The Metabolism of Butyric and Related Acids in Animal Tissue

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Butyric acid is known to undergo a number of metabolic reactions in animal tissue, viz. oxidation to acetoacetic and β -hydroxybutyric acids; formation of carbohydrate [Cross & Holmes, 1937; Blixenkron-Møller, 1938], and complete oxidation. The intermediate steps in these reactions are, however, still largely unknown. In the present paper experiments on the oxidation in animal tissue of butyric acid and a number of hitherto unexamined 4-carbon monocarboxylic acids will be reported. The metabolism of the latter was studied with the object of ascertaining whether they might be intermediates in the metabolism of butyric acid. It will be shown that vinylacetic, *dl*- α - γ -dihydroxybutyric, γ -hydroxybutyric, and in some tissues *trans*- γ -hydroxycrotonic acids are oxidized in animal tissues; *dl*- β - γ -dihydroxybutyric acid is little oxidized in kidney cortex, but causes the formation of a β -ketonic acid in starved rat and pigeon liver; tetrolic and tetric acids are not oxidized in animal tissues, whilst vinylglycollic acid is an inhibitor of tissue respiration.

EXPERIMENTAL

Preparations

γ -Hydroxybutyric acid was prepared according to Fichter & Herbrand [1896]. b.p. 202°. The preparation was free from succinic acid.

Vinylacetic acid [Linstead, Noble & Boorman, 1933]. b.p. 72.5–75°/13 mm.; 39.5 mg. required 4.54 ml. 0.1 N NaOH; calc. 4.60 ml.

Tetrolic acid [Feist, 1906]. m.p. 75.5°.

dl- α - γ -Dihydroxybutyric acid [Nef, 1910]. The lactone of the acid, together with lactic acid, was extracted with ether from a mixture of saccharinic acids obtained by treating lactose with 8 N NaOH. The acids were distilled at 5–6 mm., b.p. 115–120°; the distillate was neutralized with NaHCO₃, and the α -hydroxybutyrolactone was extracted with ether. The extracted lactone was converted into the phenylhydrazide (m.p. 130°), the hydrazide was decomposed with Ba(OH)₂, the phenylhydrazine extracted with ether and the lactone extracted after acidifying with H₂SO₄; 42.1 mg. required on heating 4.05 ml. 0.1 N NaOH, calc. 4.04 ml.

dl- β - γ -Dihydroxybutyric acid [Nef & Dar Juan, 1910]. 89.2 mg. required on heating 4.25 ml. 0.2 N NaOH, calc. 4.37 ml.

trans- γ -Hydroxycrotonic acid [Rambaud, 1935]. m.p. 107.5°.

Vinylglycollic acid was isolated from the higher boiling fractions after the distillation of its methyl ester [van der Sleen, 1902]. b.p. 127.5°/11 mm.; 100.8 mg. required on

heating 4.90 ml. 0.2 N NaOH, calc. 4.95 ml. An attempt to isolate the crystalline Ba-salt [van der Sleen, 1902] was unsuccessful.

Tetric acid was prepared by Dr H. A. Krebs according to Wolff & Schwabe [1896].

The other substrates were commercial preparations. The substrates were used as Na-salts.

Methods

1. The experiments were carried out on slices of kidney tissue from well-fed animals, unless it is stated that fasting animals were used. The oxidation of the various substrates was studied by the method of Meyerhof & Lohmann [1926] (see Kleinzeller [1943]). The formation of bicarbonate from crotonic and vinylacetic acids, as measured by this method, was compared with the disappearance of double bonds in the presence of these acids; the two methods were found to give similar results (Tables 6 and 7). As there are no specific methods available for estimating the other substrates, the assumption that the formation of bicarbonate is a measure of the utilization of these substrates is necessarily hypothetical.

2. Acetoacetic acid was estimated by the method of Edson [1935], which is specific for β -ketonic acids.

3. Estimation of crotonic and vinylacetic acids: The method is based on the estimation of H₂ necessary for the quantitative catalytic hydrogenation of the double bond. Both crotonic and vinylacetic acids are quantitatively hydrogenated under the conditions used for the estimation of acetic acid [Johnson, 1939]. The conical manometric cups were filled as follows:

Main compartment: 2.0 ml. water, 0.5 ml. 5% H₂SO₄, 0.5 ml. catalyst suspension containing about 5 mg. Pd catalyst (Membranfilter Ges. Göttingen, 5% Pd on BaSO₄), finely ground and saturated with H₂.

Centre well: 0.1 ml. 20% NaOH, 0.1 ml. 20% pyrogallol acid, small glass bead.

Side-arm: 1 ml. test solution (acidified).

The cups were filled with H₂, and put into a bath at 40°. Equilibrium was quickly attained. Since no H₂ uptake was observed in the blank (without addition of substrate), it could be generally omitted. Recovery of added material was satisfactory (Table 1).

Table 1. *Estimation of crotonic and vinylacetic acids*

Acid added (μ l.)	μ l. H ₂ uptake after			
	5 min.	10 min.	15 min.	20 min.
448 crotonic	381	447	448	446
*448 vinylacetic	410	421	421	421
*465 vinylacetic	405	450	455	456

* The somewhat low recovery of vinylacetic acid may be due to loss of this volatile acid during preparation of the solution.

In manometric experiments the following procedure was adopted: the contents of the cup were deproteinized with $\frac{1}{2}$ vol. of 5% HPO_3 and filtered; a measured portion was then transferred into a Kutscher Steudel extractor, acidified, and extracted with ether. The bulb of the extractor contained 1 ml. M NaHCO_3 to neutralize the extracted free acid and thus to prevent losses due to their volatility. The extraction was generally complete after 1 hr. (Table 2). After the extraction the ether was distilled off, and a measured portion of the acidified extract was used for the estimation.

Table 2. *Extraction of crotonic and vinylacetic acids*

(Volume of solution in extractor: 20 ml. Approximate rate of distillation of ether: 15 ml./min.)

Acid added ($\mu\text{l.}$)	Acid found ($\mu\text{l.}$) after extraction		
	0-30 min.	30-60 min.	Total
896 crotonic	881	16.5	897.5
834 vinylacetic	830	5	835

The method is not specific, for many unsaturated compounds, and also keto-acids, will be estimated in this way. The disappearance or formation of unsaturated compound will be denoted by the symbol Q_Δ , i.e. $\mu\text{l. H}_2$ absorbed/mg. dry weight of tissue/hr.

RESULTS

Oxidation of 4-carbon monocarboxylic acids in kidney cortex. It is known that kidney cortex oxidizes butyric, crotonic, β -hydroxybutyric, acetoacetic [Jowett & Quastel, 1935; Quastel & Wheatley, 1935] and *threo*- $\alpha\beta$ -dihydroxybutyric acid [Weil-Malherbe, 1938; see, however, Hoff-Jørgensen, 1940].

In the present experiments the oxidation in guinea-pig and rat kidney cortex of various substrates at a concentration of 0.0156M was tested by means of measurements of the O_2 uptake and

the bicarbonate formation; the results are summarized in Tables 3 and 4. It will be seen that butyric, crotonic, vinylacetic, β -hydroxybutyric, γ -hydroxybutyric, and *dl*- $\alpha\gamma$ -dihydroxybutyric acids are oxidized in guinea-pig and rat kidney cortex. These substrates considerably increase Q_{O_2} and $Q_{\text{bic.}}$ above those of a control. *trans*- γ -Hydroxycrotonic acid is oxidized in rat kidney only. The following substrates are slowly oxidized in kidney slices of both species: *dl*- $\beta\gamma$ -dihydroxybutyric acid, tetrolic, tetronic, and α -hydroxybutyric acids. Vinylglycollic acid inhibits tissue respiration, and $Q_{\text{bic.}}$ was found to be negative. The high rate of oxidation of γ -hydroxybutyric, *dl*- $\alpha\gamma$ -dihydroxybutyric acid, and in rat kidney *trans*- γ -hydroxycrotonic acid, suggests that the hydroxyl group is oxidized with the formation of succinic, malic, and fumaric acids respectively. The recent observation by Lipmann & Perlmann [1942] that tetrolic acid is not attacked in kidney tissue is confirmed.

A study was then made of the rates of oxidation in rat kidney of butyric and related acids by measurement of Q_{O_2} and $Q_{\text{bic.}}$ In further experiments, again using rat kidney, as it oxidizes butyric acid at a higher rate than guinea-pig kidney (see Table 3), the disappearance of crotonic and vinylacetic acids and the formation of unsaturated compounds during the oxidation of butyric acid and β -hydroxybutyric acid were measured. As shown in Table 5, the latter acid is oxidized at a slower rate than the former, confirming the observation of Dakin [1922] on the behaviour of these substrates when administered to dogs. It seems unlikely, therefore, that β -hydroxybutyric acid can be an intermediate on the main pathway of the oxidation of butyric acid in rat kidney slices. It will be noted that vinylacetic acid was oxidized at a rate slightly higher than that of either butyric or

Table 3. *Oxidation of 4-carbon monocarboxylic acids in guinea-pig and rat kidney cortex*

Acid added as substrate (0.0156M)	Formula	Kidney cortex from			
		Guinea-pig		Rat	
		No. of exps.	$Q_{\text{bic.}}$	No. of exps.	$Q_{\text{bic.}}$
None	—	12	0.00-1.97	5	0.10-1.97
Butyric	$\text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{COOH}$	4	1.53-2.19	5	3.46-6.70
Crotonic	$\text{CH}_3\cdot\text{CH}:\text{CH}\cdot\text{COOH}$	4	1.98-3.17	3	2.31-5.59
Vinylacetic	$\text{CH}_2:\text{CH}\cdot\text{CH}_2\cdot\text{COOH}$	4	3.13-5.10	3	5.57-9.85
Tetrolic	$\text{CH}_3\cdot\text{C}:\text{C}\cdot\text{COOH}$	2	0.40; 1.84	1	-0.9
α -Hydroxybutyric	$\text{CH}_3\cdot\text{CH}_2\cdot\text{CHOH}\cdot\text{COOH}$	1	1.77	—	—
β -Hydroxybutyric	$\text{CH}_3\cdot\text{CHOH}\cdot\text{CH}_2\cdot\text{COOH}$	3	3.22-4.30	2	4.44; 4.82
γ -Hydroxybutyric	$\text{CH}_2\text{OH}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{COOH}$	7	2.75-4.20	3	5.20-8.10
<i>trans</i> - γ -Hydroxycrotonic	$\text{CH}_2\text{OH}\cdot\text{CH}:\text{CH}\cdot\text{COOH}$	2	1.21; 0.28	3	5.50-10.0
<i>dl</i> - $\alpha\gamma$ -Dihydroxybutyric	$\text{CH}_2\text{OH}\cdot\text{CH}_2\cdot\text{CHOH}\cdot\text{COOH}$	3	2.90-3.78	3	5.70-9.90
<i>dl</i> - $\beta\gamma$ -Dihydroxybutyric	$\text{CH}_2\text{OH}\cdot\text{CHOH}\cdot\text{CH}_2\cdot\text{COOH}$	4	0.30-3.10	1	1.4
Tetronic	$\text{CH}_2\text{CO}\cdot\text{CH}_2\cdot\text{CO}$	2	1.65; 2.43	1	-0.4
Vinylglycollic	$\text{CH}_2:\text{CH}\cdot\text{CHOH}\cdot\text{COOH}$	3	-0.35--2.60	1	-3.2

Table 4. *Oxidation of 4-carbon monocarboxylic acids in kidney cortex*

Exp.	Substrate added (0.0156M)	Q_{O_2}	$Q_{blc.}$
Guinea-pig	1 None	-14.3	+ 0.3
	Butyrate	-22.8	+ 1.57
	Crotonate	-22.3	+ 1.98
	γ -Hydroxybutyrate	-18.6	+ 4.2
	<i>trans</i> - γ -Hydroxycrotonate	-18.4	+ 1.2
	<i>dl</i> - β -Dihydroxybutyrate	-17.3	+ 3.1
	Vinylglycollate	- 7.2	- 0.35
2	None	-14.0	+ 1.15
	γ -Hydroxybutyrate	-16.6	+ 4.05
	<i>dl</i> - α -Dihydroxybutyrate	-14.35	+ 3.78
3	None	-11.2	+ 0.32
	Crotonate	-17.7	+ 2.06
	<i>trans</i> - γ -Hydroxycrotonate	-14.5	+ 0.32
4	None	-14.3	+ 0.32
	α -Hydroxybutyrate	-17.3	+ 1.77
	Tetronate	-14.8	+ 1.65
5	None	-12.2	+ 1.28
	Butyrate	-19.4	+ 2.19
	Tetrolate	-12.6	+ 1.84
6	None	-14.6	+ 0.75
	<i>dl</i> - β -Dihydroxybutyrate	-14.9	+ 1.08
	Vinylglycollate	- 5.7	- 2.6
7	None	-12.1	+ 1.67
	Vinylacetate	-22.1	+ 3.13
	<i>dl</i> - α -Dihydroxybutyrate	-16.7	+ 2.83
Rat	8 None	-23.2	+ 1.12
	Butyrate	-32.2	+ 3.9
	α -Hydroxybutyrate	-22.6	—
	γ -Hydroxybutyrate	-27.7	+ 8.15
	<i>trans</i> - γ -Hydroxycrotonate	-25.2	+10.0
	<i>dl</i> - α -Dihydroxybutyrate	-25.7	+ 7.52
	<i>dl</i> - β -Dihydroxybutyrate	-25.9	+ 1.4
	Tetrolate	-15.6	- 0.9
	Tetronate	-26.5	- 0.4
	Vinylglycollate	- 5.8	- 3.2
9	None	-18.8	+ 1.97
	Butyrate	-29.4	+ 3.46
	Crotonate	-21.3	+ 2.31
	γ -Hydroxybutyrate	-29.9	+ 5.25
	<i>trans</i> - γ -Hydroxycrotonate	-24.6	+ 9.05
	<i>dl</i> - α -Dihydroxybutyrate	-27.4	+ 9.9

observation. Under anaerobic conditions vinylacetic acid disappears in rat kidney; Lipmann & Perlmann [1942] found under similar conditions a disappearance of crotonic acid.

The amount of unsaturated compound found when butyric acid is oxidized in rat kidney does not increase after the first hour, so that Q_{Δ} decreases, as shown in Table 7. As Jowett & Quastel [1935] have already shown, only small amounts of acetoacetic acid are formed in rat kidney in the presence of butyric acid, and the unsaturated compound formed is therefore unlikely to be acetoacetic acid.

Table 5. *Rates of oxidation of butyric and related acids in rat kidney*

No.	Substrate added (0.0156M)	Q_{O_2}	$Q_{blc.}$	Q_{Δ}
1	None	-22.8	+1.85	—
	Butyrate	-26.5	+6.70	—
	Crotonate	-25.2	+5.49	—
	Vinylacetate	-27.4	+9.85	—
	β -Hydroxybutyrate	-28.8	+4.82	—
2	None	-21.2	+0.12	+0.12
	Butyrate	-29.8	+5.15	+3.13
	Crotonate	-26.0	+3.36	-3.54
	Vinylacetate	-27.4	+5.57	-7.96
	(Vinylacetate; anaerobic)	—	—	-2.89)
	β -Hydroxybutyrate	-28.8	+4.44	+1.92

Table 6. *Oxidation of vinylacetic acid in rat kidney*

(705 μ l. vinylacetic acid added. The results refer to the whole period of the experiment.)

Duration of exp. (hr.)	Without substrate			With vinylacetate		
	Q_{O_2}	$Q_{blc.}$	Q_{Δ}	Q_{O_2}	$Q_{blc.}$	Q_{Δ}
1	-23.4	+1.88	+0.37	-29.1	+5.79	-6.30
2	-22.2	+1.43	+0.15	-27.7	+5.10	-4.35
4	-23.1	+1.80	+0.04	-27.7	+4.54	-5.45

Table 7. *Oxidation of butyric acid in rat kidney*

(The results refer to the whole period of the experiment.)

Duration of exp. (hr.)	Without substrate			With 0.0156M butyrate		
	Q_{O_2}	$Q_{blc.}$	Q_{Δ}	Q_{O_2}	$Q_{blc.}$	Q_{Δ}
1	-22.4	+0.39	+0.69	-32.6	+4.66	+6.0
2	—	—	—	-27.6	+4.08	+1.92
4	-20.4	+0.59	+0.26	-27.5	+3.39	+0.56

crotonic acid. Crotonic acid was generally found to be oxidized in rat kidney at a lower rate than butyric acid (see also Exp. 9, Table 4). According to these results vinylacetic acid could possibly be an intermediate in the oxidation of butyric acid in rat kidney but this needs to be ascertained by further investigations.

The rate of disappearance of double bonds in the presence of crotonic and vinylacetic acids is of the same order as the rate of bicarbonate formation from these acids (Tables 5 and 6). Butyric and β -hydroxybutyric acids yield an unsaturated compound, but as the method of estimating such compounds is not specific, no conclusions on the nature of the product formed can be drawn from this

Effect of malonate. 0.031M malonate inhibits the oxidation of all the substrates examined in guinea-pig and rat kidney (Table 8). A slight formation of bicarbonate in the presence of malonate was observed in rat kidney when *trans*- γ -hydroxycrotonic acid was the substrate. The inhibition of the oxidation of butyric acid by malonate has already been reported by Jowett & Quastel [1935].

Table 8. *Effect of malonate on the oxidation of 4-carbon monocarboxylic acids in kidney cortex*

Exp. no.	Substrate added (0.0156 M)	Substrate alone		Substrate + 0.031 M malonate	
		Q _{O₂}	Q _{bic.}	Q _{O₂}	Q _{bic.}
Guinea-pig					
1	None	-14.5	0.0	-10.2	+1.1
	Butyrate	-20.0	+2.1	-12.3	+0.9
	β -Hydroxybutyrate	-18.3	+3.3	-12.0	+1.4
	γ -Hydroxybutyrate	-20.0	+3.0	-11.0	+0.3
2	None	-11.4	+0.3	—	—
	Crotonate	-17.7	+2.1	-9.3	+0.3
	<i>trans</i> - γ -Hydroxy-crotonate	-14.6	+0.3	-8.5	0.0
	<i>dl</i> - $\beta\gamma$ -Dihydroxy-butyrate	-13.7	+0.3	-9.4	—
3	None	-12.9	+0.86	—	—
	Tetronate	-14.9	+2.43	-10.5	+0.41
	Tetrolate	-13.0	+0.42	-9.4	+0.22
4	None	-14.3	+1.97	-10.6	+0.74
	Vinylacetate	-22.8	+3.34	-12.1	+0.78
	γ -Hydroxybutyrate	-19.3	+3.59	-11.9	+1.27
Rat					
5	None	—	—	-7.1	-0.26
	γ -Hydroxybutyrate	-27.4	+6.9	-7.2	-0.76
	<i>dl</i> - $\alpha\gamma$ -Dihydroxy-butyrate	-26.7	+5.7	-8.5	+0.17
6	None	-18.8	+1.97	-8.4	-2.31
	Crotonate	-21.3	+2.31	-9.6	-1.53
	<i>trans</i> - γ -Hydroxy-crotonate	-24.6	+9.05	-9.4	+1.97

Table 9. *Formation of β -ketonic acids from 4-carbon monocarboxylic acids in liver tissue*

(3 ml. phosphate saline; tissue; 0.0156M substrate; O₂; 60 min.)

Exp. no.	Substrate added	Q _{O₂}	Q _{β-ket. acid}
Pigeon liver			
1	None	-10.5	+0.55
	Butyrate	-11.1	+2.00
	Crotonate	-11.8	+2.2
	α -Hydroxybutyrate	-13.1	0.00
	β -Hydroxybutyrate	-12.3	+2.4
	γ -Hydroxybutyrate	-15.2	+0.95
	<i>trans</i> - γ -Hydroxycrotonate	-11.8	+0.65
	<i>dl</i> - $\beta\gamma$ -Dihydroxybutyrate	-13.0	+1.22
	Tetrolate	-11.0	+0.85
	Tetronate	-11.1	+0.61
	Vinylglycollate	-7.7	0.00
Rat liver			
2	None	-10.7	+0.82
	Butyrate	-12.6	+2.87
	Crotonate	-14.5	+5.58
	Vinylacetate	-14.6	+6.52
	<i>dl</i> - $\alpha\gamma$ -Dihydroxybutyrate	-13.5	+0.90
	<i>dl</i> - $\beta\gamma$ -Dihydroxybutyrate	-13.4	+2.16
	γ -Hydroxybutyrate	-14.4	+0.82
3	None	-10.8	+0.75
	Butyrate	-12.8	+2.47
	Crotonate	-13.2	+4.43
	Vinylacetate	-13.6	+5.00
	β -Hydroxybutyrate	-13.1	+6.15

Formation of β -ketonic acids from butyric and related acids in liver. It is known that acetoacetic acid is formed in the liver from butyric, β -hydroxybutyric [Embsden, Salomon & Schmidt, 1906], and crotonic [Friedmann, 1908] acids. Lipmann & Perlmann [1942] have shown that tetrolic acid does not give rise to acetoacetic acid in rat liver. A study was made of the formation, in the liver tissue of starving pigeons and rats, of β -ketonic acids from certain 4-carbon monocarboxylic acids not hitherto examined (see Table 9). β -Ketonic acids are formed from butyric, crotonic, β -hydroxybutyric, vinylacetic, and, to a small extent, from *dl*- $\beta\gamma$ -dihydroxybutyric acids. None of the other substrates tested was appreciably ketogenic.

In the rat liver vinylacetic acid readily gives rise to acetoacetic acid. $Q_{\text{acetoac.}}$ exceeded, in our experiments, that from butyric and crotonic acids, but was lower than that from β -hydroxybutyric acid.

No β -ketonic acid is formed from vinylacetic or *dl*- $\beta\gamma$ -dihydroxybutyric acid under anaerobic conditions (Table 10).

Table 10. *Formation of β -ketonic acids in starved rat liver under aerobic and anaerobic conditions*

Substrate (0.0156M)	O ₂		N ₂
	Q _{O₂}	Q _{β-ket. acid}	Q _{β-ket. acid}
0	-9.95	+0.86	0.00
Crotonate	-11.5	+3.08	0.42
Vinylacetate	-14.0	+5.85	0.15
<i>dl</i> - $\beta\gamma$ -Dihydroxy-butyrate	-12.5	+2.53	0.36

DISCUSSION

Blixenkrone-Møller [1938] found that β -hydroxybutyric acid does not give rise to carbohydrate in the perfused liver of cats, whilst butyrate does, and concluded that β -hydroxybutyric acid is not an intermediate in the formation of carbohydrate from butyric acid. He therefore suggested that butyric acid is oxidized in animal tissue both at the β -carbon atom, yielding ketone bodies, and at the γ -carbon atom, yielding succinic acid, which is then further oxidized.

The results of our experiments suggest that β -hydroxybutyric acid is not an intermediate on the main pathway of the oxidation of butyric acid in rat kidney. It would appear therefore that the formation of ketone bodies represents an alternative path in the oxidation of butyric acid in the animal. It is interesting to note, in view of Blixenkrone-Møller's suggestion of γ -oxidation of butyric acid, that γ -hydroxybutyric acid is oxidized at a higher rate than butyric acid in both guinea-pig and rat kidney slices.

Under our experimental conditions, vinylacetic acid is oxidized in kidney slices at a higher rate than butyric acid. With vinylacetic acid a higher rate of β -ketonic acid formation than with butyric acid as substrate is also observed in starved rat liver. The compound thus shows physiological properties similar to those of butyric acid, and it seems possible that vinylacetic acid is an intermediate in the breakdown of butyric acid.

In our experiments a β -ketonic acid is formed in liver tissue from starving animals, in the presence of *dl*- β -dihydroxybutyric acid. Tetronic acid, which would be expected to arise from the oxidation of the β -hydroxyl of *dl*- β -dihydroxybutyric acid, does not behave as a β -ketonic acid (it does not yield any CO_2 on treatment with aniline citrate: personal communication from Dr H. A. Krebs) and consequently it cannot be the β -ketonic acid formed in the presence of *dl*- β -dihydroxybutyric acid.

The β -ketonic acid might be acetoacetic acid formed from substrates present in the liver tissue; such an effect would be similar to that observed when malonic acid is added to liver tissue [Jowett & Quastel, 1935]. *dl*- β -Dihydroxybutyric acid, however, increases Q_{O_2} , whilst malonate inhibits it. The possibility of the formation of acetoacetic acid from this substrate by dehydration seems unlikely in view of the observation that no β -ketonic acid is formed under anaerobic conditions from this substrate. On the other hand, it seems possible that a structural isomer of tetronic acid, hitherto unknown, may be formed, viz. the *trans*-isomer (I) of the enolic form of tetronic acid as opposed to the *cis*-isomer (lactone form, II):

SUMMARY

1. The oxidation, in kidney slices of well-fed guinea-pigs and rats, of the Na-salts of butyric, crotonic, vinylacetic, tetrolic, α -hydroxybutyric, β -hydroxybutyric, γ -hydroxybutyric, *trans*- γ -hydroxycrotonic, *dl*- α -dihydroxybutyric, *dl*- β -dihydroxybutyric, tetronic, and vinylglycollic acids was studied by comparing the Q_{O_2} and the formation of bicarbonate in the presence of these substrates with that of a control experiment without substrate. Of the above substrates butyric, crotonic, vinylacetic, β -hydroxybutyric, γ -hydroxybutyric, *dl*- α -dihydroxybutyric acids are rapidly oxidized in the kidney of both species. *trans*- γ -Hydroxycrotonic acid is oxidized in rat kidney only. Vinylglycollic acid inhibits tissue respiration. α -Hydroxybutyric, tetrolic, *dl*- β -dihydroxybutyric and tetronic acids are not appreciably oxidized.

2. From rates of bicarbonate formation in the presence of butyric, crotonic, vinylacetic, and β -hydroxybutyric acids in rat kidney slices, it is concluded that β -hydroxybutyric and crotonic acids are oxidized more slowly than is butyric acid. In the presence of vinylacetic acid Q_{bic} is higher than it is in the presence of butyric acid.

3. In rat kidney slices formation of an unsaturated compound in the presence of butyric and β -hydroxybutyric acids occurs under aerobic conditions.

4. Under anaerobic conditions vinylacetic acid disappears in rat kidney slices.

5. 0.031 M malonate inhibits, in the kidney of both species, the oxidation of all the substrates tested.

6. Vinylacetic acid and *dl*- β -dihydroxybutyric acid are ketogenic in liver slices from starved pigeons and rats.

7. It is suggested that vinylacetic acid may play a part in the intermediate metabolism of butyric acid in animal tissue.

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REFERENCES

- Blixenkrone-Møller, N. [1938]. *Hoppe-Seyl. Z.* **252**, 117; 137.
 Cross, M. C. A. & Holmes, E. [1937]. *Brit. J. exp. Path.* **18**, 370.
 Dakin, H. D. [1922]. *Oxidations and Reductions in the Animal Body*, 2nd ed. London: Longmans, Green.
 Edson, N. L. [1935]. *Biochem. J.* **29**, 2082.
 Embden, G., Salomon, H. & Schmidt, Fr. [1906]. *Beitr. chem. Physiol. Path.* **8**, 129.
 Feist, F. [1906]. *Liebigs Ann.* **345**, 104.
 Fichter, Fr. & Herbrand, A. [1896]. *Ber. dtsch. chem. Ges.* **29**, 1192.
 Friedmann, E. [1908]. *Beitr. chem. Physiol. Path.* **11**, 371.
 Hoff-Jørgensen, E. [1940]. *Hoppe-Seyl. Z.* **266**, 56.
 Johnson, W. A. [1939]. *Biochem. J.* **33**, 1046.
 Jowett, M. & Quastel, J. H. [1935]. *Biochem. J.* **29**, 2143, 2159, 2181.
 Kleinzeller, A. [1943]. *Biochem. J.* **37**, 674.
 Linstead, R. P., Noble, E. G. & Boorman, E. J. [1933]. *J. chem. Soc.* p. 557.
 Lipmann, F. & Perlmann, G. E. [1942]. *Arch. Biochem.* **1**, 41.
 Meyerhof, O. & Lohmann, K. [1926]. *Biochem. Z.* **171**, 381.
 Nef, J. U. [1910]. *Liebigs Ann.* **376**, 1.
 — & Dar Juan, T. [1910]. *Liebigs Ann.* **376**, 35.
 Quastel, J. H. & Wheatley, A. H. M. [1935]. *Biochem. J.* **29**, 2773.
 Rambaud, R. [1935]. *Bull. Soc. chim. Fr.* (5), **1**, 1317.
 van der Sleen, M. G. [1902]. *Rec. Trav. chim. Pays-Bas*, **21**, 209.
 Weil-Malherbe, H. [1938]. *Biochem. J.* **32**, 1033.
 Wolff, L. & Schwabe, C. [1896]. *Liebigs Ann.* **291**, 231.