CCCXXX. STUDIES IN FAT METABOLISM.

IV. ACETOACETIC ACID BREAKDOWN IN THE KIDNEY.

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THE following paper is concerned with the breakdown of acetoacetic acid in the kidney. A preliminary study of this phenomenon has already been made [Jowett and Quastel, 1935, 3] and the experimental methods used have already been described in detail [Parts I, II, III, Jowett and Quastel, 1935, 1, 2, 3]. Both the Barcroft and Warburg apparatus have been used.

Acetoacetic acid has been determined manometrically as usual (see Part I), kidney cortex slices being removed from the medium at the end of a period of 2 hours at 37°. The initial concentration of sodium acetoacetate (prepared by the method of Llunggren [1924], alcohol being removed by vigorous aeration at reduced pressure) was determined in each experiment, a control vessel containing acetoacetate without tissue being kept in the bath at 37° for the duration of the experiment.

Aerobic breakdown of acetoacetic acid in the kidney.

It was demonstrated in the preceding paper (Part III) that the kidney cortex destroys acetoacetic acid at a considerable rate, Q_{Ac}^1 reaching a value of -4.0 with the guinea-pig and -6.0 with the rat. In Table I the results of 20 experiments with each animal are reported. It will be seen that with an initial con-

Table I.

Sodium acetoacetate solution = 0.0058 M.						
Ringer solution, $NaHCO_3 = 0.025 M$. 95% $O_2 + 5\% CO_2$.						
Tissue	Cyanide M	Glucose M	No. of exps.	Mean value of $-Q_{Ac}$	Range of values of $-Q_{Ac}$	Av. deviation from mean value
Guinea-pig kidney	Nil	Nil	20	3.4	1.9-4.8	± 0.6
"	Nil	0.009	6	3.6	2.5-4.2	± 0.4
"	0.004	Nil	9	1.4	0.6-1.9	± 0.4
"	0.004	0.009	9	2.3	1.5-3.4	± 0.5
Rat kidney	Nil	Nil	20	4.5	2.3-5.7	± 0.7
"	Nil	0.009	5	5.0	4.1-5.9	± 0.5
"	0.004	Nil	11	1.05	0.0-2.7	± 0.5
"	0.004	0.009	9	1.9	1.0-3.0	± 0.5
β -Glycerophosphate M/15 buffer. O_2 .						
Guinea-pig kidney	Nil	Nil	4	3.9	2.5-4.5	± 0.7
Rat kidney	Nil	Nil	3	4.9	4.4-5.4	± 0.4

¹ As explained in an earlier paper, the symbol Q_{Ac} , when it has a positive value, expresses the rate of appearance of acetoacetic acid, and when it has a negative value, expresses the rate of disappearance of acetoacetic acid, as ml. of gas (at N.T.P.) per hour per mg. dry weight of tissue.

centration of sodium acetoacetate of 0.0058 *M* and at p_H 7.4 in a bicarbonate-Ringer solution, the mean Q_{Ac} is -3.4 for guinea-pig kidney and -4.5 for rat kidney. There is a relatively large range of variation, the average deviation from the mean value being ± 0.6 and ± 0.7 respectively. It is likely that the state of nutrition of the animal plays an important part in determining the magnitude of Q_{Ac} . It should be noted however that in all experiments reported on here apparently well-nourished animals were used.

In β -glycerophosphate buffer in presence of oxygen, the breakdown of acetoacetic acid proceeds at much the same rate as in a bicarbonate-Ringer solution.

Anaerobic breakdown of acetoacetic acid.

Acetoacetic acid is broken down under anaerobic conditions by kidney tissue (see Part III). The results of a number of experiments with rat and guinea-pig tissues in presence of 95 % N_2 + 5 % CO_2 are shown in Table II. The rate of breakdown is roughly half that under aerobic conditions, but here again there is a fairly wide range of variation probably determined by nutritional conditions.

Table II.

Sodium acetoacetate solution = 0.0058 *M*.

Ringer solution, $NaHCO_3$ = 0.025 *M*. 95 % N_2 + 5 % CO_2 .

Tissue	No. of exps.	Mean value - Q_{Ac}	Values of - Q_{Ac} obtained
Guinea-pig kidney	4	2.4	2.7, 2.1, 2.8, 1.9
Rat kidney	8	2.3	2.1, 2.1, 2.1, 2.0, 2.8, 1.8, 2.1, 2.5

Action of potassium cyanide on acetoacetic acid breakdown.

Cyanide (0.004 *M*; p_H 7.4) reduces acetoacetate breakdown by kidney under aerobic conditions to the anaerobic level. Values of - Q_{Ac} in presence of nitrogen are scarcely affected by cyanide at this concentration.

Table III. *Action of potassium cyanide on acetoacetate breakdown.*

Sodium acetoacetate solution = 0.0058 *M*.

Ringer solution, $NaHCO_3$ = 0.025 *M*.

Exp.	Tissue	- Q_{Ac} .		- Q_{Ac} .	
		95 % O_2 + 5 % CO_2		95 % N_2 + 5 % CO_2	
		No cyanide present	Cyanide present (0.004 <i>M</i>)	No cyanide present	0.004 <i>M</i> cyanide present
1	Rat kidney	—	—	1.9	1.8
2	"	5.1	1.3	—	—
3	"	—	—	—	2.0
4	"	—	—	2.1	—
5	"	4.9	1.5	—	—
6	Guinea-pig kidney	2.7	1.3	—	—
7	"	1.9	0.6	—	—
8	"	—	—	—	1.7
9	"	4.3	—	2.4	—
10	"	3.0	1.5	—	—
11	"	—	1.9	—	—
12	"	—	1.8	—	—
13	"	—	—	2.1	—
Glycerophosphate buffer. O_2 .					
14	Rat kidney	0.004 <i>M</i> cyanide present Q_{O_2} = 2.3; - Q_{Ac} = 2.5			
15	"	No cyanide Q_{O_2} = 25.2; - Q_{Ac} = 5.4			

Moreover, the effect of cyanide is much the same in a β -glycerophosphate buffer as in a bicarbonate-Ringer solution. Typical results are shown in Table III. The results of a number of experiments with guinea-pig and rat kidneys are also shown in Table I. There it will be observed that the mean values of Q_{Ac} , in presence of cyanide, are definitely lower than those found in nitrogen. This is due to the fact that, in a number of early experiments, remarkably low figures for $-Q_{Ac}$ for kidneys in presence of cyanide were found; such low figures were not subsequently encountered.

Action of glucose on acetoacetic acid breakdown.

A. *Under aerobic conditions.* Glucose, in a bicarbonate medium at p_H 7.4 and in presence of 95% O_2 +5% CO_2 , seems to have a small accelerating

Table IV. *Action of glucose on acetoacetate breakdown.*

Sodium acetoacetate = 0.0058 M .

Ringer solution, $NaHCO_3$ = 0.025 M .

$-Q_{Ac}$.					
95% O_2 + 5% CO_2					
Exp.	Tissue	Glucose		95% N_2 + 5% CO_2	
		No glucose added	present (0.009 M)	No glucose added	0.009 M glucose present
1	Guinea-pig kidney	3.1	4.0	—	—
2	"	3.0	3.8	—	—
3	"	2.9	3.4	—	—
4	"	1.9	2.5	—	—
5	"	—	—	2.0	2.1
6	Rat kidney	3.3	4.6	—	—
7	"	2.3	4.1	—	—
8	"	—	—	1.9	2.3
9	"	—	—	2.8	2.9
10	"	5.0	5.7	2.5	2.6

Table V. *Action of glucose on acetoacetate breakdown in presence of potassium cyanide.*

Sodium acetoacetate, 0.0058 M .

Ringer solution, $NaHCO_3$ = 0.025 M .

$-Q_{Ac}$.					
95% O_2 + 5% CO_2 , HCN = 0.004 M					
Exp.	Tissue	Glucose		95% N_2 + 5% CO_2 , HCN = 0.004 M	
		No glucose	0.009 M glucose	No glucose	0.009 M glucose
1	Guinea-pig kidney	1.9	1.9	—	—
2	"	1.8	2.4	—	—
3	"	1.0	2.3	—	—
4	"	0.6	1.5	—	—
5	"	2.2	2.3	—	—
6	"	—	—	1.7	2.3
7	Rat kidney	0.4	1.7	—	—
8	"	1.0	1.7	—	—
9	"	0.0	1.5	—	—
10	"	2.7	3.0	—	—
11	"	1.2	1.4	—	—
12	"	1.0	2.2	—	—
13	"	—	—	1.8	2.2
14	"	—	—	1.0	2.0
15	"	—	—	1.0	2.1
16	"	—	—	1.8	1.9

action on the rate of breakdown of acetoacetic acid in kidney. The results of a number of experiments are shown in Table I and some typical figures are given in Table IV (see also Part III). The effect of glucose addition is real but obviously variable, depending on the activity of the tissue in absence of added glucose.

B. *Under anaerobic conditions.* Glucose has but little effect on the $-Q_{Ac}$ found under anaerobic conditions (95 % N_2 + 5 % CO_2) with rat or guinea-pig kidney. Typical results are shown in Table IV.

C. *In presence of cyanide.* It will be seen from results given in Tables I and V that glucose accelerates acetoacetate breakdown in media containing potassium cyanide (0.004 M ; p_H 7.4), this acceleration obtaining whether conditions are aerobic or anaerobic. A close examination of the figures given in Table V shows however that the acceleration by glucose is only perceptible when the value of $-Q_{Ac}$, in absence of added glucose, is well below the normal anaerobic value (see Exps. 3, 4, 7, 8, 9, 11, 14, 15). Glucose addition seems to secure the value of $-Q_{Ac}$ at some maximum value; should the tissue alone exhibit this value, the addition of glucose has little or no further effect.

Action of pyruvic acid on acetoacetic acid breakdown.

Pyruvic acid, at a concentration of 0.009 M , behaves similarly to glucose. It has no appreciable effect, at this concentration, on $-Q_{Ac}$ aerobically, but in presence of cyanide it will raise any abnormally low value of $-Q_{Ac}$ to the normal anaerobic value of about 2.0. It seems to have no effect (anaerobically) on a tissue which gives this value in absence of added pyruvate.

The experiment was tried of adding a mixture of glucose and sodium pyruvate to the tissue. This, as is well known, secures conditions for maximum glycolysis under anaerobic conditions or in presence of cyanide. The addition of pyruvate (0.009 M) brings about relatively high values for $-Q_{Ac}$ in presence of cyanide (see Tables VI and VII), but the rates of breakdown of acetoacetic acid are not abnormally high and are of the same order of magnitude as those obtained under normal anaerobic conditions. This work, as a whole, indicates that the presence of a mixture of glucose and pyruvate helps to establish more definitely the

Table VI. *Action of pyruvic acid on acetoacetic breakdown.*

Sodium acetoacetate = 0.0058 M ; sodium pyruvate = 0.009 M .

Glucose = 0.009 M ; HCN = 0.004 M .

95 % O_2 + 5 % CO_2 . Ringer solution, $NaHCO_3$ = 0.025 M .

Exp.	Tissue	$-Q_{Ac}$.				
		Acetoacetate alone	Acetoacetate + glucose + pyruvate	Acetoacetate + HCN	Acetoacetate + HCN + pyruvate	Acetoacetate + HCN + glucose + pyruvate
1	Guinea-pig kidney	2.7	2.8	1.3	—	2.0
2	"	—	—	1.0	1.8	2.3
3	"	—	—	1.0	1.7	2.7
4	"	—	—	1.8	1.9	2.7
5	"	—	—	0.6	1.2	1.5
6	Rat kidney	5.1	5.2	1.3	—	2.5
7	"	4.3	5.2	1.3	—	1.9
8	"	—	—	1.1	1.5	—
9	"	—	—	0.0	2.0	—
10	"	—	—	0.0	1.7	1.9

Table VII. *Effects of change of concentration of sodium acetoacetate on $-Q_{Ac}$.*Rat kidney. Ringer solution, $\text{NaHCO}_3 = 0.025 M$. 95 % $\text{O}_2 + 5\%$ CO_2 .

Initial concentration of acetoacetate	Exp. 1 Kidney alone	Exp. 2 Kidney + glucose (0.009 M)	Exp. 3 Kidney + HCN (0.004 M)	Exp. 4 Kidney + glucose (0.009 M) + HCN (0.004 M)	Exp. 5 Kidney + pyruvate (0.009 M) + HCN (0.004 M)	Exp. 6 Kidney + pyruvate (0.009 M) + glucose (0.009 M) + HCN (0.004 M)
0.0023 M	3.4	3.7	1.1	1.7	—	1.8
0.0047 M	4.3	4.0	1.3	1.9	1.7	2.4
0.007 M	4.8	4.5	1.5	1.7	1.9	3.2

optimum conditions for the rate of breakdown of acetoacetic acid under anaerobic conditions. The effects of varying the initial concentration of acetoacetate are shown in Table VII.

β -Hydroxybutyric acid production by guinea-pig kidney.

It has already been shown (see Part II) that acetoacetic acid in the liver gives rise to β -hydroxybutyric acid. This transformation occurs also in the kidney. The results of a number of experiments carried out to determine the conditions effecting this transformation are shown in Table VIII. The rate of appearance of β -hydroxybutyric acid (expressed as ml. of gas at N.T.P. per hour per mg. dry tissue) is given by the symbol Q_{oxy} .

Table VIII. *Production of β -hydroxybutyric acid (Q_{oxy}) in presence of guinea-pig kidney from acetoacetic acid.*Ringer solution, $\text{NaHCO}_3 = 0.025 M$. 95 % $\text{O}_2 + 5\%$ CO_2 .

Glucose = 0.009 M; sodium pyruvate = 0.009 M; HCN = 0.004 M.

Exp.		$+Q_{oxy}$	$-Q_{Ac}$
1	Sodium acetoacetate (0.007 M)	0.84	—
	“ + glucose	0.85	—
2	“ + glucose + pyruvate	0.66	—
	“ + glucose + pyruvate	0.69	—
3	Sodium acetoacetate (0.0058 M)	0.99	—
	“ + HCN	1.34	—
4	“ + HCN	0.83	—
	“ + HCN	1.10	—
5	Sodium acetoacetate (0.012 M)	1.19	—
	“ + glucose + pyruvate	1.25	—
	“ + HCN	2.03	—
	“ + HCN + glucose + pyruvate	3.18	—
6	Sodium acetoacetate (0.007 M) + HCN	1.25	2.4
	“ + HCN + glucose + pyruvate	2.84	3.2
7	Sodium acetoacetate (0.007 M) + HCN	1.16	1.49
	“ + HCN + glucose + pyruvate	2.25	2.76
8	Sodium acetoacetate (0.007 M) + HCN	1.08	—
	“ + HCN + glucose	1.33	—
	“ + HCN + glucose + pyruvate	1.48	—

Details of the micro-estimation (gravimetric) of β -hydroxybutyric acid are given in Part II. It is necessary in estimating β -hydroxybutyric acid production from acetoacetic acid in presence of glucose, cyanide *etc.* to carry out control estimations in absence of tissue. All figures quoted in Table VIII have been corrected for the “blanks”.

The following results are of note:

1. The value of Q_{oxy} under normal aerobic conditions is unaffected by the presence of glucose or of a mixture of glucose (0.009 *M*) and pyruvate (0.009 *M*) (Exps. 1, 2, 5).

2. The value of Q_{oxy} is definitely increased in presence of cyanide (0.004 *M*) (Exps. 3, 4, 5).

3. The addition of glucose, or of glucose together with pyruvate, to kidney tissue in presence of cyanide increases the value of Q_{oxy} (Exps. 5, 6, 7, 8). The value of Q_{oxy} may be increased under these conditions by as much as 100%. Moreover, under these optimum conditions for the breakdown (in presence of cyanide) of acetoacetic acid, Q_{oxy} becomes comparable with $-Q_{Ac}$, i.e. the breakdown of acetoacetic acid, under these conditions, can be almost quantitatively accounted for by transformation into β -hydroxybutyric acid.

It is clear from these results that glucose (or glucose + pyruvate) affects greatly the transformation of acetoacetic acid into β -hydroxybutyric acid, this being readily observed in the presence of cyanide when the respiration of the tissue is practically entirely eliminated.

Under normal aerobic conditions production of β -hydroxybutyric acid cannot account for more than one-quarter to one-third of the acetoacetic acid broken down; presumably most of the remainder of the acetoacetic acid broken down is oxidised to CO_2 and H_2O .

Effects of glutathione and ascorbic acid on acetoacetic acid breakdown by kidney.

Results of the action of glutathione and ascorbic acid on acetoacetic acid breakdown are shown in Table IX.

Table IX. *Effects of glutathione and ascorbic acid on acetoacetate breakdown.*

Sodium acetoacetate = 0.0058 *M*.

Ringer solution, $NaHCO_3$ = 0.025 *M*. 5% CO_2 , 95% N_2 or 95% O_2 .

GSH (glutathione) = 3.3 mg./ml. (except in Exp. 1 where it is 2.3 mg./ml.).

Ascorbic acid = 3.3 mg./ml. (except in Exp. 8 where it is 2.5 mg./ml.).

Exp.	Tissue	$-Q_{Ac}$			Gaseous phase
		Acetoacetate alone	Acetoacetate + GSH	Acetoacetate + ascorbic acid	
1	Guinea-pig kidney	2.1	2.8	1.8	N_2/CO_2
2	"	4.2*	4.2*	—	O_2/CO_2
	"	2.5*	3.1*	—	N_2/CO_2
3	"	2.2*	3.2*	—	N_2/CO_2
4	"	1.7†	3.1†	2.2†	N_2/CO_2
5	Rat kidney	2.8	3.5	—	N_2/CO_2
	"	2.9*	3.5*	—	N_2/CO_2
6	"	2.0†	4.1†	—	N_2/CO_2
7	"	5.8	6.2	—	O_2/CO_2
8	"	2.7	4.3	2.8	N_2/CO_2
9	"	1.8	3.2	2.2	N_2/CO_2

* Glucose added at a concentration of 0.009 *M*.

† Glucose (0.009 *M*) and HCN (0.004 *M*) added.

Under normal aerobic conditions the addition of glutathione to acetoacetic acid in presence of kidney causes little or no change in its rate of breakdown (Exps. 2, 7).

Under anaerobic conditions however the presence of glutathione causes a pronounced acceleration of the rate of disappearance of acetoacetic acid, this

being the case whether glucose is added or not. The presence of cyanide (Exps. 4, 6) does not diminish this effect of glutathione. In Exp. 6 an acceleration of the order of 100 %, due to glutathione, is recorded.

There can be no doubt from these results that the presence of glutathione in the tissue must be a factor contributing to the anaerobic disappearance of acetoacetic acid.

A few experiments carried out with ascorbic acid show no definite effect of this substance on the rate of anaerobic change of acetoacetic acid in the kidney (Exps. 4, 8, 9).

Effects of added fatty acids on acetoacetic acid breakdown.

The addition of sodium salts of fatty acids to kidney reduces the apparent rate of breakdown of acetoacetate. Only acetate, propionate and butyrate have been investigated so far and their effects are specially noticeable with guinea-pig kidney. The results are noted in Table X.

Table X. *Effects of added fatty acids on acetoacetic acid breakdown.*

Exp.	Tissue	Sodium acetoacetate = 0.0058 <i>M</i> .	Q_{O_2}	Q_{Ac}
		Glycerophosphate buffer. O_2 .		
1	Guinea-pig kidney	Acetoacetate	17.9	-4.5
		„ + acetate 0.033 <i>M</i>	18.3	-2.0
		Bicarbonate-Ringer solution. 95 % O_2 + 5 % CO_2 .		
2	„	Acetoacetate	—	-3.9
		„ + acetate 0.016 <i>M</i>	—	-2.7
3	Rat kidney	Acetoacetate	—	-5.7
		„ + acetate 0.016 <i>M</i>	—	-4.6
		95 % N_2 + 5 % CO_2 .		
4	Guinea-pig kidney	Acetoacetate	—	-2.1
		„ + acetate 0.016 <i>M</i>	—	-1.6
		95 % O_2 + 5 % CO_2 .		
5	„	Acetoacetate + HCN (0.002 <i>M</i>)	—	-1.8
		„ + HCN + acetate 0.016 <i>M</i>	—	-2.1
6	„	Acetoacetate	—	-3.9
		„ + propionate 0.016 <i>M</i>	—	-1.6
7	„	Acetoacetate	—	-3.9
		„ + propionate 0.016 <i>M</i>	—	-2.4
8	Rat kidney	Acetoacetate	—	-4.8
		„ + propionate 0.033 <i>M</i>	—	-4.8
		„ + propionate 0.1 <i>M</i>	—	-4.3
		Glycerophosphate buffer. O_2 .		
9	Guinea-pig kidney	Acetoacetate	17.7	-4.3
		Propionate 0.016 <i>M</i>	14.0	0.0
		Acetoacetate + propionate 0.016 <i>M</i>	15.7	-1.6
		95 % N_2 + 5 % CO_2 .		
10	„	Acetoacetate	—	-2.0
		„ + propionate 0.016 <i>M</i>	—	-1.7
		Glycerophosphate buffer. O_2 .		
11	„	Acetoacetate	16.6	-4.3
		Butyrate 0.016 <i>M</i>	19.2	+1.2
		Acetoacetate + butyrate 0.016 <i>M</i>	16.0	-1.8
		95 % N_2 + 5 % CO_2 .		
12	Guinea-pig kidney	Acetoacetate	—	-2.0
		„ + butyrate 0.016 <i>M</i>	—	-1.5

The inhibitory effects of acetate and butyrate (Exps. 1, 2, 11) might be explained as due to the ketogenic effects of these acids; for it would be expected that the enzyme dealing with acetoacetic acid would be saturated when in presence of the concentration of acetoacetate used in these experiments and hence would not be able to deal effectively with any acetoacetic acid produced by acetic and butyric acids.

The inhibitory effects of propionate (Exps. 6, 7, 9) cannot however be explained in this way; the explanation for this inhibition must await further investigation.

It is of interest that the propionate inhibition which is so pronounced with guinea-pig kidney seems only to occur at relatively high concentrations with rat kidney (Exp. 8).

Under anaerobic conditions, or in presence of cyanide, the inhibitions of acetate, propionate and butyrate are diminished or eliminated (Exps. 4, 5, 10, 12).

Effect of malonic acid on acetoacetic acid breakdown.

It was shown in the preceding paper (Part III) that the presence of malonate increases the rates of spontaneous acetoacetic acid production by rat and guinea-pig livers. It will now be shown that malonate exercises a large inhibitory effect on the breakdown of acetoacetic acid in the presence of rat kidney.

Typical results are shown in Table XI. Four experiments demonstrating the action of 0.016 *M* malonate are shown in Series 1, and another four demonstrating the action of 0.008 *M* malonate in Series 2. The former concentration of

Table XI. *Effect of malonic acid on acetoacetic acid breakdown.*

Rat kidney. Acetoacetate = 0.0058 <i>M</i> . 95% O ₂ + 5% CO ₂ . Bicarbonate-Ringer solution, NaHCO ₃ = 0.025 <i>M</i> .		- Q_{Ac}			
		Exp. A	Exp. B	Exp. C	Exp. D
Series 1. Acetoacetate		4.68	5.67	5.08	5.13
„	+ sodium malonate 0.016 <i>M</i>	2.04	1.41	2.00	1.89
Series 2. Acetoacetate		4.82	5.66	5.80	5.45
„	+ sodium malonate 0.008 <i>M</i>	2.65	3.42	3.00	3.52
Exp. 1. 95% N ₂ + 5% CO ₂ . Acetoacetate		2.1			
„	+ sodium malonate 0.016 <i>M</i>	1.7			
Exp. 2. Rat liver. 95% O ₂ + 5% CO ₂ .					
Tissue alone					Q_{Ac} + 0.26
„	+ sodium malonate 0.04 <i>M</i>				+ 1.2
„	+ acetoacetate 0.0058 <i>M</i>				- 1.3
„	+ acetoacetate 0.0058 <i>M</i> + malonate 0.04 <i>M</i>				+ 0.8

malonate gives an average inhibition of 63%, the latter of 42%, of the rate of breakdown of acetoacetate with rat kidney. This effect occurs only aerobically, there being little inhibitory effect of malonate in presence of nitrogen (Exp. 1, Table XI). A similar inhibitory action of malonate occurs also with rat liver (Exp. 2), and doubtless this phenomenon explains the action of malonate in apparently increasing acetoacetic acid formation with liver slices (see Part III).

Some possible interpretations of the malonate inhibition of aerobic acetoacetic acid breakdown may now be considered.

The first obvious possibility is that malonate competes with acetoacetic acid for the enzyme involved, just as it has been shown [Quastel and Wooldridge, 1928] that malonate competes with succinate for succinic dehydrogenase. It is unlikely, however, that this view is wholly correct because the addition of sodium fumarate to the acetoacetate-malonate system partially neutralises the malonate effect (Exps. 1, 2, Table XII). Should it be held that the alleviating action of

Table XII.

Rat kidney. Acetoacetate = 0.0058 *M*. 95% O₂ + 5% CO₂: bicarbonate-Ringer solution.

Exp.		- <i>Q</i> _{Ac}
1	Acetoacetate	5.66
	„ + fumarate 0.016 <i>M</i>	5.94
	„ + malonate 0.008 <i>M</i>	3.42
	„ + fumarate 0.016 <i>M</i> + malonate 0.008 <i>M</i>	4.76
2	Acetoacetate	5.80
	„ + fumarate 0.033 <i>M</i>	5.92
	„ + malonate 0.008 <i>M</i>	3.00
	„ + fumarate 0.033 <i>M</i> + malonate 0.008 <i>M</i>	5.05
3	Acetoacetate	4.68
	„ + lactate 0.018 <i>M</i>	5.27
	„ + malonate 0.016 <i>M</i>	2.04
	„ + lactate 0.018 <i>M</i> + malonate 0.016 <i>M</i>	4.08
4	Acetoacetate	5.31
	„ + malonate 0.008 <i>M</i>	3.61
	„ + malonate 0.008 <i>M</i> + lactate 0.018 <i>M</i>	5.88
	„ + malonate 0.008 <i>M</i> + alanine 0.019 <i>M</i>	5.79
5	Acetoacetate	5.18
	„ + malonate 0.008 <i>M</i>	2.73
	„ + malonate 0.008 <i>M</i> + lactate 0.018 <i>M</i>	4.47
	„ + malonate 0.008 <i>M</i> + alanine 0.019 <i>M</i>	3.50
6	Acetoacetate	4.41
	„ + malonate 0.008 <i>M</i>	2.12
	„ + malonate 0.008 <i>M</i> + lactate 0.018 <i>M</i>	4.11
	„ + malonate 0.008 <i>M</i> + alanine 0.018 <i>M</i>	3.52
7	Acetoacetate	5.13
	„ + glucose 0.009 <i>M</i>	5.32
	„ + malonate 0.016 <i>M</i>	1.89
	„ + malonate 0.016 <i>M</i> + glucose 0.009 <i>M</i>	2.48
8	Acetoacetate	5.65
	„ + malonate 0.008 <i>M</i>	3.04
	„ + malonate 0.008 <i>M</i> + lactate 0.018 <i>M</i>	4.91
	„ + malonate 0.008 <i>M</i> + glucose 0.009 <i>M</i>	2.92

fumarate is due to its competition with malonate for the acetoacetic enzyme, then it follows that fumarate itself should inhibit acetoacetate breakdown. This does not occur; in fact, fumarate exercises a very slight accelerating action (Exps. 1, 2, Table XII).

The next possibility is that acetoacetate oxidation is accomplished by a succinic-fumaric-oxaloacetic system acting catalytically in the sense described by Szent-Györgyi and his colleagues [1935]. On this view acetoacetate would be oxidised by oxaloacetate, the latter molecule becoming reduced to fumarate and succinate. These substances would re-form oxaloacetate under normal aerobic conditions. In the presence of malonate, the oxidation of succinate is inhibited and therefore the succinic-oxaloacetic system is no longer reversible; such oxaloacetic or fumaric acid as is present in the tissue becomes ultimately reduced to succinic acid. In this manner malonate would be held to inhibit the oxidation of acetoacetate. Addition of fumarate neutralises the malonate inhibition simply by re-forming the fumaric-oxaloacetic system.

According to this theory, no substance should neutralise the malonate inhibition except one which gives rise to the succinic-fumaric-oxaloacetic catalytic system. Thus it might be expected that the addition of malic or aspartic acid (which gives rise to oxaloacetic acid on oxidation) would neutralise the malonate effect. But it would not be expected that substances such as lactic acid or alanine could accomplish such a neutralisation. This, however, is what in fact occurs. Exps. 3, 4, 5, 6, 8, Table XII show that both lactate and alanine in a Ringer medium diminish the malonate inhibition; neither of these substances shows any marked acceleration of acetoacetate breakdown in the absence of malonate.

Such results as these do not mean, of course, that the second possibility, just outlined, is incorrect; but they do mean that a separate explanation would have to be found for the partial neutralising effects of lactate or alanine. Our experiments on this point are so far not very numerous, but sufficient perhaps has been done to justify our putting forward the following suggestion.

Let us assume that the oxidation of acetoacetate in the tissue under aerobic conditions is a coupled one, the reaction depending on the oxidation of other substrates in the cell. The assumption may also be made, in order to give definition to this view, that the coupling is effected in the cell by hydrogen peroxide this substance being normally produced in the oxidation of cell substrates. We have then the following processes:

- (1) cell substrates + $O_2 \rightarrow H_2O_2$ + products of substrate oxidation,
- (2) acetoacetic acid + $H_2O_2 \rightarrow$ oxidation products of acetoacetic acid.

In presence of a given concentration of acetoacetate the velocity of process (2) will be determined by the amount of "active" oxygen supplied by the hydrogen peroxide after activation by its appropriate enzymes (peroxidase, catalase). It is not unreasonable to suppose that when the cell is working under optimum aerobic conditions the enzymes dealing with hydrogen peroxide are saturated and process (2) proceeds at a constant rate. The addition of further oxidisable substrates which might increase the available amount of H_2O_2 in the cell would not necessarily increase the rate of process (2). If however a substance is added to the cell which diminishes the velocity of process (1), it follows that the amount of H_2O_2 produced may fall below the saturation concentration of its activating enzyme and the velocity of process (2) will diminish. The addition to the cell, at this point, of substances restoring the velocity of process (1) should also have the effect of restoring the velocity of process (2).

This view seems to account for the facts obtained so far. Lactate, alanine and fumarate raise the respiration of kidney slices and therefore may, as possible H_2O_2 formers, neutralise, or partially neutralise, malonate inhibition. Glucose, which seems not to affect markedly the respiration of kidney, has but little neutralising effect on malonate inhibition at the concentration at which it has been tried (Exps. 7, 8, Table XII).

Effect of benzoic acid on acetoacetic acid breakdown.

Sodium benzoate is a much more vigorous inhibitor of acetoacetate breakdown in the guinea-pig kidney than in rat kidney. A few typical results are shown in Table XIII. It will be observed that with guinea-pig kidney, 0.005 *M* sodium benzoate brings about 40% inhibition of the rate of breakdown of acetoacetate (Exp. 4). Under anaerobic conditions this concentration of benzoate has but little effect.

It is of interest that the inhibitory action of benzoate on acetoacetic acid decomposition is considerably smaller than on the oxidation of the lower fatty acids by liver (see Part II).

Table XIII. *Effect of sodium benzoate on acetoacetic acid breakdown.*

Acetoacetate = 0.0058 <i>M</i> .		
Exp. 1. Rat kidney. Ringer solution, 95 % O ₂ + 5 % CO ₂ .		
Acetoacetate		4.4
„ + benzoate 0.02 <i>M</i>		4.1
„ + benzoate 0.01 <i>M</i>		4.1
Exp. 2. Rat kidney. Ringer solution, 95 % O ₂ + 5 % CO ₂ .		
Acetoacetate		4.8
„ + benzoate 0.02 <i>M</i>		3.8
„ + benzoate 0.01 <i>M</i>		4.4
„ + benzoate 0.005 <i>M</i>		4.7
Exp. 3. Guinea-pig kidney. Ringer solution, 95 % O ₂ + 5 % CO ₂ .		
Acetoacetate		4.8
„ + benzoate 0.02 <i>M</i>		1.6
„ + benzoate 0.01 <i>M</i>		2.5
Exp. 4. Guinea-pig kidney. Ringer solution, 95 % O ₂ + 5 % CO ₂ .		
Acetoacetate		4.3
„ + benzoate 0.005 <i>M</i>		2.6
Ringer's solution, 95 % N ₂ + 5 % CO ₂ .		
Acetoacetate		2.4
„ + benzoate 0.005 <i>M</i>		1.9

Effects of iodoacetic and arsenious acids on acetoacetic acid breakdown.

Both iodoacetate and arsenite greatly inhibit the decomposition of acetoacetic acid by kidney. Typical results are shown in Table XIV. Arsenite at a concentration of 2.5×10^{-4} *M* inhibits the breakdown of acetoacetic acid in guinea-pig kidney by over 70 %, and iodoacetate at a concentration of 3.3×10^{-4} *M* brings about an inhibition of the same order.

Table XIV. *Action of iodoacetic and arsenious acids on acetoacetic acid breakdown.*

Acetoacetate concentration = 0.0058 <i>M</i> . 95 % O ₂ + 5 % CO ₂ .				
Ringer solution, NaHCO ₃ = 0.025 <i>M</i> .				
Exp.	Tissue	- <i>Q</i> _{Ac}		Concentration of iodoacetic acid (Na salt)
		No iodoacetic acid added	Iodoacetic acid present	
1	Guinea-pig kidney	3.7	1.2	<i>M</i> /3000
2	„	4.6	0.0	„
3	„	3.4	1.2	„
4	„	4.3	1.1	„
5	Rat kidney	4.6	1.8	<i>M</i> /2250
6	„	5.0	2.6	„
7	„	5.0	2.6	„
Exp.	Tissue	- <i>Q</i> _{Ac}		Concentration of sodium arsenite
		No arsenite added	Arsenite present	
8	Guinea-pig kidney	- 4.1	- 0.7	<i>M</i> /1000
9	„	- 4.1	- 1.2	<i>M</i> /4000

Effects of minced kidney and of kidney extracts on acetoacetic acid breakdown.

It is of interest that the effect of mincing kidney (rat or guinea-pig) is to diminish but not to eliminate acetoacetate decomposition. The following results were obtained:

Acetoacetate concentration = 0.0058 *M*. Ringer solution, 95 % O₂ + 5 % CO₂.

		- <i>Q</i> _{Ac}
Rat kidney	Intact slices	4.7
	Minced tissue	2.9
Guinea-pig kidney	Intact slices	3.8
	Minced tissue	1.3

On making an extract of kidney, by grinding it with sand in four times its volume of *M*/50 phosphate buffer (*p*_H 7.4) and centrifuging, it was found that the opalescent centrifugate possessed some power of breaking down acetoacetic acid. The following results were obtained with rat kidney:

Exp.	ml.		O ₂ consumed in 2 hr. (37°) μl.	Acetoacetic acid found after 2 hr. μl.
1	2	Kidney extract	134.9	Nil
	2	Kidney extract + acetoacetate (377 μl.)	162	324
<i>i.e.</i> increase in O ₂ uptake = 27.1 μl. and acetoacetate decomposed = 53 μl.				
Exp.	ml.		O ₂ consumed μl.	Acetoacetic acid found μl.
2	2	Kidney extract	118	Nil
	2	Kidney extract + acetoacetate (379 μl.)	143	322
<i>i.e.</i> increase in O ₂ uptake = 25 μl. and acetoacetate decomposed = 57 μl.				

DISCUSSION.

It is obvious from the facts given in this paper that acetoacetic acid undergoes at least two changes in the kidney:

- (a) an anaerobic transformation;
- (b) an aerobic oxidation.

The two processes are quite distinct as is clear from the fact that inhibitors (*e.g.* malonate, propionate, benzoate, cyanide), which may greatly affect the aerobic change, have but little effect on the anaerobic change.

The anaerobic change consists principally of a transformation into β-hydroxybutyric acid; this is shown by the fact that kidney, in presence of cyanide and glucose, shows a production of β-hydroxybutyric acid which is of the same order as the rate of breakdown of acetoacetic acid. The anaerobic process of breakdown or the process in presence of cyanide is accelerated to some extent by glucose and to a larger extent by glutathione. The rates of aerobic breakdown of acetoacetic acid are not nearly so much affected either by glucose or by glutathione. This may be explained partly by the fact that β-hydroxybutyric acid is oxidised by kidney; a few experiments with guinea-pig kidney have indicated that the rate of breakdown of β-hydroxybutyric acid is of the same order as that of acetoacetic acid.¹ We may assume that normally in the kidney cell there exists an equilibrium between acetoacetic and β-hydroxybutyric acids. This equilibrium is determined on the one hand by the source of active hydrogen (glucose, pyruvate, glutathione) and on the other hand by the supply of active oxygen.

¹ The facts given in this paper do not invalidate the possibility that acetoacetic acid may only suffer oxidation after prior reduction to β-hydroxybutyric acid.

It is of particular interest that glutathione should markedly affect the rate of anaerobic transformation of acetoacetic acid. The fact that no stimulation occurs aerobically may be taken to mean that the effect of the glutathione is not due to an activation of the enzyme concerned with acetoacetic acid breakdown, and it is likely therefore that the anaerobic effect is due to a reduction of ketonic acid by the sulphhydryl compound. Should this be confirmed by further experiment, the phenomenon will provide an interesting example of the participation of glutathione in a ketonic acid-hydroxy-acid equilibrium.

Production of β -hydroxybutyric acid does not account for more than one-quarter to one-third of the acetoacetic acid broken down under aerobic conditions. The rest of the acetoacetic acid broken down is probably mostly oxidised to CO_2 and H_2O . This oxidation is, in the rat kidney, greatly inhibited by the addition of malonate and the malonate inhibition is neutralised, or partly neutralised, in a Ringer medium, by the further addition of substances burned by the kidney. Such substances are fumarate, lactate and alanine. Glucose, which does not increase kidney respiration appreciably, does not neutralise the malonate inhibition. It is suggested that an explanation of these facts lies in assuming that the oxidation of acetoacetate is a coupled one, the reaction depending on the oxidation of other cell substrates. Granting the truth of this assumption, which explains adequately the facts in this paper, there seems no reason to adopt the view that malonate inhibition of acetoacetate breakdown might be due to the inhibition of a succinic acid catalytic system. Such a view will not explain the neutralising effects of lactate and alanine.

That the malonate inhibition may be due to the inhibition of a process involving succinic acid formation and oxidation seems feasible, but that it should be taken to indicate the presence of a catalytic system controlling the entire respiration of the tissue is doubtful, if only because of the facts already cited. It has already been pointed out [Jowett and Quastel, 1935, 3] that malonate acts very differently on the respirations of guinea-pig liver and rat liver and a similar difference occurs with the kidneys. Malonate inhibition of acetoacetate breakdown is considerably less at the concentrations tried with guinea-pig kidney than with rat kidney.

SUMMARY.

1. Acetoacetic acid is broken down under both aerobic and anaerobic conditions by rat and guinea-pig kidneys (cortex slices).

2. The presence of cyanide reduces the rate of aerobic breakdown of acetoacetic acid to the anaerobic level.

3. Production of β -hydroxybutyric acid accounts for only one-third to one-quarter of the acetoacetic acid decomposed under aerobic conditions. The presence of cyanide increases β -hydroxybutyric acid production, and the rate of the latter in presence of cyanide is increased by addition of glucose, or of a mixture of glucose and sodium pyruvate.

4. The anaerobic transformation of acetoacetic acid is greatly increased by the addition of glutathione but not by ascorbic acid. The aerobic change is unaffected by glutathione.

5. Salts of acetic, propionic and butyric acids inhibit aerobic oxidation of acetoacetic acid (guinea-pig kidney). They have little effect on the anaerobic process.

6. Sodium malonate greatly inhibits aerobic oxidation of acetoacetic acid (rat kidney). This inhibition may be neutralised, or partially neutralised, when experiments are carried out in a Ringer medium by the addition of sodium

fumarate, sodium lactate or alanine. It is suggested that the oxidation of acetoacetic acid is a coupled one, depending upon the oxidation of other cell substrates.

7. Benzoic acid has a much greater inhibitory effect on acetoacetate breakdown in presence of guinea-pig kidney than in presence of rat kidney. Iodoacetic and arsenious acids are highly inhibitory.

8. Minced kidney and kidney extracts show an ability to break down acetoacetic acid.

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