Microbiological Studies of *Coli-aerogenes* Bacteria*

Part XI. Influence of the Amount of Available Oxygen upon α -Ketoglutarate-fermentation

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Investigations were carried out on the effect of the amount of available oxygen upon the metabolisms of carbonaceous substances in *coli-aerogenes* bacteria. When *E. coli* was grown in the presence of glucose, a gentle aeration (a small supply of air) suppressed the accumulation of lactate against increasing the productions of acetate, carbon dioxide and of the bacterial cell. α -Ketoglutarate-fermentation occurred only under a large supply of air. When the cells of *coli-aerogenes* were incubated under the low levels of oxygen, not only glucose, C₃- (pyruvic, lactic), C₄-dicarboxylic and C₆-tricarboxylic acids but also acetic acid were mainly oxidized to carbon dioxide and water, but little or no formation of α -ketoglutaric acid occurred. The production of α -ketoglutaric acid from various organic acids except C₆-tricarboxylic acids also took place only under the high levels of available oxygen. It was concluded that the direction of oxidative metabolism of substrate-carbon was changeable according to variation of the environmental oxygen level. Relationships among respiration, oxidative fermentation and oxidative assimilation were investigated under various levels of available oxygen.

INTRODUCTION

In the previous papers of this series, the authors have found that lactic acid-fermentation, which occurs anaerobically, by a number of *coli-aerogenes* bacteria is converted into α -ketoglutaric acid-fermentation under aerobic conditions¹⁻³. The oxidative metabolisms in the bacteria have, furthermore, been studied with varied substrates including carbohydrates, C₂-, C₃-, C₄- and C₆- acids, and consequently the mode of degradations of C₆-tricarboxylic acids was propounded and discussed⁴⁻¹¹.

The effects of oxygen-supply on the fate of

substrate-carbon in the metabolism of bacteria have been studied by several workers¹³⁻¹⁷. In the preceding paper¹², the authors have also demonstrated that when *Escherichia coli* is aerobically cultivated with a gentle stream of sterile air in the presence of glucose, ammonium-nitrogen and other mineral salts, a large amount of acetic acid is obtained in place of lactic and α -ketoglutaric acids as the fermentation-product¹².

* A part of this paper was read before the symposia on Enzyme Chemistry (January, 1957) and the Meeting of the Japanese Biochemical Society (July, 1957).

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FIG. 1. Diagram of 10-liter Swinging Tank (K-Type Fermentor)

- a. Periodic Agitator (Clock and anti-clockwise motion with angle $\pi/6$ to π)
 b. Cooling water Pipe, c. Thermometer, d. Air Outlet,
 e. Air Inlet, f. Raw Material Inlet, g. Fermented Liquor Outlet

The influence of the amount of available oxygen upon the fate of substrate-carbon in *coli-aerogenes* are again described in the present paper.

EXPERIMENTAL AND RESULTS

Methods. Many experiments described in this paper were carried out with *Escherichia coli* (G-2 strain). *Aerobacter aerogenes* (B-2 strain) was also used in some experiments. Analytical procedures were performed by the methods mentioned in the previous papers^{1,6}.

Fermentations were run in 10-liter stainless steel tank (Fig. 1) equipped with propellers. This fermentor has been termed a swinging (K-type) tank by the authors²³. Air was passed through a sterile cotton filter and introduced into a cultural medium through a sparger beneath an agitator with three propellers. Fermentation-tem-

perature was controlled at 30°C by circulating water through the jacket. Some experiments were also carried out with a shaker or the conventional Warburg-respirator as already mentioned in the previous papers.

Aeration Efficiency. The transfer of oxygen from the gas phase to the liquid phase is generally recognized to be the limiting factor in aeration of cultures. The aeration efficiency is referred to in this paper as sulfite oxidation value, expressed as mM O₂ per liter per min. Effective aeration values were determined by the oxidation of sulfite in the presence of copper according to the method of Cooper et al.¹⁸. Effective aeration rates of some aeration devices employed in the authors' laboratory are shown in Table I. In the case of the fermentation-tanks with 5~10 liters of medium, it will be seen that aeration without any agitation gives very poor aeration efficiencies, and in order to obtain high aeration efficiencies, aeration must be accompanied with agitation of the medium. Shake flasks with 15~100 ml of a medium on a shaker were observed to be effective (maximal sulfite value; about 2.9 mM O₂ per liter per min.), whereas the conventional Warburg-apparatus revealed poor aeration efficiencies (0.15~0.35 mM O₂ per liter per min.).

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TABLE I. EFFECTIVE AERATION RATES OF SOME AERATION PROCEDURES

Vessel used	Volume of medium (ml)	Aeration procedure	Aerating rate of air (vol per vol per min.)	Effective aeration (mM O ₂ per L per min.)
(a) Conventional Warburg vessel	2.5~5.0	Shaken on Warburg respirator	—	0.15~0.35
(b) 500 ml shaking flask	15~100	Shaken on shaker at 110~130 r. p. m.	—	0.93~2.92
(c) Conventional submerged fermentor	5000~7000	120 r. p. m. agitator plus sparger	$\begin{cases} 0.50 \\ 1.00 \end{cases}$	$\begin{cases} 0.02 \\ 0.03 \end{cases}$
(d) K-type fermentor (swinging fermentor)	7000	300 r. p. m. agitator plus sparger	1.00	0.28
	8000~10000	Aeration only with sparger	1.00	0.08~0.11
		63 r. p. m. agitator plus sparger	$\begin{cases} 0.50 \\ 1.00 \\ 0.25 \\ 0.13 \end{cases}$	$\begin{cases} 0.25 \\ 0.40 \\ 0.12 \\ 0.09 \end{cases}$
		100 r. p. m. agitator plus sparger	0.20	0.47~0.52
		110 r. p. m. agitator plus sparger	0.55	0.91
		132 r. p. m. agitator plus sparger	0.45	1.46
		138 r. p. m. agitator plus sparger	$\begin{cases} 0.55 \\ 1.00 \end{cases}$	$\begin{cases} 1.62 \\ 2.20 \end{cases}$
		168 r. p. m. agitator plus sparger	1.00	3.00

TABLE II. OXIDATIVE FERMENTATION BY *E. coli* UNDER LOW AND HIGH LEVELS OF AVAILABLE OXYGEN

Time of fermentation (hours) at 30°C	23	38	48	64
Methods of aerobic cultures				
Aerating rate of air (vol per vol per min.)	1.0	1.0	1.0	1.0
Agitation	—	—	After 38 hours' incubation agitator (120 r. p. m.) was operated	
Consumption of glucose (mM/dl)	15.10	18.40	18.40	18.40
(%)	82.2	100.0	100.0	100.0
Acetic acid found (mM/dl)	8.10	11.00	—	8.00
(Molar yield)	0.536	0.597		0.435
Lactic acid found (mM/dl)	10.70	13.50	10.50	7.400
(Molar yield)	0.710	0.734	0.571	0.402
Pyruvic acid found (mM/dl)	1.50	0	0	0
(Molar yield)	0.099			
α -Ketoglutaric acid found (mM/dl)	Trace	0	0.51	1.55
(Molar yield)			0.028	0.084
Bacterial cells found (mg/dl)	192	272	296	326

Note: Fermentation without any aeration and agitation was as follows:

Time of incubation (hours)	Glucose consumed (mM/dl)	Acetic acid found (Molar yield)	Lactic acid found (Molar yield)	Bacterial cells found (mg/dl)
64	10.8	0.485	0.950	35

Oxidative Fermentation in Fermentor. Table II shows the effects of aeration with a stream of sterile air and agitation upon the fermentations of glucose in *E. coli* (G-2 strain). The medium contained 3.31% (18.4 mM/dl) glucose, 0.1% each $(\text{NH}_4)_2\text{SO}_4$ and $(\text{NH}_4)_3\text{HPO}_4$, 0.1% KH_2PO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2% NaCl and 5 p.p.m. $\text{Fe}_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$ in tap water to which sterilized CaCO_3 corresponding to 75% of glucose was added at the time of inoculation. Ten liter of the medium was taken in a fermentor and sterilized. After inoculation with 200 ml of 60 hours' culture under aerobic conditions at 30°C of the organism on glucose-medium, the incubation was carried out at 30°C under the conditions as indicated in Table II. During the first phase of fermentation, the medium was aerated only by a bubbling stream of air, without any agitation by propellers; this period of fermentation was about 38 hours with a medium containing 3.31% glucose. In the second phase of fermentation, i.e., when glucose was completely consumed, an agitator was operated in the fermentor to increase the efficiency of aeration. An experiment of anaerobic culture was made with the same medium for the sake of comparison. The chemical changes occurring during fermentation is shown in Table II. Lactate was observed to be produced with glucose even under such conditions that the medium was aerated with a stream of air but without any agitation, while the yield of the bacterial cell was increased under the conditions. It has, however, been found that the formation of α -ketoglutarate occurs when the fermented liquid is again incubated by the combination of aeration with a stream of air and agitation by propeller.

Similar experiments were carried out with a medium containing 2.97% (16.5 mM) glucose, 0.42% Na-glutamate $\cdot \text{H}_2\text{O}$, 0.1% KH_2PO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 500 γ % $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2% NaCl and 2% CaCO_3 . In the first stage of fermentation, the medium was aerated at 30°C by a stream of air at rate of volume per volume per minute, without any agitation, and this time of incubation was about 70 hours. In the second stage (after 70 hours' incubation), the agitator was operated to increase the efficiency of aeration. The results of experiment are shown in Table III, from which the following facts will be indicated. The consumption of glucose was observed to proceed at a much faster rate in aerated culture than in anaerobic culture without any aeration. In the case of aerated culture with a stream of air, the molar yields of acetate and lactate after 38 hours' incubation, were about 0.91 and 0.67, respectively, and also those after 60 hours were about 1.21 and 0.42. In these cases, however, α -ketoglutarate was never formed in a noticeable amount. On the other hand, the molar yields of acetate and lactate under anaerobic conditions were about 0.71 and 0.84, respectively after 87 hours' incubation. The yield of the bacterial cell synthesized was ascertained to be exceedingly high even in a gentle aeration as shown in Table III. Thus, it is clear that when a small amount of oxygen is supplied to the medium, α -ketoglutarate-fermentation does not occur, but there are the increasing yields of acetate and bacterial cells against the decreasing yield of lactate. It should be noted, here, that *coli-aerogenes* bacteria are capable of inducing α -ketoglutarate-fermentation only under a high level of available oxygen

TABLE III. FERMENTATION OF GLUCOSE BY *E. coli* UNDER ANAEROBIC AND AEROBIC CONDITIONS

Time of fermentation at 30°C (hours)		38		60		87	
Kinds of cultures		Anaerobic		Aerobic		Anaerobic	
Aerating rate of air (vol per vol per min.)		—		1.0		—	
Agitation		—		—		After 70 hours, agitator (140r.p.m.) was operated	
Consumption of glucose	(mM/dl)	6.30	16.50	10.10	16.50	13.30	16.50
	(%)	38.2	100.0	61.3	100.0	80.7	100.0
Acetic acid found	(mM/dl)	—	15.00	—	20.00	9.40	15.00
	(Molar yield)	—	0.910	—	1.212	0.706	0.910
Lactic acid found	(mM/dl)	—	11.00	10.00	7.00	11.10	3.60
	(Molar yield)	—	0.668	0.991	0.424	0.836	0.218
Pyruvic acid found	(mM/dl)	Trace	0.34	0.20	Trace	Trace	0
	(Molar yield)	Trace	0.021	0.020	Trace	Trace	0
α -Ketoglutaric acid found	(mM/dl)	0	Trace	0	Trace	0	1.24
	(Molar yield)	0	Trace	0	Trace	0	0.075
Bacterial cells found	(mg/dl)	—	219	—	—	35	296

TABLE IV. INFLUENCE OF AGITATION AND AERATION UPON α -KETOGLUTARATE-FERMENTATION BY *E. coli*

Amounts of nitrogen added			Agitator speed (r. p. m.)	Aerating rate of air (vol per vol per min.)	Time of incubation (hours)	Glucose consumed (g/dl)	Pyruvic acid found (g/dl)	α -Ketoglutaric acid found (% on glucose consumed)
(NH ₄) ₂ SO ₄ (g/dl)	NH ₄ Cl (g/dl)	(NH ₄) ₂ HPO ₄ (g/dl)						
(with 10 liter-fermenter)								
0	0	0.10	60	1.0	46	2.90	0.41	7.9
0	0	0.15	60	1.0	37	3.89	0.77	Trace
0.05	0	0.10	100	0.35	47	2.83	0	31.7
0.10	0	0.10	160	1.0	50	4.46	0	50.3
0.10	0	0.10	160	1.0	38	2.48	0	54.5
0.10	0	0.10	170	1.0	42	3.95	0	56.2
0.10	0.10	0.10	180	1.0	39	4.15	0	55.0
0.20	0.10	0.10	180	1.0	109	10.47	0	32.4
0.20	0.10	0.10	180	1.0	87	8.80	0	35.5
0.20	0.10	0.10	180	1.0	108	10.55	0	38.7
(with 500ml-shaking flask)								
0	0	0.15	shaken on shaker		42	2.35	0	36.2
0	0	0.15	shaken on shaker		85	3.45	0	49.2
0.10	0	0.15	shaken on shaker		85	4.90	0	45.5
0.10	0	0.10	shaken on shaker		168	8.83	0	45.6

TABLE V. PRODUCTION OF PYRUVIC AND α -KETOGLUTARIC ACIDS FROM GLUCOSE BY *E. coli*

	Experiment I			Experiment II			
	14	24	37	14	20	36	46
Time of incubation (hours)							
Agitator speed (r. p. m.)	60	60	60	120	120	120	120
Aerating rate of air (vol per vol per min.)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Glucose consumed (mg/ml)	15.6	30.3	38.9	20.0	28.7	40.3	44.4
Pyruvic acid found (mg/ml)	2.7	5.2	7.7	1.3	Trace	0	0
α -Ketoglutaric acid found (mg/ml)	Trace	Trace	Trace	1.4	7.0	16.9	19.0

Medium contained 5.4% glucose, 0.15% (NH₄)₂HPO₄, 0.05% (NH₄)₂SO₄ and other mineral salts.

TABLE VI. SYNTHESIS OF CELL OF *E. coli* IN EARLY STAGE OF α -KETOGLUTARATE-FERMENTATION

Time of incubation (hours)	Glucose consumed (g/dl)	Bacterial cells synthesized (g/dl)	α -Ketoglutaric acid found (g/dl)	Pyruvic acid found (g/dl)	Agitator speed (r.p.m.)	Aerating rate of air (V per V per min.)
22	2.330	0.339	1.088	0	180	1.0
37	2.990	0.380	1.535	0	180	1.0
22	1.52	0.257	0.405	0	100	1.5
38	2.15	0.368	0.897	0	100	1.5

(high efficiency of aeration).

Therefore, in the case of α -ketoglutarate-fermentation on a large scale (with a large fermentor), aeration must be accompanied with agitation of the medium; the resulting aeration-efficiencies depend upon both air-flow and agitation (see Table I).

Table IV shows the effects of agitator-speed and

aeration-rate upon the oxidative fermentation of glucose by *E. coli*. Incubations were carried out in both aeration with air-flow and agitation by propellers. The media contained 3~11% glucose, the amounts of ammonium-nitrogen and other mineral matters as already mentioned. It is worth to note that a much lower yield of α -ketoglutaric acid was generally observed when

the accumulation of pyruvic acid was increasing (see Table V). α -Ketoglutaric acid-fermentation took place under the effective aeration rates of about 0.7~3.0 mM O_2 per liter per minute. Increasing the effective aeration rate from about 0.7 to 3.0 mM O_2 per liter per minute resulted in accelerating the fermentation rate. Table VI shows the cell-synthesis in *E. coli* inducing α -ketoglutarate-fermentation. It is evident that α -ketoglutarate-fermentation is always accompanied with the remarkable production of the bacterial cell, although the fate of glucose-carbon is greatly dependent upon the C/N value of a culture medium as previously reported. It may now be suggested that both pyruvate and α -ketoglutarate are the starting substance in the oxidative assimilation in microorganisms. Some workers have indicated the principal role of pyruvate in oxidative assimilation^{13,19}.

Manometric Experiments. From the results hitherto mentioned, the following facts have been found; if *E. coli* is incubated in the presence of glucose under such low levels of available oxygen as to give effective aeration of about 0.1~0.4 mM oxygen per liter per minute, there are the increasing amounts of acetate and bacterial cells against the decreasing amount of lactate, while α -ketoglutarate-fermentation takes place under high levels of oxygen (about 0.7~3.0 mM O_2 per L per min.). It

is now of interest to ascertain the mode of oxidation of various substrates with a Warburg technique. Experiments were at first carried out with glucose in the following manner: the reaction mixture (2.5 ml) contained 100~500 μ M phosphate buffer (pH:5.6), 4 μ M $MgSO_4$, 10~50 μ M glucose and 10~40 mg washed cells of *E. coli* (G-2 strain) grown aerobically on glucose, and in the centre well, 0.2 ml H_2O or 15% KOH. Incubations were carried out at 30°C in air. The efficiency of aeration of the Warburg manometer employed here was ascertained to be about 0.15~0.35 mM oxygen per liter per minute. The results of experiments are given in Table VII, from which it will be seen that the bacterial cells oxidize most of glucose to carbon dioxide and water with a great readiness, but there is little or no formation of α -ketoglutarate to be observed. The complete oxidation and the oxidative assimilation of glucose may proceed according to the following equations^{20,21}:

Complete oxidation

Oxidative assimilation

Similar experiments were carried out with pyruvate; Warburg vessel contained 100~500 μ M phosphate buffer (pH:6.0) 4 μ M $MgSO_4$, 10~40 mg washed cells of *E. coli* grown aerobically on glucose and the indicated

TABLE VII. MANOMETRIC OBSERVATION ON OXIDATION OF GLUCOSE BY WASHED CELLS OF *E. coli* GROWN AEROBICALLY ON GLUCOSE

Exp. No.	Glucose consumed (μ M)	O_2 consumed (μ M)	CO_2 evolved (μ M)	α -Ketoglutaric acid found (μ M)
I	10.0	42.5	41.5	0
II	10.0	35.5		0
III	10.0	46.5	43.0	0
IV	20.0	90.0	88.5	0
V	50.0	170.5		0.6

Incubation for 2~6 hours.

TABLE VIII. MANOMETRIC OBSERVATION ON OXIDATION OF PYRUVATE BY WASHED CELLS OF *E. coli* GROWN AEROBICALLY ON GLUCOSE

Exp. No.	Pyruvic acid		O_2 consumed (μ M)	CO_2 evolved (μ M)	α -Ketoglutaric acid found (μ M)
	added (μ M)	consumed (μ M)			
I	10.0	10.0	20.5	21.0	Trace
II	10.0	10.0	16.7	17.0	0
III	20.0	20.0	31.1	30.5	0.5
IV	60.0	60.0	87.5		1.5
V	100.0	100.0	130.5		2.5

Incubation for 2~8 hour.

amounts of Na-pyruvate, and in the centre well, 0.2 ml H₂O or 15% KOH and in the side arm, 0.2 ml 4N H₂SO₄; total volume, 2.5 ml; incubation at 30°C in air. Table VIII shows that the pyruvate is mainly oxidized to carbon dioxide and water, although a part of substrate-carbon may be assimilated by the organism. In the previous papers of this series, the authors have demonstrated that C₃-acids such as pyruvate and lactate are a potent precursor in the formation of α -ketoglutarate under the high levels of oxygen (also see Table I~III). It will, however, be seen in Table VIII that when the oxidation of pyruvate proceeds at such low levels of available oxygen that aeration is carried out with a Warburg-respirator, little or no formation of α -ketoglutarate occurs.

The bacteria of *coli-aerogenes* are well known to oxidize acetate to carbon dioxide and water^{7, 20-22}. The organism used in this work was also ascertained to reveal an exceedingly high ability of oxidizing acetate to carbon dioxide and water. The inhibiting effects of arsenite on the oxidation of pyruvate and acetate are shown in Table IX, in which experiments were carried out with

TABLE IX. INFLUENCE OF ARSENITE UPON OXIDATION OF PYRUVATE AND ACETATE BY WASHED CELL OF *E. coli* GROWN AEROBICALLY ON GLUCOSE

Substrates added (μ M)	Na-arsenite added (M/l)	O ₂ consumed (μ M)
Pyruvate		
10.0	0	16.7
10.0	4×10^{-2}	1.8
Acetate		
10.0	0	15.5
10.0	4×10^{-2}	0

Each vessel contained 150 μ M phosphate buffer (pH 6.2), 4 μ M MgSO₄, 5 mg washed cells of *E. coli* (G-2 strain) and stated amounts of Na-arsenite and substrates; total volume 2.5ml; incubation for 2 hours at 30°C in air.

a glucose-grown cell in the similar manner as already mentioned. Table X also shows the oxidative activities towards various substrates of *coli-aerogenes*. The equations for the complete oxidations and oxidative assimilations of several organic acids by washed cells of *E. coli* may be summarized according to Clifton et al.^{20, 21} as follows:

TABLE X. OXIDATION OF VARIOUS ORGANIC ACIDS BY *Coli-aerogenes* BACTERIA

Bacteria used	Washed cells of <i>E. coli</i> grown aerobically on glucose-medium (Qo ₂)	Dried cells of <i>E. coli</i> grown aerobically on acetate-medium (Qo ₂)	Dried cells of <i>A. aerogenes</i> grown aerobically on citrate-medium (Qo ₂)
Substrates			
Acetate	38	30	30
Pyruvate	50	45	40
DL-Lactate	60		
DL-Malate	65	48	55
Fumarate	55	40	48
Succinate	68	55	63
α -Ketoglutarate	Ground cell 6	5	7
Citrate	Ground cell 7	6	38

DISCUSSION

It has frequently been observed by many workers that molecular oxygen which is available, produces a striking effect upon the mode of metabolisms of substrates in micro-organisms. In the case of respiration, it has been recognized that the presence of free oxygen is indispensable for the operation of respiratory system and the metabolic rate depends upon the amount of available oxygen. On the other hand, the tricarboxylic acid cycle is generally believed to be the most fundamental mechanism for the oxidation of acetic acid.

From the results of investigations mentioned both in previous and present papers, the authors have indicated the following important points. (1) A gentle aeration suppresses the formation of lactate but increases the production of acetate, carbon dioxide and the bacterial cell, when *E. coli* is grown in the presence of glucose. (2) When the bacterial cells of *coli-aerogenes* are incubated with a gentle aeration, i.e., under low levels of available oxygen, not only carbohydrates, C₃-, C₄- and C₆- acids but also C₂- acid (acetate) are mainly oxidized to carbon dioxide and water, while, α -keto-

under the low levels of oxygen. Thus, the metabolisms of substrate-carbon in *coli-aerogenes* may be approximately summarized as follows:

(I-a) Lactic acid-fermentation

(I-a) Lactic acid-fermentation

(I-b) Fermentative assimilation of substrate-carbon

(II-a) Acetic acid-fermentation

(II-b) Oxidative assimilation of substrate-carbon

(II-c) Complete oxidation of substrate-carbon

(III-a) α -Ketoglutaric acid-fermentation

$$\text{C}_6\text{H}_{12}\text{O}_6 \xrightarrow{+10/8 \text{ O}_2} 2/3 \text{C}_5\text{H}_6\text{O}_5 + 8/3 \text{CO}_2 + 4\text{H}_2\text{O}$$

(III-c) Oxidative assimilation of substrate-carbon

(IV) Oxidative fermentation in the presence of inhibitors

(IV-a) Pyruvic acid-fermentation

(IV-b) Acetic acid-fermentation

Results hitherto mentioned may throw some light on the problem of the evolution of metabolic pattern in living organism.

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Studies on Oxidative Fermentation

Part XIX. Catabolism of K-5-Ketogluconate by *Acetobacter suboxydans*. II*

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Oxidation products of 5-ketogluconic acid by intact cells of *Acetobacter suboxydans* ATCC 621 were investigated further. Besides acetic, succinic, α -ketoglutaric and glycolic acids reported previously, a little amount of pyruvic acid was newly recognized in the oxidation products of K-5-ketogluconate. Members of the tricarboxylic acid cycle except oxalacetic acid, and acetic, L-tartaric and glycolic acids were not oxidized, while glycolaldehyde was oxidized quantitatively to glycolic acid by this organism. From these results, oxidation pathway of 5-ketogluconic acid was discussed.

It is well known that glucose is oxidized to 2-ketogluconic acid (2-KGA), 5-ketogluconic acid (5-KGA) or both of them via gluconic acid by *Acetobacter* and allied oxidative bacteria. It is considered to be important to clarify the further oxidative pathways of these ketogluconic acids. Several papers¹⁻⁶⁾ have appeared on the metabolic route of 2-KGA, however, little attention

has been paid to the oxidative route of 5-KGA. The authors studied this problem to elucidate metabolic pathway of 5-KGA and in the previous paper⁷⁾ it was ascertained that 5-KGA was oxidized by intact cell suspension of *Acetobacter suboxydans* ATCC 621, and acetic, succinic, glycolic acids and α -ketoglutaric acid (α -KGA) were confirmed as the oxidation products. The experiments in this paper were carried out in order to detect pyruvic acid, (supposed to be a precursor of acetic, succinic acids and α -KGA) and to clarify the roles of these organic acids in metabolism of 5-KGA.

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