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for 1 cc. *M*/45 phosphate *read* 1 cc. *M*/15 phosphate

CCXXXVI. OXIDATION OF FATTY ACIDS IN THE LIVER[1].

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SINCE the classical experiments of Embden, Knoop and Dakin over 25 years ago, little work has been carried out on the oxidation of fatty acids in the body. This has been due perhaps to the difficulties inherent in perfusion technique. The advent of the comparatively modern manometric methods of Barcroft and Warburg has made it possible to study fatty acid oxidation, and the various factors influencing it, in isolated organs in a strictly quantitative manner. We propose to describe in this paper the results of experiments carried out with these methods using thin tissue slices, usually not exceeding 20 mg. dry weight.

Methods.

Tissue slices about 0·4 mm. thick were prepared from fresh guinea-pig liver according to Warburg's methods. The slices were bathed in 0·9 % saline for a few minutes before immersion in the vessels of the manometer. Both the Warburg and Barcroft apparatus have been used. The dry weights of the tissue slices varied from 8 to 20 mg.

The medium in which the tissue slices were immersed consisted of 1·5 cc. Locke solution, 1 cc. $M/45$ phosphate buffer solution p_H 7·4 and 0·5 cc. of a solution of the fatty acid neutralised with sodium hydroxide (to p_H 7·4). In control experiments the solution of the fatty acid was replaced by 0·9 % saline.

Air was displaced by oxygen in all experimental vessels as soon as the tissue slices had been added to the media in the manometric vessels. CO_2 was absorbed by rolls of filter-paper, moistened with 6 % KOH, contained in the inner tubes of the vessels.

After attainment of temperature equilibrium readings of O_2 uptake were taken every 15 minutes for 3 hours. At the end of this period the slices were removed from the vessels, washed in distilled water and dried at 105°, after which they were weighed. The solutions in the vessels were investigated either qualitatively by colour tests or quantitatively by methods which will be described later.

In determining Q_{O_2} (*i.e.* mm.^{3} of O_2 absorbed per mg. dry weight tissue per hour) the reading over the first 15 minutes was usually neglected, and the average oxygen uptake was calculated over the remaining period of the experiment.

In all experiments described in this paper guinea-pig liver was used. The animals were mostly young (about 11 oz. weight) and well fed (bran, oats and greens). The animal was well bled before the liver was removed. Tissue slices were taken usually within 15 minutes of the death of the animal.

[1] The substance of this note was read before the Third International Congress for Cytology in August, 1932.

Oxidation of fatty acids etc. *by the liver.*

Representative values of the respiration of guinea-pig liver and of the development of acetone or acetoacetic acid (as indicated by the nitroprusside reaction), in the presence of the first eight members of the fatty acid series, are shown in Table I. The 2nd and 3rd columns of this Table give the Q_{O_2} for an

Table I. *Oxidation of fatty acids* etc. *by guinea-pig liver. Initial* $p_H = 7{\cdot}4$.

Substrate	Q_{O_2} 0·017 *M*	Nitroprusside reaction	Q_{O_2} 0·067 *M*	Nitroprusside reaction
None	4·5	Nil	.	.
Formic acid	4·2	Nil	.	.
Acetic acid	7·4	+ + +	5·5	+ +
Propionic acid	10·0	Nil	7·0	Nil
Butyric acid	12·0	+ + + +	10·0	+ + + +
n-Valeric acid	12·8	Trace	12·2	Trace
n-Hexoic acid	13·6	+ + + +	5·0	+ +
n-Heptoic acid	13·8	+	2·8	Nil
n-Octoic acid	13·0	+ + + +	2·8	Nil
Crotonic acid	8·2	+ + + +	8·2	+ + + +
*Iso*crotonic acid	.	.	7·6	+ + + +
Lactic acid	6·5	?	.	.
Pyruvic acid	5·0	+	.	.
Glucose	4·3	Nil	.	.
Glycerol	5·2	Nil	.	.

initial concentration of 0·017 *M*, and comparative estimates of the intensities of the nitroprusside reaction yielded by the substrates at this concentration; the 4th and 5th columns give the Q_{O_2} for an initial concentration of substrate of 0·067 *M* and comparative estimates of the nitroprusside reactions yielded at this concentration.

The normal Q_{O_2} of guinea-pig liver (*i.e.* in the absence of any added substrate) is given as 4·5. This is an average of 25 observations, the limits of variation being 2·7 and 6·0. The remaining figures quoted in Table I are representative of the results of at least three experiments.

The following facts are of note.

(1) All fatty acids with the exception of formic acid increase markedly the respiration of liver. Acetic acid has the least effect.

(2) There exists an optimum concentration for each fatty acid, above which any increase in concentration leads to a fall in Q_{O_2} and also to the development of acetone bodies, as indicated by the nitroprusside reaction. This is particularly noticeable with higher members of the series. Table II shows the variation of Q_{O_2} with concentration of butyric acid.

Table II. *Oxidation of butyric acid by guinea-pig liver. Variation of* Q_{O_2} *with concentration of fatty acid.*

	Q_{O_2}
Liver alone	4·5
+ butyric acid 0·0017 *M*	6·6
+ „ 0·0035 *M*	11·0
+ „ 0·0170 *M*	12·0
+ „ 0·0670 *M*	10·0

The rate of O_2 uptake by liver in the absence of added substrates becomes constant after about the first 30 minutes.

Similarly in the presence of fatty acids at the initial concentration of 0·017 *M* the rate of O_2 uptake is linear. With fatty acids at higher concentrations there is a considerable departure from linearity, this becoming particularly observable with *n*-hexoic and higher fatty acids. In Table III are recorded the rates of

Table III. *Rate of O_2 uptake by guinea-pig liver in presence of fatty acids 0·067 M. Initial $p_H = 7·4$.*

Fatty acid	Q_{O_2} after 30 mins.	Q_{O_2} after 60 mins.	Q_{O_2} after 90 mins.	Q_{O_2} after 120 mins.
Control (no fatty acid added)	6·6	4·7	4·7	4·7
n-Valeric	13·7	13·0	12·2	12·2
n-Hexoic	9·8	6·3	4·4	3·8
n-Heptoic	7·0	3·5	2·5	1·5

respiration of guinea-pig liver, alone and in the presence of *n*-valeric, *n*-hexoic and *n*-heptoic acids, at particular periods during the experiment. Thus with valeric acid the rate of respiration (Q_{O_2}) after 60 minutes was 13·0 and had only fallen to 12·2 after 120 minutes. With *n*-hexoic acid, however, the rate of respiration was 6·3 after 60 minutes and had fallen to 3·8 after 120 minutes.

The existence of an optimum concentration for each fatty acid for maximum respiration, and the departure from the linear rate of respiration of liver in the presence of relatively high concentrations of the higher fatty acids, are probably to be attributed to increased surface adsorption of the fatty acid leading to an interference with the access of oxygen or of an oxygen carrier.

(3) All the fatty acids with an even number of carbon atoms, including acetic acid, give rise to comparatively large quantities of acetone (or acetoacetic acid). Those fatty acids with an odd number of carbon atoms give rise to little or no acetone (or acetoacetic acid). This result is in complete accord with the results of early perfusion experiments.

(4) Both the unsaturated acids, crotonic and *iso*crotonic acids, are vigorously oxidised with the production of acetone (or acetoacetic acid).

(5) Glucose appears not to be oxidised by liver and not to give any increase in respiration [see also Dickens and Greville, 1933]. Lactic acid definitely increases the Q_{O_2} [see Dickens and Šimer, 1930]—so do pyruvic acid and glycerol, but none of these substances leads to so great an increase in respiration as the fatty acids at equivalent concentrations. Of all substances so far investigated the fatty acids appear to be the most vigorously attacked.

Oxidation of butyric acid by the liver.

The question arose as to how far butyric acid suffered oxidation in the liver. This problem was investigated in the following ways.

(*a*) Determinations were made of the increased CO_2 output by the liver in presence of butyric acid.

(*b*) Estimates were made of the yield of acetoacetic acid and acetone produced by butyric acid in liver.

1. *CO_2 output by liver in presence of butyric acid.* O_2 uptakes of guinea-pig liver alone and in the presence of butyric acid 0·017 *M* were determined in a bicarbonate-phosphate-Locke medium of the following composition: 1·5 cc. Locke solution, 0·25 cc. phosphate buffer *M*/15 p_H 7·4, 0·47 cc. sodium bicarbonate solution 0·16 *M*, 0·28 cc. saline. To this was added sodium butyrate solution to make a total volume of 3 cc. Air was displaced by an $O_2 + 5$ % CO_2 mixture and determinations of changes of volume were obtained using a Barcroft

differential manometer. No KOH was placed in the inner tubes of the manometer vessels.

The changes of volume obtained were the differences between O_2 absorbed and CO_2 produced and could be compared with experiments carried out under similar conditions but where provision was made to absorb all CO_2 produced. In such experiments the bicarbonate was replaced by Locke solution, the O_2-CO_2 mixture by O_2, and KOH was placed in the inner tubes of the vessels.

In a number of such experiments it was shown that little or no extra CO_2 was produced by the combustion of butyric acid in liver. A typical result is shown in Table IV, the increase in Q_{O_2} due to butyric acid being 5·7 and the

Table IV.

	Q_{O_2}	Q_{CO_2}
Guinea-pig liver	4·2	3·7
,, + butyric acid 0·017 *M*	9·9	4·0
Increase	5·7	0·3

	Exp.	Q_{O_2}	$Q_{\text{acetone bodies}}$ (iodoform method)	$Q_{\text{acetoacetic acid}}$ (aniline method)
Guinea-pig liver + butyric acid 0·017 *M*	1	9·4	2·8	.
	2	9·9	.	2·5
	3	13·3	4·2	.
	4	13·3	.	4·2

Exp.	Increase in Q_{O_2} due to butyric acid	$Q_{\text{acetoacetic acid}}$
1	4·4	2·5
2	8·7	4·2

increase in Q_{CO_2} (mm.^{3} CO_2 produced per hour per mg. dry weight of tissue) only 0·3.

There is thus reason to believe that although a vigorous oxidation of butyric acid occurs in the liver this does not result in an extensive breakdown to CO_2. Presumably, therefore, if acetoacetic acid is a product of oxidation of butyric acid in the liver, this does not break down markedly in this organ to acetone and CO_2. This conclusion can be put to the test by direct analysis.

2. *Estimations of acetone and acetoacetic acid.* Two methods have been employed.

(*a*) *Iodoform method.* In this method 2·5 cc. of the solution containing the acetone bodies were placed in a large boiling-tube contained in a water-bath and 1 cc. *N* H_2SO_4 was added. Air was led through the solution into a large tube containing 5 cc. 5*N* NaOH and 5 cc. *N*/20 iodine solution. The water in the bath was brought to the boil, air being drawn slowly through the solution containing the acetone bodies into the alkaline iodine solution. In usually 30–45 minutes all the acetone (together with that contained in the acetoacetic acid) had been taken over into the iodine solution, less than 0·5 cc. liquid remaining in the tube in the water-bath. The alkaline iodine solution, containing an iodoform precipitate, was carefully neutralised (at 0°) and the liberated iodine titrated with *N*/20 sodium thiosulphate solution. This method was found to be accurate only for quantities of acetone corresponding to 1 cc. or less of *N*/20 iodine solution. It does not of course differentiate between any volatile compounds which can combine with iodine in alkaline solution.

(*b*) *Aniline hydrochloride method.* This method is identical in principle with that recently used by Krebs [1933] and by Postern [1933] in the estimation of oxaloacetic acid. It differs somewhat in detail from that of these workers. The method depends upon the fact that one molecule of acetoacetic acid is broken down in presence of aniline to yield one molecule of CO_2 which can be estimated manometrically. We have adopted the following procedure.

2·5 cc. of the solution containing acetoacetic acid are placed in the left-hand vessel of a Barcroft manometer and to this is added 1 cc. $M/5$ acetate buffer p_H 3·8. A small cup, containing 0·1 g. aniline hydrochloride, is hung by means of a platinum hook on the inner tube of the Barcroft vessel. In the right-hand vessel are placed 2·5 cc. saline together with 1 cc. $M/5$ acetate buffer p_H 3·8. The manometer is placed in a bath at 37° and allowed to shake at the usual rate for 10 minutes after which the taps are closed and the manometer again allowed to shake. Usually no change in the manometer levels occurs within 10–15 minutes, showing little or no spontaneous breakdown of acetoacetic acid under these conditions. The cup containing the aniline hydrochloride is displaced by a sharp shake of the manometer and in a few minutes there is rapid change in the manometer levels as the aniline reacts with the acetoacetic acid. Readings are taken every 5 minutes. The reaction appears to be complete in 30 minutes but we usually take readings for a further 30 minutes. We have not dealt with quantities of acetoacetic acid appreciably greater than that corresponding to an evolution of 250 mm.^{3} CO_2.

This method, which is quick and gives reproducible results, estimates acetoacetic acid and not acetone or acetaldehyde. Were oxaloacetic acid present in small quantity it would be, in all probability, fully decomposed under these experimental conditions before the aniline hydrochloride was added [see Postern, 1933].

The acetone bodies, estimated either by the iodoform method or by the aniline hydrochloride method, are calculated in terms of mm.^{3} produced per mg. dry weight of tissue per hour, and are thus given in units directly comparable with the Q_{O_2}. Representative results are shown in Table IV. Taking two experiments in which respirations of guinea-pig liver in presence of sodium butyrate 0·017 M were identical, $Q_{\text{acetone bodies}}$ (iodoform method) proved to be identical with $Q_{\text{acetoacetic acid}}$ (aniline method). This indicates that no appreciable quantities of acetone or acetaldehyde are formed in the combustion of butyric acid by liver; otherwise the $Q_{\text{acetone bodies}}$ would be greater than $Q_{\text{acetoacetic acid}}$.

If butyric acid is burned completely to acetoacetic acid it would be expected that the $Q_{\text{acetoacetic acid}}$ would be equal to the increase in Q_{O_2} due to the butyric acid. This does not appear to be the case. Two typical results are shown in Table IV where it will be seen that the $Q_{\text{acetoacetic acid}}$ is about half the value of the increase in Q_{O_2}.

It was conceivable that some of the butyric acid was oxidised to succinic acid, which would then be rapidly oxidised by liver to *l*-malic acid. The latter is only very slowly oxidised by liver and it would be anticipated that *l*-malic acid would accumulate and be observable polarimetrically if succinic acid were formed. Polarimetric examinations, however, gave negative results, so that it must be concluded that oxidation of butyric acid to succinic and *l*-malic acids does not take place to any marked extent in the liver.

It is more likely that the extra oxygen taken up, and not accounted for by the acetoacetic acid, is to be found in β-hydroxybutyric acid, which we have not yet estimated. It has been shown by Snapper and Grünbaum [1927, 1, 2] in perfusion experiments that liver has a marked reducing action on acetoacetic acid. These writers have also shown, by perfusion experiments, that the power of surviving liver to break down acetoacetic acid is small, a conclusion with which the results given above are in agreement.

The action of propionic acid on the oxidation of butyric acid in the liver.

It was shown, in Table I, that propionic acid is oxidised by guinea-pig liver but that it does not give rise to acetoacetic acid.

If propionic acid be added to butyric acid, the formation of acetoacetic acid by the latter in presence of liver is markedly reduced, though there is little change in the uptake of oxygen. Typical results are shown in Table V. The

Table V. *Effect of propionic acid on oxidation of butyric acid by liver.*

			Q_{O_2}	$Q_{\text{acetoacetic acid}}$
Liver + butyric acid	0·017 *M*		13·3	4·2
,,	+ propionic acid	0·0017 *M*	14·3	3·6
,,	+ ,,	0·01 *M*	15·6	1·8
,,	+ ,,	0·017 *M*	14·7	0·7

addition of propionic acid to butyric acid at equivalent concentrations (0·017 *M*) lowers the $Q_{\text{acetoacetic acid}}$ by 84 %. It is very likely that the effect of propionic acid is due to simple competition with butyric acid for the active surfaces involved in fatty acid oxidation. Another explanation, however, may be that propionic acid is converted in the liver into lactic acid or glucose which, according to clinical observation, exert antiketogenic effects.

This conception was put to the test by examining the effects of glucose and lactic acid on the acetoacetic acid production from butyric acid.

Effects of glucose and lactic acid on the oxidation of butyric acid in the liver.

According to current conceptions the production of acetoacetic acid represents an incomplete oxidation of butyric acid; in presence of glucose the oxidation of the fatty acid should be increased and the production of acetoacetic acid decreased. Experiment (see Table VI) shows no effect, within experimental

Table VI. *Effects of antiketogenic substances on oxidation of butyric acid by liver.*

			Q_{O_2}	$Q_{\text{acetoacetic acid}}$
Glucose:				
Liver + butyric acid	0·017 *M*		9·9	2·5
,,		+ glucose 0·017 *M*	9·9	2·8
Liver + butyric acid	0·0017 *M*		6·6	+ + + +
,,		+ glucose 0·017 *M*	6·9	+ + + +
Liver + crotonic acid	0·017 *M*		7·1	+ + + +
,,		+ glucose 0·017 *M*	6·7	+ + + +
Lactic acid:				
Liver + butyric acid	0·017 *M*		10·0	1·9
,,		+ lactic 0·017 *M*	10·8	1·7

error, of glucose or of lactic acid at the concentrations used, on Q_{O_2} of liver in the presence of butyric acid or of crotonic acid, or on the acetoacetic acid production from these substances.

The effect of glucose, therefore, as observed clinically, cannot be due to this substance *per se* in the liver. There is certainly no evidence under these physiological conditions that glucose combines with or removes acetoacetic acid; compare also the results of Wigglesworth [1924], who arrived at a similar conclusion.

Effect of glycogen on oxidation of butyric acid in the liver.

On testing glycogen[1], however, a different picture presents itself. Glycogen (see Table VII) brings about a marked decrease in the production of acetoacetic

Table VII. *Effect of glycogen on oxidations of liver.*

	Q_{O_2}	$Q_{\text{acetoacetic acid}}$
Liver + butyric acid 0·017 *M*	9·9	2·5
,, + glycogen (0·5 %)	7·0	1·0
Liver + butyric acid 0·017 *M*	9·9	2·8
,, + glycogen (0·27 %)	7·8	1·8
Liver + crotonic acid 0·017 *M*	9·5	3·1
,, + glycogen (0·25 %)	7·8	1·8
Liver + *n*-hexoic acid 0·017 *M*	12·4	3·0
,, + glycogen (0·5 %)	7·8	1·6

acid, but, contrary to current ideas, no increased combustion of the fatty acid. There is in fact a definite decrease in the Q_{O_2} in the presence of glycogen. This occurs with crotonic acid as well as with butyric acid. The results would be consistent with the hypothesis that glycogen induces a "sparing" of fat oxidation, in a sense similar to protein sparing, but further experiment will be necessary to establish this. The investigation so far does not support the conception that the presence of carbohydrate stimulates or completes fatty acid oxidation in the liver.

Effects of increased phosphate concentration on the oxidation of butyric acid in the liver.

As stated earlier the oxidation of butyric acid is carried out in a Locke-phosphate buffer solution, the phosphate buffer being *M*/45 (p_H 7·4). On increasing the latter concentration to *M*/15 (p_H 7·4), a marked fall occurs in Q_{O_2} and acetoacetic acid production. Typical results are shown in Table VIII. This

Table VIII. *Effect of increase in phosphate concentration on butyric acid oxidation.*

	Phosphate concentration	Q_{O_2}	$Q_{\text{acetoacetic acid}}$
Liver alone	*M*/45	4·8	0·20
,, + butyric acid 0·017 *M*	,,	10·0	2·00
Liver alone	*M*/15	2·0	0·10
,, + butyric acid 0·017 *M*	,,	4·3	0·73

result is not due to any marked change in p_H. It seemed possible that the effect of the relatively high concentration of phosphate was due to removal of calcium ions from the medium and to an alteration of the ionic balance as a whole. To test this, phosphate buffer was replaced by sodium glycerophosphate[2] solution brought to p_H 7·4. Such a solution acts as a very good buffer at this p_H. It was found that both *M*/45 and *M*/15 glycerophosphate Locke media resulted in the production of the normal figures for Q_{O_2} and $Q_{\text{acetoacetic acid}}$ (see Table IX).

[1] The glycogen used was a commercial (B.D.H.) sample.

[2] A commercial (B.D.H.) sample.

Table IX.

	Conc. of glycerophosphate	Q_{O_2}	$Q_{\text{acetoacetic acid}}$
Liver alone	$M/45$	4·8	0·10
„ + butyric acid (0·017 M)	„	10·0	3·61
„ alone	$M/15$	5·4	0·10
„ + butyric acid (0·017 M)	„	12·3	4·31

Increase of glycerophosphate concentration did not result in a fall of Q_{O_2} or of $Q_{\text{acetoacetic acid}}$; there was evidence rather of a small increase in these values.

Using $M/15$ sodium glycerophosphate (p_H 7·4) as buffer, replacement of Locke solution by saline resulted in a decided fall in Q_{O_2} and acetoacetic acid production (Table X).

Table X.

		Q_{O_2}	Nitroprusside reaction	Final p_H
Liver alone	Locke sol. present	6·0	Trace	7·4
„ + butyric acid 0·017 M	„	12·0	+ + + +	7·4
„ alone	Saline present	4·7	Trace	7·4
„ + butyric acid 0·017 M	„	7·1	+	7·4

These results indicate the importance either of calcium ions, or of a proper balance of ions involving calcium, for the effective oxidation of butyric acid in the liver. The inhibitory effects of increased concentrations of phosphate ions may be due to removal of free calcium ions, though it is also possible that phosphates themselves may play a definite rôle in the mechanisms which regulate fatty acid oxidation.

Comparison of liver, brain and kidney.

The effects of liver, brain and kidney on butyric and crotonic acid oxidation were compared using the tissue slice method. Typical results are shown in Table XI.

Table XI.

		Q_{O_2}	Nitroprusside reaction
Minced liver		2·1	Nil
„	+ butyric acid 0·017 M	2·7	Nil
„	+ crotonic acid 0·017 M	2·6	Nil
Intact liver slices		4·5	Nil
„	+ butyric acid 0·017 M	12·0	+ + + +
„	+ crotonic acid 0·017 M	8·2	+ + + +
Brain (slices)		3·1	Nil
„	+ butyric acid 0·017 M	3·0	Nil
„	+ crotonic acid 0·017 M	2·0	Nil
Kidney (slices)		12·2	Nil
„	+ butyric acid 0·017 M	21·3	Trace
„	+ crotonic acid 0·017 M	20·3	Trace

Brain cannot oxidise butyric or crotonic acid; kidney oxidises both these substances but produces little or no acetone or acetoacetic acid (a result in confirmation of the work of Snapper and Grünbaum [1927, 2]). It is easy to show that in contrast to the liver, kidney rapidly breaks down acetoacetic acid but the mechanism of this decomposition has still to be discovered.

Effects of minced liver.

Minced liver has practically no power to oxidise butyric or crotonic acid (Table XI). It was "minced" by squeezing the liver through a sieve of 1 mm. mesh. No obvious damage to the individual cells had occurred. Yet this rupture of the cell organisation as a whole had destroyed the ability of the organ to accomplish one of its most active metabolic processes. In this sense liver offers a distinct contrast to brain. This organ (again in contrast to the liver) oxidises glucose freely, and minced or chopped brain is also very effective [Quastel and Wheatley, 1932]. It is apparent that with fatty acid oxidation in the liver a number of regulating factors are concerned, one at least of which (? a co-enzyme) depends for its stability or effectiveness on the integrity of the cell organisation as a whole.

SUMMARY.

1. A study has been made of the oxidation of fatty acids by guinea-pig liver using the Warburg tissue slice method. The following results have been obtained.

(*a*) All fatty acids excluding formic acid increase markedly the respiration of liver; acetic acid has the least effect.

(*b*) An optimum concentration for each fatty acid exists, above which any increase in concentration leads to a fall in Q_{O_2} and the production of acetone bodies. The rate of O_2 uptake is constant except with relatively high concentrations of the higher fatty acids, when the rate of O_2 uptake falls off rapidly.

(*c*) In agreement with the results of perfusion experiments, all fatty acids with an even number of C atoms (including acetic acid) give rise to acetone (acetoacetic acid) production. Those fatty acids with an odd number of C atoms produce little or no acetone bodies.

(*d*) The unsaturated acids, crotonic and *iso*crotonic acids, are vigorously oxidised to give rise to acetoacetic acid.

(*e*) The fatty acids are more vigorously attacked, for equivalent concentrations, than lactic acid, pyruvic acid or glycerol.

2. A manometric method of estimating acetoacetic acid is described.

3. Acetoacetic acid is not broken down appreciably to acetone and CO_2 in the liver; it is apparently the final oxidation product of butyric acid in this organ.

4. The addition of propionic acid to butyric acid in presence of liver lowers $Q_{\text{acetoacetic acid}}$ (mm.^{3} acetoacetic acid produced per hour per mg. dry weight of tissue) without affecting markedly the Q_{O_2} (mm.^{3} O_2 absorbed per hour per mg. dry weight of tissue).

5. Neither glucose nor lactic acid affects the Q_{O_2} or $Q_{\text{acetoacetic acid}}$ observed for butyric acid in presence of liver. There is no evidence that glucose combines with or removes acetoacetic acid under the conditions of these experiments.

6. Glycogen reduces the Q_{O_2} and $Q_{\text{acetoacetic acid}}$ due to butyric acid.

7. Increase of phosphate concentration reduces Q_{O_2} and $Q_{\text{acetoacetic acid}}$, possibly by removing free Ca ions. Phosphate buffer may be effectively replaced by glycerophosphate buffer; increase in concentration of this does not lead to a fall in Q_{O_2} or $Q_{\text{acetoacetic acid}}$. Replacement of Locke solution by saline results in a diminished oxidation of butyric acid by liver.

8. Brain slices do not oxidise butyric or crotonic acid; kidney slices oxidise butyric and crotonic acids but little or no acetone or acetoacetic acid is formed.

9. Minced liver cannot oxidise fatty acids.

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