CCLVII. STUDIES IN FAT METABOLISM.

III. THE FORMATION AND BREAKDOWN OF ACETOACETIC ACID IN ANIMAL TISSUES.

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THIS paper is concerned with measurements of the rates of acetoacetic acid formation from fatty acids in tissues other than liver. The oxidation of fatty acids in the liver has been dealt with in Parts I and II of this series [Jowett and Quastel, 1935, 1, 2]. The paper also describes the effect of sodium malonate in increasing acetoacetic acid formation in the liver—an effect which is largely due to its inhibitory action on acetoacetic acid breakdown—and the effects of atoxyl and quinine in depressing the “spontaneous” acetoacetic acid formation in this organ. Measurements have been made of the rate of destruction of acetoacetic acid by tissues.

Acetoacetic acid production in tissues other than liver.

There is much evidence that the liver is the most important organ for fat metabolism in the body. It has been suggested that other organs may also oxidise fat, but the evidence in favour of this view is apparently not very strong.

Formation of ketone bodies by other organs does not appear to have been shown by perfusion methods. Embden and Kalberlah [1906] found no formation of acetone or acetoacetic acid by dog muscle, lung or kidney. Snapper *et al.* [1926] found no ketone body formation on perfusing kidneys with butyric acid, and neither kidney nor lung oxidised β -hydroxybutyric acid to acetoacetic acid.

On the other hand Himwich *et al.* [1931] found that in intact phlorhizinised and depancreatized dogs the muscles and the organs drained by the portal vein sometimes liberated acetone bodies into the blood and sometimes removed them from the blood.

A number of experiments² has been carried out on the respiration and acetoacetic acid production by tissues other than liver. The experiments have been sufficient to show some new facts.

In the absence of added fatty acids, kidney and rat brain and spleen show no measurable production of acetoacetic acid (Table I), for values of Q_{Ac} less than 0.2 are within the experimental error. On the other hand rat testis gives a low but measurable production of acetoacetic acid.

In the presence of added fatty acids—butyric and hexanoic acids—all the tissues examined, except brain, produce small but definite quantities of acetoacetic acid, and the production of acetoacetic acid is usually accompanied by a definite increase in respiration. We have therefore grounds for believing that kidney, spleen and testis have a definite fat metabolism. No evidence of acetoacetic acid production under anaerobic conditions has been found.

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² Details of the experimental methods used in this paper are given by Jowett and Quastel [1935, 1, 2].

Table I. *Respiration and acetoacetic acid production by tissues in presence of fatty acids.*

Oxygen. Glycerophosphate buffer.

Exp.	Tissue		Substrate				
			Nil	Butyrate 0.01 M	Crotonate 0.01 M	β -Hydroxy- butyrate 0.01-0.02 M	Hexanoate 0.007 M
1	Guinea-pig kidney	Q_{O_2}	17.2	—	—	26.6	—
		Q_{Ac}	0.10	—	—	2.49	—
2	Guinea-pig kidney	Q_{O_2}	18.8	23.2	—	—	24.7
		Q_{Ac}	0.10	0.58	—	—	0.48
3	Rat kidney	Q_{O_2}	13.5	18.2	—	—	18.0
		Q_{Ac}	0.14	0.23	—	—	0.30
4	Rat kidney	Q_{O_2}	26.4	33.8	—	—	33.8
		Q_{Ac}	0.10	0.34	—	—	0.36
5	Rat spleen	Q_{O_2}	10.6	13.9	—	—	13.3
		Q_{Ac}	0.13	0.35	—	—	0.42
6	Rat spleen	Q_{Ac}	—	—	—	1.4	—
7	Rat testis	Q_{O_2}	8.36	8.46	8.29	9.67	—
		Q_{Ac}	0.42	0.85	0.74	2.22	—
8	Rat brain cortex	Q_{O_2}	—	—	—	7.5†	—
		Q_{Ac}	—	0.0*	—	2.16†	—

* In presence and absence of glucose.

† In absence of glucose. In presence of glucose the values $Q_{O_2}=12.2$, $Q_{Ac}=1.38$ were found.

All the tissues examined produce acetoacetic acid from *dl*- β -hydroxybutyric acid. This is further evidence, in addition to that given in Part I, that the mechanism of oxidation of β -hydroxybutyric acid is quite different from that of saturated fatty acids, for brain will oxidise β -hydroxybutyric acid but not butyric acid.

The effect of potassium and calcium ions on Q_{Ac} from β -hydroxybutyric acid.

In an experiment (see Table II) on the effect of varying the potassium and calcium ion concentrations on the rate of formation of acetoacetic acid from β -hydroxybutyric acid by guinea-pig kidney, we found a similar result to one

Table II. *dl*- β -Hydroxybutyrate 0.01 M.

Guinea-pig kidney	K^+	0	0.0005	0.002
	Ca^{++}	0	0.00025	0.001
	Q_{O_2}	16.0	19.1	21.9
	Q_{Ac}	4.07	3.16	3.18

formerly found with guinea-pig liver (Part I), namely that the rate is highest in absence of potassium and calcium. As will be shown later, potassium and calcium ions stimulate the removal of acetoacetic acid by guinea-pig kidney, and we consider therefore that the gross rate of production of acetoacetic acid in the present experiment is probably unaffected by the ionic environment, although the nett (observed) rate is affected.

It may be remarked here that kidney, testis and spleen all have the power of destroying acetoacetic acid, and the observed rates of production of acetoacetic acid from fatty acids are of course lowered for this reason below the rates that they would show if the destruction of acetoacetic acid were prevented.

Fat metabolism of tissues and respiratory quotients.

It is interesting to correlate the probable possession of fat metabolism by kidney, spleen and testis with the fact that all these tissues have respiratory quotients below unity [Dickens and Šimer, 1930; 1931]. The possession of an R.Q. below 1, or even below 0.8, cannot of itself inform us in the case of isolated tissues of the nature of the metabolism. The end-products of metabolism are largely known with the intact organism, and the R.Q. of the organism can therefore be interpreted to some extent. With individual organs, it is improbable that each organ is a self-contained unit which metabolises only carbohydrate, protein and fat, and produces the same end-products as does the whole organism. It is more likely that an extensive chemical symbiosis is to be found among the various tissues of an organism, and that metabolic processes begun in one tissue are frequently completed in another.

However, now that it has been shown that kidney, spleen and testis can oxidise fatty acids, the fact that these tissues have an R.Q. below unity in presence of glucose is probably an indication that these tissues do metabolise fatty substances. We may note on the other hand that brain, which, according to our findings (Table I) and those of Quastel and Wheatley [1933], probably does not oxidise fatty acids, has an R.Q. of unity.

The respiratory quotient of fat oxidation in the liver.

If tristearin is oxidised completely to carbon dioxide and water, the process has a respiratory quotient of 0.70. The higher fats generally give not dissimilar quotients for complete oxidation, and for this reason it is customary to assign to the oxidation of fat in the body an R.Q. of 0.7.

Fat is not completely oxidised in the liver, the process being completed elsewhere in the body. For we have found with rat liver slices a rate of production of acetoacetic acid, in the absence of added fatty acids, which indicates that the oxidation of fat is far from complete. Probably other products of incomplete oxidation are formed—acetone, β -hydroxybutyric acid and others—but we have no data concerning them.

We have therefore insufficient information to enable us to calculate the R.Q. of fat oxidation in the liver, but it appears that even calculations based on the available information are sufficient to show that the R.Q. of the process is probably much lower than 0.7.

Let us assume that tristearin is the fat burned, and attempt to account for the values $Q_{O_2} = 1.0$ and $Q_{Ac} = 1.1$ which we have found for rat liver.

(1) Let us first assume that the respiration is entirely due to oxidation of fat. Then we can account approximately for the acetoacetic acid production by the equation:

For then if $Q_{O_2} = 1.0$, $Q_{Ac} = 1.04$ and the R.Q. of the process is 0.57. If other products of incomplete combustion are formed as well, the R.Q. will be lower than 0.57.

(2) It is unlikely that fat is the only substrate burned in the liver of a normally fed animal. We will therefore make an alternative calculation on the basis that half the oxygen respired oxidises fat, and that the other half oxidises carbohydrate completely. This leads approximately to the equation:

Then for the fat oxidation $Q_{O_2}=5$, $Q_{Ac}=0.99$ and the R.Q. is 0.46. For the whole respiration of the liver we have then the values $Q_{O_2}=10$, and R.Q.=0.73.

Many such calculations of varying degrees of probability could be made. The two calculations given show however that, if the acetoacetic acid produced by rat liver arises from a fat of the type common in foodstuffs, then the R.Q. of the fat oxidation is almost certainly below 0.6, and may be much lower. How much lower it is depends on how far oxidation of other substances takes place, and on the quantity and nature of other products of incomplete combustion of fat.

Another probable result of these calculations is that, on the same assumptions, a higher fatty acid gives rise to more than one molecule of acetoacetic acid.

It may be noted, as an experimental point in connection with determination of the R.Q. and acid production with slices of rat liver, that the breakdown of acetoacetic acid in acid solutions has not been allowed for in former work, and that the existing values in the literature are therefore somewhat in error. Gemmill and Holmes [1935] have noted this point, but have not corrected for it.

The destruction of acetoacetic acid by tissues.

Some experiments have been carried out in which the rate of destruction of acetoacetic acid by tissue slices has been measured. In these experiments, which are to be regarded therefore as of a preliminary nature, the fate of the acetoacetic acid has not been determined. It has been found by perfusion methods [Snapper and Grünbaum, 1927, 1] that the liver reduces acetoacetic acid to β -hydroxybutyric acid, while the kidney [Snapper and Grünbaum, 1927, 2] effects the same reduction but mainly brings about a disappearance of ketone bodies. The nature of the action of other tissues has not been determined.

The acetoacetic acid has been determined in these experiments as usual by the manometric method (see Part I) after removing the tissue-slices at the end of the period of two hours in the bath at 37°. A "control" vessel containing sodium acetoacetate without tissue has always been similarly kept in the bath to obtain an initial value for the acetoacetic acid content. A correction for the amount of acetoacetic acid removed with the tissue slices has been applied on the basis that equal distribution occurs between tissue and medium. The correction may be faulty and may lead to an appreciable error when the rate of destruction is low.

The experiments show that guinea-pig kidney destroys acetoacetic acid at a considerable rate under the usual aerobic conditions.

A new finding is that the kidney retains some power to destroy acetoacetic acid when the conditions are anaerobic. The anaerobic rate of disappearance is some 30–40% of the aerobic rate. Under anaerobic conditions the presence of acetoacetate enhances the small acid production that is shown by guinea-pig kidney in absence of added glucose. Whether the effect is due to a stimulation of glycolysis or whether the destruction of acetoacetic acid results in appearance of a new acid group must be left for further investigation to decide.

Lactate and glucose increase to some extent the rate of disappearance of acetoacetic acid, both aerobically and anaerobically, but their effects are not very marked.

When the potassium and calcium ions normally present in the media are absent, the rate of destruction of acetoacetic acid is lessened. It will be remembered that it has already been suggested that this effect accounts for an apparent stimulation of acetoacetic acid production from β -hydroxybutyric acid by absence of potassium and calcium ions. These results are all noted in Table III.

Table III. *Guinea-pig kidney.*(A) *Destruction of acetoacetic acid.*In glycerophosphate buffer or Ringer's solution (5% CO₂, 0.025 *M* NaHCO₃).

Medium	Gas	Initial conc. acetoacetate <i>M</i>	<i>Q</i> _{Ac}			
Glycerophosphate buffer	O ₂	0.0033-0.0046	-3.83,	-3.19,	-3.40,	-2.92, -3.53, -4.05
Glycerophosphate buffer	N ₂	0.0033	-1.31,	-1.30		
Ringer's solution	O ₂	0.0029-0.0033	-3.39,	-2.65,	-2.33	
Ringer's solution	N ₂	0.0032-0.0033	-0.73,	-1.42,	-1.28,	-1.00

(B) *Effect of concentration of acetoacetate.*

Exp.	Glycerophosphate. Oxygen.			
1	Initial conc. acetoacetate (<i>M</i>)	0.0020	0.0035	0.0050
	<i>Q</i> _{Ac}	-1.32	-3.43	-3.92
2	Initial conc. acetoacetate (<i>M</i>)	0.0019	0.0037	0.0056
	<i>Q</i> _{Ac}	-2.89	-3.38	-3.68

(C) *Effect of lactate and glucose.*

Ringer's solution.

Exp.	Gas	Initial conc. acetoacetate (<i>M</i>)	<i>Q</i> _{Ac} in presence of		
			No addition	<i>dl</i> -Lactate 0.01 <i>M</i>	Glucose 0.2%
3	N ₂	0.0032	-1.28	-1.48	-1.52
4	O ₂	0.0031	-2.65	-3.17	-3.20
5	O ₂	0.0029	-2.33	-2.56	-2.95

(D) *Effect of acetoacetic acid on anaerobic acid production.*

Exp.	<i>Q</i> _{CO₂} ^{N₂} . Ringer's solution	
	In absence of acetoacetate	In presence of acetoacetate
6	0.05	0.81
7	0.15	0.87
8*	1.21	1.67

* Chromous chloride present in side-tube, to ensure complete anaerobiosis.

(E) *Effect of potassium and calcium ions on acetoacetic acid disappearance.*

Exp.				
9	K ⁺	0	0.0005	0.002
(Oxygen. Glycerophosphate buffer.)	Ca ⁺⁺	0	0.00025	0.001
Initial conc. of acetoacetate 0.0046 <i>M</i>	<i>Q</i> _{Ac}	-2.26	-2.93	-3.41

It has been found (see Table IV) that other tissues also bring about disappearance of acetoacetic acid. Of those examined rat kidney is the most active. Spleen is less so, and testis and liver are relatively inactive.

The power of tissues to remove acetoacetic acid lessens the nett formation of acetoacetic acid which results from oxidation of added fatty acids. In the case of kidney the effect will be great, but in the case of liver it is less. According to the work of Snapper and Grünbaum [1927, 1] liver does not destroy ketone bodies to any definite extent, and its main action on acetoacetic acid will be to

Table IV.

(A) *Destruction of acetoacetic acid.*

Glycerophosphate buffer. Oxygen.

Exp.	Tissue	Initial conc. (<i>M</i>) acetoacetate	<i>Q</i> _{Ac}
1	Guinea-pig liver	0.0038	-0.57
2	Guinea-pig liver	0.0037	-0.26
3	Rat testis	0	+0.56
	Rat testis	0.0048	-0.28
4	Rat spleen	0.0046	-2.25
5	Rat kidney	0	0.0
	Rat kidney	0.0046	-6.00

(B) *Effect of β -hydroxybutyrate.*

Guinea-pig liver.

Initial conc. acetoacetate (<i>M</i>)	0.0037	0.0037	0
Initial conc. β -hydroxybutyrate (<i>M</i>)	0	0.01	0.01
<i>Q</i> _{Ac}	-0.26	+0.50	+1.36

convert it into β -hydroxybutyric acid; the rate of this process is quite appreciable, particularly when fatty acids are present as sources of acetoacetic acid (see Part II).

When both acetoacetic acid and β -hydroxybutyric acid are present in the medium, they will be interconverted. The experiment given in Table IV, together with others already quoted in Part II, suggests that the rates of interconversion are of the same order of magnitude.

The effects of malonate on respiration and acetoacetic acid production.

None of the substances hitherto examined has shown any very definite accelerating effect on the production of acetoacetic acid by the liver.

Malonate, however (which has been used in all these experiments at a concentration of 0.04–0.05 *M*) increases the rate of acetoacetic acid production by

Table V. *Effects of malonate on respiration.*

Exp.	Liver	Fatty acid	No added fatty acid		Added fatty acid	
			(a)	(b)	(c)	(d)
			Malonate present		Malonate present	
Values of Q_{O_2} .						
1	Rat	Acetate 0.01 M	11.48	9.23	14.05	9.29
2	Rat	Acetate 0.02 M	10.64	7.77	13.31	7.94
3	Guinea-pig	Propionate 0.01 M	5.97	6.58	9.77	8.58
4	Rat	Butyrate 0.015 M	11.73	9.19	15.25	11.53
5	Rat	Butyrate 0.015 M	10.95	7.80	14.28	10.91
6	Guinea-pig	Butyrate 0.015 M	4.12	5.04	6.91	7.07
7	Guinea-pig	Butyrate 0.015 M	4.50	4.65	9.96	8.25
Values of Q_{Ac} .						
1	Rat	Acetate 0.01 M	1.10	2.65	2.25	3.37
2	Rat	Acetate 0.02 M	1.46	2.41	2.99	2.76
3	Guinea-pig	Propionate 0.01 M	0.07	0.38	0.09	0.19
4	Rat	Butyrate 0.015 M	1.18	2.48	4.52	5.07
5	Rat	Butyrate 0.015 M	1.13	2.01	4.60	4.84
6	Guinea-pig	Butyrate 0.015 M	0.12	0.47	1.69	2.46
7	Guinea-pig	Butyrate 0.015 M	0.14	0.74	3.93	3.78

rat and guinea-pig liver in the absence of added substrates. With guinea-pig liver Q_{Ac} rises from about 0.2 to 0.68 (mean of 8 experiments, average deviation ± 0.12 , range 0.38–0.82), and with rat liver Q_{Ac} rises from 1.1 to 2.38 (3 experiments, range 2.01–2.65). These experiments were made, as usual, under aerobic conditions and in presence of glycerophosphate.

The respiration of rat liver in presence of malonate undergoes a progressive lowering. Guinea-pig liver is oppositely affected, the respiration being maintained at its original level by malonate (Fig. 1). The reasons for these effects are still speculative.

It seemed possible that malonate might be increasing Q_{Ac} by undergoing decarboxylation to acetate, followed by formation of acetoacetate from acetate. No evidence that decarboxylation of malonate takes place was found on measuring the acid-base change undergone by rat liver in presence of malonate in Ringer's solution.

In absence of malonate the value +1.9 was found for Q_S , and in its presence the value +2.1. We therefore reject the idea that the extra acetoacetic acid produced in presence of malonate arises by chemical transformation of the malonate ion.

We prefer instead to suggest that malonate has an inhibitory action on the decomposition of acetoacetic acid by liver and in this way enhances the observed Q_{Ac} values.

Experiments which show that this suggestion is correct have been made and will be reported upon in a later communication. A considerable inhibition of acetoacetic acid breakdown by malonate in presence of rat liver has been found.

Evidence has been obtained that malonate has some inhibitory action on the oxidation of fatty acids. The evidence rests mainly on the effect of malonate on the increase in respiration which fatty acids bring about.

Reference to Table V shows that in the case of acetate this increase is practically abolished by malonate, and with propionate and butyrate the increase is diminished. In the case of propionate the evidence from the respiration receives confirmation from an experiment on the acid production by propionate in presence of guinea-pig liver: malonate lowers Q_S from -2.35 to -1.15 .

The data obtained concurrently on the acetoacetic acid production in presence of malonate are less easy to interpret quantitatively, the evidence of a decrease in Q_{Ac} being less definite, since it is uncertain whether additivity or competition is to be assumed between the Q_{Ac} in absence of fatty acid and the acetoacetic acid due to its presence.

Since malonate inhibits acetoacetic acid breakdown by liver there is no difficulty in understanding that a partial inhibition of fatty acid oxidation need not lead to a diminution of nett acetoacetic acid production.

These results provide evidence that malonate, which was known previously only to inhibit the oxidation of succinic acid [Quastel and Whetham, 1925; Quastel and Wheatley, 1931] has also an inhibitory action on the oxidation of

Fig. 1. The effect of malonate on respiration.

certain fatty acids and on the breakdown of acetoacetic acid. It is noteworthy that these substances all possess a terminal $-\text{CH}_2\text{COOH}$ group.

From the low yield of ketone bodies obtained with guinea-pig liver from butyric acid, it is probable that part of the butyric acid undergoes oxidation to products other than acetoacetic acid, and the possibility presented itself that part of the butyric acid is oxidised through succinic acid as an intermediary. Malonate might then serve to prevent further oxidation of the succinic acid. No evidence in favour of this view was found on measuring the acid-base change with butyrate and guinea-pig liver, for we found in absence of malonate that Q_S had the value -0.4 , and in presence of malonate -0.2 . If in the latter case a dibasic acid were formed, and in the former it were oxidised to carbon dioxide and water, the first value of Q_S would be definitely more negative than the second.

The effect of poisons on the acetoacetic acid production by rat liver.

It has already been mentioned (Part II) that in the absence of added substrates rat liver shows an appreciable production of acetoacetic acid, Q_{Ac} having a mean value of 1.1 , and it is possible therefore to study the effect of conditions on this "spontaneous" acetoacetic acid production.

The acetoacetic acid production, as already mentioned, is inhibited by benzoate, and it is also inhibited by arsenite. Both substances also inhibit the acetoacetic acid production from added fatty acids.

It occurred to us that, if the "spontaneous" acetoacetic acid production were due to oxidation of fatty acids liberated by hydrolysis of fats or phosphatides, it might be inhibited in a specific manner by substances which inhibit lipase action. We have therefore examined the effects of quinine and atoxyl.

In order to produce inhibitory effects on the acetoacetic acid production from liver slices it was necessary to use very much higher concentrations of these substances than are inhibitory to purified esterase preparations.

It was found (Table VII) that at a concentration of quinine which inhibited the spontaneous acetoacetic acid production, the acetoacetic acid production from added fatty acid was inhibited to about the same extent. On the other hand, with atoxyl (Table VI) the inhibition of acetoacetic acid production from added fatty acid was much less than that in the absence of added fatty acid. The action of atoxyl is therefore much more specific than that of quinine on "spontaneous" acetoacetic acid production.

The effects of quinine and atoxyl on the esterase activity of rat liver were then compared. According to Rona and Lasnitzki [1926], the esterase of tissue slices diffuses into the medium to some extent, and the esterase activity is also dependent on the thickness of the slices. We therefore tried to choose slices of uniform thickness, but the accuracy of these experiments is not great.

In each experiment the poison was present in the manometer vessel from the beginning, and after some time at 37° 0.15 ml. of tributyrin was added to the 3.2 ml. of Ringer's solution in which the tissue slices were being shaken. The tributyrin only dissolved in part, and approximately saturated the Ringer's solution. The rate of gas output was approximately constant for some time, and the rate during this period was used as a measure of the esterase activity.

In one experiment the tissue-slices were removed before adding tributyrin, and the esterase activity of the residual Ringer's solution was measured. (In Tables VIII and IX the esterase activity is stated in terms of the weight of tissue removed.) It was found that the activity in the Ringer's solution was diminished by atoxyl to the same extent as the activity in the presence of tissue slices. This simplifies the interpretation of the other experiments.

Table VI. *Inhibitory effects of atoxyl.*

Rat liver.					
A. On acetoacetic acid formation.					
Exp.	Oxygen. Glycerophosphate buffer.				
1	Conc. atoxyl (<i>M</i>)	0	0.002	0	0.002
	Conc. butyrate (<i>M</i>)	0	0	0.01	0.01
	Q_{O_2}	11.30	11.00	14.98	14.71
	Q_{Ac}	1.40	1.03	5.68	5.40
	% decrease in Q_{Ac}	—	27	—	5
2	Conc. atoxyl (<i>M</i>)	0	0.003	0	0.003
	Conc. butyrate (<i>M</i>)	0	0	0.01	0.01
	Q_{O_2}	10.88	10.20	13.71	13.03
	Q_{Ac}	1.59	1.07	5.36	4.63
	% decrease in Q_{Ac}	—	33	—	14
3	Conc. atoxyl (<i>M</i>)	0	0.012	0	0.012
	Conc. butyrate (<i>M</i>)	0	0	0.02	0.02
	Q_{O_2}	9.93	6.20	14.02	9.15
	Q_{Ac}	0.95	0.19	4.72	2.91
	% decrease in Q_{Ac}	—	80	—	38
4	Conc. atoxyl (<i>M</i>)	0	0.012	0	0.012
	Conc. decoate (<i>M</i>)	0	0	0.0025	0.0025
	Q_{O_2}	9.46	5.50	11.81	7.47
	Q_{Ac}	1.03	0.24	3.02	1.77
	% decrease in Q_{Ac}	—	76	—	41
B. On esterase action.					
Exp.	Ringer's solution $N_2 + 5\% CO_2$. 0.025 <i>M</i> $NaHCO_3$.				
1	Conc. atoxyl (<i>M</i>)	0 0.002		0 0.002	
		Tissue removed		Tissue present	
	Time when tissue removed (min.)	30			
	Time when tributyrin added (min.)	50		45	
	$Q_{CO_2}^{N_2}$	42	21	164	84
	% inhibition	50		52	
Tissue present. Tributyrin added after 20 min.					
2	Conc. atoxyl (<i>M</i>)	0	0.003	0.006	0.012
	$Q_{CO_2}^{N_2}$	127	47	52	33
	% inhibition	63		59	74

A comparison of the data in the tables shows that at concentrations of atoxyl and quinine which inhibit acetoacetic acid formation considerably, the esterase activity (measured under our rather arbitrary conditions) is also inhibited. The inhibition by quinine of esterase is not quite so great as that of the acetoacetic acid production, whilst in the case of atoxyl the inhibition of esterase activity is of the same order as that of the spontaneous acetoacetic acid production.

Taking the results altogether, we may conclude that the action of quinine is not connected with an effect on lipase activity.

Atoxyl, on the other hand, probably exerts its effect on the spontaneous acetoacetic acid production principally by depressing lipase activity. For here we have the facts (1) that the effect of atoxyl on "spontaneous" acetoacetic acid production is greater than on Q_{Ac} with added fatty acids, and the effect is probably therefore of another kind than with quinine, and (2) the effect on esterase action is of the same order as on spontaneous acetoacetic acid production.

Table VII. *Inhibitory effects of quinine.*

Rat liver.					
A. On acetoacetic acid formation.					
Oxygen. Glycerophosphate buffer.					
Exp.					
1	Conc. of quinine (<i>M</i>)	0	0.0004	0.0008	0.0016
(No added substrates)	Q_{O_2}	9.71	9.42	8.54	6.21
	Q_{Ac}	1.26	0.79	0.61	0.40
2	Conc. of quinine (<i>M</i>)	0	0.002	0	0.002
	Conc. of butyrate (<i>M</i>)	0	0	0.01	0.01
	Q_{O_2}	10.67	8.43	13.42	8.98
	Q_{Ac}	0.91	0.25	3.42	0.65
	% decrease in Q_{Ac}	—	73	—	81
B. On esterase action.					
Ringer's solution, $N_2 + 5\%$ CO_2 . 0.025 <i>M</i> $NaHCO_3$.					
Tissue-slices present. Tributyrin added after 25 min. in bath.					
	Conc. quinine (<i>M</i>)	0	0.002		
	$Q_{CO_2}^{N_2}$	125	87		
	% inhibition		30		

We are therefore led to the tentative conclusion that the "spontaneous" acetoacetic acid production is bound up with lipase activity. This in turn makes it probable that the origin of the acetoacetic acid is from fats or phosphatides, which must be hydrolysed before they are accessible to oxidation with production of acetoacetic acid.

As a corollary we should conclude that in the liver the concentration of free fatty acids must be low, since otherwise the liver slices could produce acetoacetic acid for some time even if the lipase were inactive. This conclusion receives some support from the finding of Weber and King [1935] that the salts of fatty acids at low concentrations inhibit liver esterase. Such an inhibitory action means that probably fatty acids cannot accumulate to any great extent.

We have made the assumption in the above arguments that liver esterase behaves in the same manner to inhibitors as does the lipase or phosphatidase responsible for the production of fatty acids in the liver. The arguments can therefore obviously only be suggestive, not conclusive.

SUMMARY.

1. Kidney, spleen and testis produce acetoacetic acid in small quantities from fatty acids, but brain gives no measurable production. All these tissues produce acetoacetic acid from *dl*- β -hydroxybutyric acid.

2. In the absence of potassium and calcium ions the rate of production of acetoacetic acid from β -hydroxybutyric acid by kidney is highest; this is due to the stimulating action of these ions on the breakdown of acetoacetic acid by kidney.

3. Kidney, spleen, testis and liver destroy acetoacetic acid in presence of oxygen, the last two organs being the least active.

4. Acetoacetic acid is also removed anaerobically by kidney.

5. Sodium malonate increases the rate of spontaneous acetoacetic acid formation by liver (*i.e.* the formation in absence of added fatty acids). Its effects on the respirations of rat and guinea-pig liver are noted. Malonate inhibits the oxidation by the liver of acetic, propionic and butyric acids and also the breakdown of acetoacetic acid.

6. Atoxyl inhibits in a specific manner the spontaneous acetoacetic acid formation by liver; quinine also does so but in a less specific way. The inhibition by atoxyl takes place at those concentrations which inhibit esterase activity of liver, and it is concluded tentatively that the spontaneous formation of acetoacetic acid is due to the oxidation of fatty acids liberated by hydrolysis from fats present in the tissue.

7. The interpretation of the R.Q. of isolated tissues is discussed. If the spontaneous acetoacetic acid formation of rat liver is due to oxidation of fat, it follows that the R.Q. of oxidation of fat by liver is less than 0.7.

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