoacetate, also inhibit acetoacetic acid disappearance; although fluoride reduces CO_2 formation in presence of pyruvate, it has only a small inhibitory action upon the rate of acetoacetic acid disappearance (Table II (*b*)). Exp. 7 (Table I) illustrates the fact that acetoacetic acid disappearance is slower in the liver of the starved rat than in the well-nourished organ. This experiment also shows that fructolysis and CO_2 formation in presence of pyruvate are less [Rosenthal, 1930; 1931]; in spite of this the substrates still increase acetoacetic acid disappearance. Further, when glucolysis is "activated" by means of 0·001 *M* pyruvate, acetoacetic acid disappearance is not increased in proportion.

These experiments lead to no final conclusions, but they suggest that there is some parallelism between liver glycolysis and acetoacetic acid reduction; the parallelism, however, is not complete in the case of pyruvate.

Aerobic disappearance of acetoacetic acid in animal tissues.

The results of investigation of aerobic disappearance of acetoacetic acid in different tissues and in presence of certain substrates are given in Table III.

Table III. *Aerobic disappearance of acetoacetic acid in presence of tissue slices.*

2–3 ml. phosphate saline, *p*H 7·4. 2 hours at 37·5°. Gas: oxygen.

Animal	Substrate	Q_{O_2}	Q_{Acac}
	M initial concentration		
	Kidney, cortex.		
Rat	Acetoacetic acid, 0·0033	−29·5	−5·02
	,, +glucose, 0·01	−30·2	−5·55
	,, +pyruvate, 0·01	−33·5	−6·37
Rat	Acetoacetic acid, 0·0033	−27·0	−3·88
	,, +glycerol, 0·01	−27·4	−3·98
Rat	Acetoacetic acid, 0·0033	−22·6	−4·33
	,, +α-glycerophosphate, 0·01	−24·6	−4·91
Rat	Acetoacetic acid, 0·0033	−23·6	−4·37
	,, +hexosediphosphate, 0·01	−29·9	−5·45
	Testis.		
Rat	Nil	− 6·8	+0·51
	Glucose, 0·01	−11·0	+0·56
	Acetoacetic acid, 0·0033	− 6·4	−0·28
	,, +glucose, 0·01	−11·3	−0·75
	,, +pyruvate, 0·01	−13·3	−0·34
	,, +fructose, 0·01	−10·0	−0·18
	,, +glycerol, 0·01	− 6·7	−0·10
Rat	Nil	− 5·2	+0·43
	Acetoacetic acid, 0·0033	− 6·1	−0·22
	,, +α-glycerophosphate, 0·01	− 6·6	−0·17
Rat	Acetoacetic acid, 0·0033	− 7·3	−0·18
	,, +hexosediphosphate, 0·01	− 6·7	0·00
	,, +α-phosphoglycerate, 0·01	− 6·5	−0·11
	,, +β-phosphoglycerate, 0·01	− 6·1	−0·05
	,, +alanine, 0·01	− 5·4	−0·13

Table III (*cont.*).

Animal	Substrate	Q_{O_2}	Q_{Acac}
	M inital concentration		
	Brain cortex.		
Guinea-pig	Glucose, 0·01	−11·7	0·00
	Fructose, 0·01	−12·0	0·00
	Pyruvate, 0·01	−14·0	0·00
	Lactate, 0·01	−12·5	0·00
	Acetoacetic acid, 0·0023	− 9·1	−1·11
	,, +glucose, 0·01	−12·1	−1·39
	,, +fructose, 0·01	−11·0	−1·04
	,, +pyruvate, 0·01	−13·9	−1·22
	,, +lactate, 0·01	−13·0	−1·05
Guinea-pig	Acetoacetic acid, 0·0023	− 8·6	−0·66
	,, +α-glycerophosphate, 0·01	− 9·7	−0·99
Pigeon	Nil	− 8·9	+0·17
	Pyruvate, 0·01	−18·1	+0·27
	Acetoacetic acid, 0·0033	−16·1	−4·48
	,, +pyruvate, 0·01	−18·6	−3·34
	,, +glucose, 0·01	−14·1	−4·72
	Glucose, 0·01	−14·6	+0·20
	Spleen.		
Guinea-pig	Acetoacetic acid, 0·003	− 6·8	−1·11
	,, +pyruvate, 0·01	− 6·9	−0·90
	Lung.		
Guinea-pig	Nil	− 7·4	+0·12
	Acetoacetic acid, 0·003	− 8·9	−1·12
	,, +pyruvate, 0·01	− 9·0	−0·77
	Pyruvate, 0·01	− 8·0	+0·13
	Pancreas.		
Pigeon	Nil	− 8·7	+0·18
	Acetoacetic acid, 0·003	− 8·5	−1·07
	Submaxillary gland.		
Guinea-pig	Nil	− 5·0	+0·12
	Acetoacetic acid, 0·003	− 7·2	−1·40
	Diaphragm.		
Rat	Acetoacetic acid, 0·0033	− 4·8	−1·35
	,, +pyruvate, 0·01	− 5·2	−1·20
	Skeletal muscle.		
Pigeon	Acetoacetic acid, 0·003	− 8·5	−0·60
	,, +pyruvate, 0·01	−11·8	−0·91
	,, +fructose, 0·01	−13·2	−0·83
	3 ml. bicarbonate-Ringer solution. Gas: 5% CO_2 and 95% O_2.		
	Liver.		
Rat	Nil	—	+0·23
	Pyruvate, 0·01	—	+1·08
	Acetoacetic acid, 0·0023	—	−0·80
	,, +pyruvate, 0·01	—	−0·59
	,, +glucose, 0·01	—	−1·06
	Glucose, 0·01	—	+0·20
	Kidney cortex.		
Rat	Acetoacetic acid, 0·0033	—	−3·57
	,, +pyruvate, 0·01	—	−4·68
	,, +α-phosphoglycerate, 0·01	—	−4·02

The experiments show that the rate of disappearance of added acetoacetic acid is much greater in kidney than in any other tissue except pigeon brain. In testis the disappearance is very small, the figures being within the experimental error, but becomes significant in presence of glucose. In slices of liver and skeletal muscle the rate is slow and unaffected by substrates. In pancreas,

diaphragm and submaxillary gland the aerobic disappearance is significantly greater than the anaerobic (see Table I); in spleen it is not. In lung acetoacetic acid disappears aerobically at about the same rate as in spleen.

In all tissues except kidney the addition of substrates produced no acceleration. In kidney pyruvate, α-glycerophosphate, hexosediphosphate, α-phosphoglycerate and glucose caused small but significant increases in rate of acetoacetic acid disappearance.

The oxidation of β-hydroxybutyric acid in various tissues.

Jowett & Quastel [1935] have shown that *dl*-β-hydroxybutyric acid is oxidized to acetoacetic acid by guinea-pig kidney and liver, and by spleen,

Table IV. *Oxidation of β-hydroxybutyric acid in various tissues.*

Phosphate saline, *p*H 7·4. In oxygen at 37·5°.

Animal	Tissue	Substrate (*M*)	Q_{O_2}	Q_{Acac}
Guinea-pig	Brain	Nil	− 6·9	0·49
		dl-β-Hydroxybutyrate, 0·01	− 9·0	1·59
		Nil	− 5·9	0·46
		l-β-Hydroxybutyrate, 0·01	− 6·9	1·92
	Liver	Nil	− 7·8	0·39
		dl-β-Hydroxybutyrate, 0·01	− 8·5	1·28
		Nil	− 8·3	0·91
		l-β-Hydroxybutyrate, 0·01	− 8·8	1·28
	Kidney	Nil	− 14·0	0·09
		dl-β-Hydroxybutyrate, 0·01	− 20·0	3·25
		Nil	− 13·2	0·00
		l-β-Hydroxybutyrate, 0·01	− 17·8	2·12
	Spleen	Nil	− 7·9	0·30
		dl-β-Hydroxybutyrate, 0·01	− 9·5	0·84
		Nil	− 8·7	0·17
		l-β-Hydroxybutyrate, 0·01	− 9·8	0·73
	Pancreas	Nil	− 2·90	0·00
		dl-β-Hydroxybutyrate, 0·01	− 2·94	0·28
	Submaxillary gland	Nil	− 5·0	0·12
		dl-β-Hydroxybutyrate, 0·01	− 8·5	0·65
	Skeletal muscle	Nil	− 2·12	0·19
		dl-β-Hydroxybutyrate, 0·01	− 2·36	0·18
	Blood 2·0 ml. (citrated)	Nil	—	0·00
		dl-β-Hydroxybutyrate, 0·01	—	0·00
Rat	Kidney	Nil	− 17·7	0·10
		l-β-Hydroxybutyrate, 0·005	− 22·3	1·43
	Lung	Nil	− 7·9	0·28
		dl-β-Hydroxybutyrate, 0·01	− 8·3	0·34
	Intestine	Nil	− 7·1	0·20
		l-β-Hydroxybutyrate, 0·01	− 6·3	0·53
	Pancreas	Nil	− 3·7	0·02
		dl-β-Hydroxybutyrate, 0·01	− 4·1	0·11
	Diaphragm	Nil	− 5·5	0·16
		dl-β-Hydroxybutyrate, 0·01	− 5·3	0·36
		Nil	− 5·3	0·25
		l-β-Hydroxybutyrate, 0·01	− 4·7	0·66
Pigeon	Pancreas	Nil	− 8·7	0·18
		dl-β-Hydroxybutyrate, 0·01	− 9·0	0·53
	Skeletal muscle	Nil	8·5	0·10
		dl-β-Hydroxybutyrate, 0·01	− 11·7	0·15

NOTE. *l*-β-Hydroxybutyrate was prepared from the Ca Zn salt by decomposition with sodium carbonate.

testis and brain cortex of the rat. We have confirmed these observations and found that the oxidation takes place at approximately the same rate in presence of the same concentration of *l*-β-hydroxybutyric acid (Table IV).

The oxidation also occurs in other tissues: in pancreas and submaxillary gland of the guinea-pig, in intestine and diaphragm of the rat and in pigeon pancreas. Though the values of Q_{Acac} are small, the differences are significant. Guinea-pig blood and rat lung slices do not appear to oxidize β-hydroxybutyric acid.

As a result of the experiments of this and preceding sections it becomes clear that the reaction, acetoacetic acid $\rightleftharpoons$ β-hydroxybutyric acid, is reversible and can occur in tissues other than liver and kidney, although the respective rates of oxidation and reduction vary widely in different tissues.

The aerobic destruction of β-hydroxybutyric acid.

By determining acetoacetic and β-hydroxybutyric acids simultaneously we have measured the real destruction of β-hydroxybutyric acid as distinct from the conversion into ketonic acid. This has been done chiefly in kidney in presence and in absence of added substrates, but a few experiments were made with other tissues (Table V).

Table V. *Aerobic breakdown of β-hydroxybutyric acid.*

Phosphate saline and oxygen. 37·5°.

	Substrate Initial conc. 0·005 *M*, β-hydroxybutyrate	Q_{O_2}	Q_{Acac}	$Q_{\beta\text{-Hydroxy}}$
Rat kidney	Nil	−17·8	0·0	0·0
	dl-β-Hydroxybutyric acid	−23·9	+2·80	−5·90
	dl-β-Hydroxybutyric acid	−31·6	+1·32	−5·34
	,, +succinate, 0·01 *M*	−45·7	+1·27	−5·73
	,, +glucose, 0·01 *M*	−33·6	+1·30	−5·30
	dl-β-Hydroxybutyric acid	−23·0	+0·45	−5·17
	,, +fructose, 0·01 *M*	−32·0	+0·20	−5·20
	dl-β-Hydroxybutyric acid +pyruvate, 0·01 *M*	−32·02	—	−5·22
	dl-β-Hydroxybutyric acid	−28·3	+0·90	−4·44
	,, +glycerol, 0·01 *M*	−32·6	+0·71	−4·93
	,, +lactate, 0·01 *M*	−29·0	+0·76	−3·64
	dl-β-Hydroxybutyric acid	−25·7	+0·95	−5·51
	,, +hexosediphosphate 0·01 *M*	−29·0	+0·90	−5·04
	10 ml. phosphate saline and O_2. Large shaking vessels.			
Rat testis	*dl*-β-Hydroxybutyric acid	—	+0·55	−1·06
	,,	—	+0·45	−1·08
	dl-β-Hydroxybutyric acid +succinate, 0·01 *M*	—	+0·67	−1·95
	,, +glucose, 0·01 *M*	—	+0·31	−1·68
Rat lung	*dl*-β-Hydroxybutyric acid	—	+0·05	−1·10
	,,	—	+0·05	−1·15

These figures confirm the findings of Quastel & Wheatley [1935], who observed that only about one-quarter of the β-hydroxybutyric acid disappearing in kidney is converted into acetoacetic acid. They show further that addition of substrates such as glucose, succinate and pyruvate does not increase the rate of breakdown. In absence of other added substrates β-hydroxybutyric acid is broken down at an appreciable rate in rat testis and lung; in the former tissue glucose does not accelerate the process.

The influence of inhibitors on ketone body disappearance.

Quastel & Wheatley [1935] have shown that sodium malonate greatly inhibits the disappearance of acetoacetic acid in kidney slices, an effect which occurs only aerobically. This we have confirmed and also the action of fumarate and lactate in preventing inhibition. The ketogenic effect of malonate has been referred to in Part III of this series (see also Jowett & Quastel [1935]), where it was shown that other dicarboxylic acids—hydroxymalonic, mesoxalic, tartaric

Table VI. *Influence of anticatalysts on the disappearance of ketone bodies in rat kidney and liver.*

2 hours at 37·5°.

Substrate	Q_{O_2}	Q_{Acac}
Kidney.		
Aerobic. Bicarbonate-Ringer solution: 5% CO_2 and 95% O_2.		
Acetoacetic acid, 0·003 *M*	—	−3·28
,, + malonate, 0·02 *M*	—	−0·27
,, + hydroxymalonate 0·02 *M*	—	−2·10
,, + NH_4Cl, 0·02 *M*	—	−3·12
,, + pyruvate, 0·01 *M*	—	−3·70
,, + pyruvate, 0·01 *M* + malonate, 0·02 *M*	—	−1·13
Acetoacetic acid, 0·003 *M*	—	−3·50
,, + oxalate, 0·003 *M*	—	−3·23
Aerobic. Phosphate saline and oxygen.		
Acetoacetic acid, 0·003	−30·6	−3·88
,, + NH_4Cl, 0·01 *M*	−29·8	−3·80
Nil	−22·2	+0·49
Malonate, 0·01 *M*	−14·7	+1·09
Hydroxymalonate, 0·01 *M*	−20·6	+0·47
Butyrate, 0·01 *M*	−29·8	+0·79
,, + malonate, 0·01 *M*	−19·4	+1·24
,, + hydroxymalonate, 0·01 *M*	−23·3	+0·52
Nil	−20·1	+0·17
Oxalate, 0·003 *M*	−18·3	+0·06
Butyrate, 0·01 *M* + oxalate, 0·003 *M*	−29·8	+0·33
Nil	−22·2	+0·32
Acetoacetic acid, 0·0023 *M*	−25·8	−3·65
NH_4Cl, 0·01 *M*	−20·3	0·00
NH_4Cl, 0·02 *M*	−16·1	0·00
Acetoacetic acid, 0·0023 *M* + NH_4Cl, 0·01 *M*	−27·4	−3·53
,, + NH_4Cl, 0·02 *M*	−23·5	−3·26
Malonate, 0·02 *M*	−9·0	+0·57
Hydroxymalonate, 0·02 *M*	−19·4	0·00
Acetoacetic acid, 0·0023 *M* + malonate, 0·02 *M*	−8·6	−0·31
,, + hydroxymalonate, 0·02 *M*	−21·9	−2·88
Nil	−20·4	+0·25
d-Tartrate, 0·01 *M*	−19·6	+0·10
Acetoacetic acid, 0·0023 *M*	−25·3	−3·33
,, + *d*-tartrate, 0·01 *M*	−24·4	−3·30
,, + *d*-tartrate, 0·02 *M*	−23·0	−3·02
Nil	−22·6	+0·23
Oxalate, 0·005 *M*	−19·8	0·00
Acetoacetic acid, 0·0023 *M*	−30·6	−4·41
,, + oxalate, 0·005 *M*	−29·1	−4·03
,, + oxalate, 0·01 *M*	−29·2	−3·90
Nil	−22·2	+0·31
Mesoxalate, 0·01 *M*	−22·6	+0·11
Mesoxalate, 0·02 *M*	−19·2	+0·09
Acetoacetic acid 0·0023 *M*	−24·3	−3·02
,, + mesoxalate, 0·01 *M*	−22·8	−3·15
,, + mesoxalate, 0·02 *M*	−18·3	−2·31

Table VI (*cont.*).

2 hours at 37·5°.

Substrate	Q_{CO_2}	Q_{Acac}
Liver.		
Anaerobic. Bicarbonate-Ringer solution; 5% CO_2 and 95% N_2.		
Acetoacetic acid, 0·0023 *M*	2·23	−1·36
,, +malonate, 0·01 *M*	3·18	−1·41
,, +hydroxymalonate, 0·01 *M*	3·47	−1·67
,, +NH_4Cl, 0·02 *M*	1·91	−1·27
Acetoacetic acid, 0·0023 *M*	1·26	−0·95
,, +oxalate, 0·003 *M*	0·99	−1·05
Nil	2·74	+0·03
NH_4Cl, 0·02	2·94	+0·02
Acetoacetic acid, 0·0023 *M*	2·04	−1·11
,, +NH_4Cl, 0·02 *M*	1·87	−1·64
,, +pyruvate, 0·01 *M*	5·56	−2·63
,, +pyruvate, 0·01 *M* +NH_4Cl, 0·02 *M*	5·79	−2·32

NOTE. Experiments with oxalate were performed in Ca-free media.

and oxalic—are ketogenic in liver. Since these substances take no direct part in fatty acid metabolism, and since they appear to inhibit some dehydrogenase systems more or less specifically, they may be regarded as anticatalysts. Ammonium chloride possibly belongs to the same class. We have examined the influence of these substances on ketone body disappearance in kidney, the results being given in Table VI.

It will be seen that malonate is a powerful and relatively specific inhibitor of respiration and of aerobic disappearance of acetoacetic acid in kidney. Hydroxymalonate, mesoxalate, tartrate, oxalate and ammonia cause relatively little depression of respiration and only a small inhibition of acetoacetic acid disappearance when they are added in appropriate concentrations. Pyruvate prevents the malonate action to some degree.

In liver under anaerobic conditions these substances do not significantly affect the rate of acetoacetic acid disappearance.

The influence of malonate and hydroxymalonate on the oxidation of β-hydroxbutyric acid was investigated (Table VII).

Table VII. *Influence of malonate and hydroxymalonate on oxidation of β-hydroxybutyric acid.*

Rat kidney cortex. Phosphate saline and oxygen.

Substrate	Q_{O_2}	Q_{Acac}	$Q_{\beta\text{-Hydroxy}}$
dl-β-Hydroxybutyric acid, 0·005 *M*	−25·0	+0·95	−6·80
,, +malonate, 0·01 *M*	−18·1	+0·67	−4·50
dl-β-Hydroxybutyric acid, 0·0075 *M*	−41·7	+3·17	—
,, +malonate, 0·01 *M*	−14·2	+3·16	—
,, +malonate, 0·02 *M*	−11·0	+3·00	—
dl-β-Hydroxybutyric acid, 0·005 *M*	−34·3	+2·21	—
,, +hydroxymalonate, 0·02 *M*	−27·8	+2·05	—

These figures show that malonate and hydroxymalonate do not inhibit the oxidation of β-hydroxybutyric acid to acetoacetic acid; and that malonate prevents the aerobic breakdown of β-hydroxybutyric acid.

Discussion.

A considerable portion of the acetoacetic acid or β-hydroxybutyric acid added to kidney slices is broken down to products no longer recognizable as ketone bodies. At least part of this disappearance is due to complete combustion, since Elliott *et al.* [1935] have found that bicarbonate is formed during oxidation of β-hydroxybutyric acid, but there may be intermediate stages.

The apparently specific action of malonate in preventing aerobic breakdown of ketone bodies acquires further significance from the fact that malonate is known to be a specific inhibitor of succinic dehydrogenase. As a tentative working hypothesis we suggest that the aerobic metabolism of ketone bodies may depend upon the oxidation of succinic acid.

Krebs [1936] has described the reduction of acetoacetic acid as a linked reaction which involves the anaerobic oxidation of carbohydrate derivatives. This provides one point of contact between fat and carbohydrate metabolism.

Another is possible. If acetic acid were formed by cleavage of ketone bodies, it might react with pyruvic acid according to Krebs's scheme:

$$\begin{matrix}\text{Acetic acid} \\ + \\ \text{pyruvic acid}\end{matrix} \rightarrow \alpha\text{-Ketoglutaric acid} \rightarrow \text{Succinic acid, etc.}$$

The continuation of ketone body breakdown would then depend upon the oxidation of succinic acid and the consequent supply of pyruvic acid.

The action of malonate could be explained as an interruption of the chain of reactions leading to pyruvic acid.

$$\text{Succinic acid} \rightarrow^{*} \text{Fumaric acid} \rightarrow \text{Malic acid} \rightarrow \text{Oxaloacetic acid} \rightarrow \text{Pyruvic acid.}$$

* Malonate inhibition.

The partial neutralization of the malonate effect by fumarate, lactate, alanine [Quastel & Wheatley, 1935] and pyruvate agrees with this hypothesis.

Preliminary measurements of acid-base changes have shown that, within the limits of error, (i) the rate of bicarbonate formation in kidney in presence of ketone bodies is equal to or greater than the rate of ketone body destruction, (ii) acetate is burnt approximately twice as rapidly as the ketone bodies. These observations are consistent with the view that ketone bodies are split into acetic acid before combustion, but they afford no proof.

Summary.

1. The metabolism of ketone bodies, both aerobic and anaerobic, has been investigated in a number of tissues by means of the slice technique. The rate of metabolism varies in different tissues.

2. Pyruvate and fructose accelerate the anaerobic disappearance of acetoacetic acid in liver but have no marked influence in other tissues except pigeon's kidney.

3. Since β-hydroxybutyric acid is oxidized to acetoacetic acid by most tissues, the reaction, acetoacetic acid $\rightleftharpoons$ β-hydroxybutyric acid, appears to be of general importance.

4. The effects of malonate, hydroxymalonate, mesoxalate, tartrate and oxalate on ketone body oxidation have been studied.

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