Aerobic Catabolism of Glucose in Lactate Forming *Rhizopus oryzae*

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Certain species of *Rhizopus* produces L (+) lactate in a high yield from glucose both aerobically and anaerobically. KITAHARA *et al.*[1] obtained 2 moles of lactate per mole of hexose consumed in the presence of $PbCO_3$ proposing that lactate fermentation by the genus *Rhizopus* is homofermentative. From the studies on the distribution of ^{14}C of the lactate derived from labeled glucose, GIBBS *et al.*[2] and MARGULIES *et al.*[3] are in agreement with the concept that the mold converts glucose into lactate under both aerobic and anaerobic conditions through the EM pathway. The latter authors ascertained, with pad culture, that neither aerobic nor anaerobic culture affects the yield of lactate.

Although the EM pathway is not restricted to anaerobic condition little information concerning the correlation between aerobiosis and lactate formation has been provided.

In the experiment undertaken to clarify the nature of the respiration of the mold, the authors[4] found that the respiration in the early stage of shaking culture was completely inhibited by KCN and that the following growth rendered the nature of the respiration to be resistant to KCN, concomitantly producing lactate abundantly. In other words, when any respiratory system other than cytochrome-involving one is operative, lactate is to be accumulated.

The studies to be reported provide an information on whether or not the C-1 ~ C-3 moiety and the C-4~C-6 moiety of glucose molecule convert, on the same level, to a common pool of triose followed by lactate formation. For this purpose, aerobic CO_2 evolution from differentially labeled glucose was observed, and ^{14}C migrations into the lactate and fumarate derived from labeled glucose or ribose in the aerobic incubation were determined. From the results were discussed the pathways of aerobic catabolism of glucose in respect to lactate and fumarate formation.

MATERIALS AND METHODS

One strain of *Rhizopus oryzae*[5] producing lactate as the principal catabolite was used throughout this work. The organism was incubated at 30°*C*. The medium contained 5*g* glucose, 0.2*g* $(NH_4)H_2PO_4$, 0.2*g* KH_2PO_4, 0.05*g* $MgSO_4 7H_2O$ and 0.001*g* $FeSO_4 7H_2O$ in 100 *ml* of tap water (*pH* 5.5) and after being autoclaved for 20 min. at 15 *lbs.*, 1*g* $CaCO_3$, sterilized separately, was added. The medium of the same components was used as agar slant for stock culture after the addition of 2.5 % agar. The culture for

obtaining mycelia was started by seeding spores harvested after one week's incubation on the agar slants and incubated for about 28 hr. on a reciprocating shaker at 110 strokes per min. until all $CaCO_3$ disappeared. The *pH* value of the medium after incubation was about 4.5. When the value was made to be below 3 by the suppressed addition of $CaCO_3$, the rate of sugar consumption in the washed-cell culture decreased.

The washed-cell incubation:

Mycelia harvested by filtration from the growing culture were washed three times with sterile water or with 1/100 *M* ascorbic acid solution, which was effective to shorten the lag-period of washed-cell culture. Washed mycelia were suspended in 0.2 *M* phosphate buffer (*pH* 6.0) containing chloramphenicol (1 : 10,000) and homogenized for 10 sec. in a Waring blendor at 20,000 rpm. An aliquot of the homogenized mycelia was pipetted into an Erlenmeyer flask containing reaction solution. Each *ml* of the reaction solution in a flask contained, in most cases, 2~5 *mg* mycelia on dry basis.

Unless otherwise specified, 300-*ml* Erlenmeyer flask containing 25 *ml* medium under a rubber-stopper was shaken for aerobic incubation. This condition gave products similar to those obtained under cotton plug. In order to determine CO_2 produced in the closed culture-system after completion of reaction, two tubes were passed through the rubber stopper, the one was an inlet of both H_2SO_4 and CO_2-free air stream and the other was an outlet of CO_2-containing polluted air which was connected to the absorbing-tubes containing sodium hydroxide. The amount of glucose per one flask of the washed cell culture was, in most cases, around 500 *mg*. After incubation at 30°*C* for 20 hr. or, at the time when the cells washed with ascorbic acid were used, for 6 hr. during which period about two thirds of the glucose added were consumed, the reaction mixture was acidified by the addition of 1/10 *N* sulfuric acid through the inlet tube, and CO_2 freed was absorbed into the alkaline traps. CO_2 absorbed was treated with saturated $BaCl_2$ solution. The resultant $BaCO_3$ was filtered on a Buchner funnel and successively washed with water, ethanol and ethylether. For the test of incorporation of $^{14}CO_2$, $Na_2{}^{14}CO_3$ solution, prepared from $Ba^{14}CO_3$ by the addition of a slight excess of Na_2SO_4, was added at the start of incubation.

Treatment of the medium cultured with washed cells:

The acidified mixture in the reaction-flask was filled up to 100 *ml* with water, and filtered from the mycelia. An aliquot of the filtrate was used for the determinations of glucose, lactate and fumarate, and another aliquot was supplied, after perfect oxidation, for the determination of radioactivity. Ether extract of one more aliquot by Soxhlet apparatus, after removal of the solvent, was treated preparatively by paperchromatography using a solvent system consisting of *n*-butanol-formic acid-water (10 : 2 : 15 V/V), and the respective bands of lactic and fumaric acids were cut out and separately eluted with hot water. The acids were also separated on a silica gel column using 5 % *n*-butanol-chloroform, 25 % *n*-butanol-chloroform and 35 % *n*-butanol-chloroform successively.[6] The respective eluates were concentrated to a definite volume and were used for the determination of the radioactivities of the respective acids. Perfect oxidation was carried out with the Van Slyke-Folch mixture, and partial degradations of lactic and fumaric acids followed "Methods in Enzymology".[7] Radioactivities of $BaCO_3$ were counted by a thin window Geiger-Müller tube (Radiation counter model 100 Kaken). They were corrected for self-absorption when necessary, and corrected also for the dilution

resulted from the addition of unlabeled carrier. Glucose and ribose were assayed according to the Somogyi method modified by KOBAYASHI and TABUCHI.[8] Lactic acid was determined according to BARKER-SUMMERSON.[9] Oxidation methods were applied for the determinations of fumaric acid[10] and alcohol.[11]

Chemicals:

$Ba^{14}CO_3$, labeled glucose and labeled ribose, were supplied by the Radiochemical Center, England, and some of D-glucose-1-^{14}C and -6-^{14}C by Daiichi Kagaku Yakuhin KK., Japan.

RESULTS

CO_2 evolution from the differentially labeled glucose.

Determination of CO_2 evolution from glucose-1-^{14}C, -2-^{14}C and -6-^{14}C makes the existence of an alternative pathway certain. However, mold is characteristic of its high endogenous respiration, which complicates the experimental study. Moreover, it is very difficult to get rid of the endogenous respiration. Therefore, using glucose-U-^{14}C, the rate of CO_2 evolution from only the exogenous glucose was determined. The results were presented in Table 1. CO_2 from the endogenous substrate is 6.4 % (=100−93.6). CO_2 from the C-1 position of glucose is 17.3 % and that from the C-6 position is 3.8 %, the former being 4.6 times as much as the latter. Operation of the pentose phosphate (PP) pathway seems to account for the larger release of C-1 as CO_2.

Table 1. CO_2 evolution from differentially labeled glucose

Glucose supplied	Apparent CO_2 evolved (molar ratio)	^{14}C recovery as $^{14}CO_2$ (%)	True CO_2 from exogenous glucose (molar ratio)	Calculated CO_2 from respective C of glucose (*m* moles)	CO_2 from respective C of glucose (%)
Glucose-U-^{14}C*1	1.08	93.6	1.01		
Glucose-1-^{14}C*2	1.04	16.2	0.97	0.17	17.3
Glucose-2-^{14}C*3	1.04	5.1	0.97	0.05	5.4
Glucose-6-^{14}C*4	1.00	3.6	0.94	0.04	3.8
Average of the above three			0.96		
Glucose-5-^{14}C		5.1*5		0.05*5	5.4*5
Glucose-3,4-^{14}C		63.6*5		0.65*5	67.9*5
Total		93.6		0.96	99.9

Incubation : 20 hr.

*1 Average of 2 experiments
*2 Average of 6 experiments
*3 Average of 5 experiments
*4 Average of 5 experiments
*5 Expected or calculated figures

The portion of the glucose metabolized via the PP pathway is, on a rough calculation, 0.13 (=0.17−0.04) *m* moles per *m* mole glucose consumed, *i. e.*, 13 %, if the CO_2 evolved is not incorporated again in the metabolites.

The result that CO_2 from the C-2 position is 5.4 % indicates that the pentose derived via the PP pathway has not been driven into the pentose phosphate cycle.

Furthermore, CO_2 from the C-3 and C-4 positions is calculated to be 67.9 %, with the fixed indication that the most vigorous CO_2 evolution is brought from the positions of C-3 and/or C-4 of glucose.

Radioactivities of the lactate produced from the differentially labeled glucose.

When lactate is formed from glucose via only the EM pathway, the radioactivities of the lactate formed from the differentially labeled glucose is to be the same, regardless of the ^{14}C position in glucose. Table 2 shows that the relative specific radioactivities of the lactate derived from glucose-1-^{14}C, -2-^{14}C and -6-^{14}C were 40.5 %, 48.8 or 51.7 % and 58.5 or 55.6 %, respectively. Futhermore, the relative specific radioactivities of the β-carbon of the lactate derived from glucose-1-^{14}C and of the α-carbon of that from glucose-2-^{14}C were 34.8 % and 42.1 or 47.9 %, respectively; while those of the β-carbon of the lactate derived from glucose-6-^{14}C were 52.8 and 51.8 %. Of particular interest is the fact that the former values are lower and the latter are a little higher than 50 %, the relative specific radioactivity of labeled carbon of the lactate derived via only the EM pathway. This means that the radioactivity of glucose-6-^{14}C migrated into lactate more predominantly than that of glucose-1-^{14}C or-2-^{14}C, though the major route of lactate formation was fixed to be the EM pathway because of the ^{14}C distributions of the lactate. The result is of significance in relation to the experimental result in Table 1. Such an unhomogeneity of the ^{14}C migration into lactate between the C-1∼C-3 moiety of glucose and the C-4∼C-6 one was assumed to have resulted from the PP pathway observed in the experiment of CO_2 evolution from the differentially labeled glucose.

The ^{14}C distribution of the lactate derived from glucose-2-^{14}C also practically excludes the possibility of the pentose phosphate cycle. It is noteworthy that the carboxyl carbon of the lactate contains a certain amount of radioactivity regardless of the ^{14}C position of glucose used. It will be discussed later.

Radioactivities of the fumarate produced from the differentially labeled glucose.

Table 2 shows also the ^{14}C migration into fumarate. The relative specific radioactivities of the fumarate were lower than 50 % irrespective of the ^{14}C position in glucose. The result may be assumed to be brought about from endogenous origin. It appeared that there is little difference of the ^{14}C migration into the fumarate between glucose-1-^{14}C and -2-^{14}C, the least migration being shown in the case when glucos-6-^{14}C was used as a substrate.

It is evident from the ^{14}C distribution of the fumarate in Table 2 that the radioactivities of the fumarate are concentrated in the methin carbons, irrespective of the ^{14}C position of glucose used, except that, when glucose-2-^{14}C was supplied, about one third of the radioactvity was found in the carboxyl carbons. This suggests that fumarate formation of the mold does not necessarily follow only one pathway.

Radioactivities of the lactate and fumarate derived from ribose-1-^{14}C.

Table 2. ^{14}C migration into lactate and fumarate from labeled glucose

Starting medium

	Glucose-1-$^{1}C^{14}$	Glucose-2-^{14}C No. 1	Glucose-2-^{14}C No. 2	Glucose-6-^{14}C No. 1	Glucose-6-^{14}C No. 2
Glucose consumed					
m mole	1.675	1.744	1.31	1.73	1.316
total cpm	21,300	129,030	79,910	153,300	230,700
sp. act.(cpm/*m* mole)	12,720	73,990	61,000	88,600	175,300
Mycelia added					
total *mg* C	46.3	60.6	27.7	41.1	27.7

Products

	Glucose-1-^{14}C	Glucose-2-^{14}C		Glucose-6-^{14}C	
Mycelia					
total mg C	53.5	57.9	32.7	45.8	33.2
total cpm	2,140	5,340	7,150	12,650	20,090
% cpm	10.0	4.1	8.9	8.3	8.7
CO_2					
m mole	2.14	2.31	0.645	1.15	0.614
total cpm	4,270	4,000	3,900	5,880	4,900
% cpm	20.0	3.1	4.9	3.8	2.1
Lactate					
m mole	1.465	1.844	1.43	1.65	1.45
molar ratio	0.87	1.06	1.09	0.95	1.1
total cpm	7,550	66,600	45,100	85,570	141,320
% cpm	35.4	51.6	56.4	55.8	61.3
sp. act.(cpm/*m* mole)	5,150	36,110	31,540	51,850	97,460
rel. sp. act.*	40.5	48.8	51.7	58.5	55.6
^{14}C distribution C-1 : C-2 : C-3	12.2 : 1.8 : 86.0	10.7 : 86.2 : 3.1	4.5 : 92.6 : 2.9	8.8 : 0.9 : 90.3	5.2 : 1.6 : 93.2
rel.sp. act. of C-3 or C-2	34.8 in C-3	42.1 in C-2	47.9 in C-2	52.8 in C-3	51.8 in C-3
Fumarate					
m mole	0.62	0.47	0.42	0.485	0.42
molar ratio	0.37	0.27	0.32	0.28	0.32
total cpm	3,450	14,850	9,260	14,180	20,580
% cpm	16.2	11.5	11.6	9.2	8.9
sp. act.(cpm/*m* mole)	5,580	31,600	22,000	29,240	49,000
rel. sp. act.*	43.9	42.7	36.1	33.0	28.0
^{14}C distribution COOH : CH	3.2 : 96.8		25.5 : 74.5		1.0 : 99.0

Incubation : 20 hr.

$$* \frac{\text{Specific activity of the respective acids}}{\text{Specific activity of glucose}} \times 100$$

With ribose-1-^{14}C as a substrate the foregoing results summarized in the fact that the pentose phosphate cycle is not operative in the mold were confirmed. The information of the radioactivities of the lactate and fumarate derived from ribose-1-^{14}C is also very valuable for the evaluation of the pathways of lactate and fumarate formation proposed with the differentially labeled glucose.

From the experimemts it may be noted that in spite of the high yield of the lactate formed per ribose consumed, as shown by molar ratio of 0.86 in Table 3, the migration of

radioactivity into the lactate was low, being 8.39 % of the glucose consumed and the relative specific radioactivity of the lactate was about one tenth of the ribose supplied; while the migration of radioactivity into the fumarate reached to 15.09 %, notwithstanding its low yield as indicated by molar ratio of 0.07, and the relative specific radioactivity was about twice as much as that of the ribose. This means that the lactate was preferentially derived from the C-3~C-5 portion of ribose while the fumarate was derived from the C-1~C-2 portion of it.

Table 3. ^{14}C migration into lactate and fumarate from ribose-1-^{14}C

	Ribose consumed	Mycelia added
Starting medium		
m mole	1.99	not measured
total cpm	83,840	
sp. act. (cpm/*m* mole)	42,130	

Products	CO_2	Lactate	Fumarate	Medium except lactate and fumarate	Mycelia
m mole	1.65	1.71	0.41		
molar ratio		0.86	0.07		
total *mg*					29.8
total cpm	5,500	7,030	12,650	49,870	8,360
% cpm	6.56	8.39	15.09	59.5	9.97
sp. act. (cpm/*m* mole)	3,330	4,110	90,360		
rel. sp. act.	7.9	9.8	214.5		
^{14}C distribution		C-1 : C-2 : C-3			
		98.5 : 0 : 1.5			

Incubation : 6 hr.
Medium : 100 *ml* in a 5*l*-flask

The radoactivities of the lactate and the fumarate might be compatible with the assumption that the ribose was cleaved into two portions at the joint of C-2 and C-3, and the C-3~C-5 portion was transferred to lactate while the C-1~C-2 portion migrated into fumarate by some condensation of two moles of the portion.

Furthermore, the ^{14}C activity in the lactate was concentrated in only the carboxyl carbon of it, with the result also expelling the formation of lactate through the pentose phosphate cycle.

CO_2 fixation in lactate and fumarate.

It has been noted that the carboxyl carbon of the lactate in Table 2 contains a certein amount of radioactivity regardless of the ^{14}C position of glucose used. The fact is not to be expected from the EM pathway. GIBBS *et al.*[2] indicated that aerobically there is an increase of radioactivity in the carboxyl carbon of lactate, presumably due to CO_2 fixation reaction. FOSTER *et al.*[12] and SAKAGUCHI *et al.*[13] also observed that some CO_2 entered into lactate molecule.

The washed-cell culture was performed in the presence of both unlabeled glucose and

$Na_2{}^{14}CO_3$ to determine the difference of the extents of $^{14}CO_2$ incorporation between lactate and fumarate. As shown in Table 4, under the condition employed, the mold incorporated into lactate and fumarate 1.8 % and 7.0 % of the $^{14}CO_2$ initially supplied, respectively, notwithstanding that the molar ratios of the both acids produced to the glucose consumed are 1.03 in the lactate and 0.13 in the fumarate. ^{14}C was situated only in the carboxyl carbon of either lactate or fumarate. It may be noted that the fumarate and the malate were not only highly labeled but were in isotopic equilibrium with each other.

In view of the high incorporation of the ^{14}C into the fumarate and malate, as the rates of CO_2-C in the fumarate and malate were shown in Table 4 to be 13.8 % and 13.6 %, respectively, in spite of the production of metabolic CO_2, it is apparent that the fumarate formation involves a net fixation of CO_2, presumably due to the Wood-

Table 4. CO_2 incorporation

Starting medium

	Glucose consumed	CO_2 supplied	Mycelia added
m mole	2.36	1.12	
total *mg* (*mg* C)			268(118)
total cpm		628,000	
sp. act (cpm/*m*mole)		560,000	

Products

	CO_2	Lactate	Fumarate	Malate	Mycelia
m mole	3.73	2.42	0.31	0.09	
molar ratio		1.03	0.13	0.038	
total *mg* (*mg* C)					250(103)
total cpm	531,600	11,300	44,100	12,500	17,900
% cpm		1.8	7.0	2.0	
sp. act.(cpm/*m*mole)		4,670	142,300	138,900	
^{14}C distribution		100 (COOH)	100 (COOH)		

Incubation : 20 hr.

Calculated table

	Lactate	Fumarate	Malate
CO_2 picked up (*m* mole) *1	0.044	0.171	0.049
CO_2-C calculated (%) *2	0.61	13.8	13.6
〃 theor.max .(%)	33.3	25.0	25.0

*1 $$\frac{m \text{ mole of initial } CO_2 \times \text{dilution factor} \times \text{total cpm of the product}}{\text{total cpm of the original } CO_2 \text{ supplied}}$$

here, dilution factor: 2.17

*2 $$\frac{m \text{ mole } CO_2 \text{ picked up into the product}}{m \text{ mole of the product} \times 3 \text{ or } 4}$$

here, 3 for lactate, 4 for fumarate and malate

Werkman reaction. On the other hand, the rate of CO_2-C in the lactate was only 0.61 %, and the ^{14}C was concentrated in only the carboxyl carbon of it. The small incorporation of external CO_2 into lactate, presumably due to some exchange mechanism, might account for the ^{14}C migration in a certain amount observed in the lactate derived from the differentially labeled glucose, since the labeled glucose evolves labeled CO_2, more or less, as shown in Table 1 and the metabolic CO_2 may be expected to be incorporated at a faster rate than external CO_2.

DISCUSSION

In regard to the aerobic formation of lactate in *Rhizopus oryzae*, the data of ^{14}C migration into the lactate from the differentially labeled glucose are in accord with the conclusion that the major route of the lactate formation is the EM pathway, as demonstrated formerly by GIBBS *et al.*[2] and subsequently by Margulies *et al.*[3]

However, the predominant evolution of CO_2 from the C-1 position over that of CO_2 from the C-6 position, and the migration of ^{14}C into the lactate from glucose-6-^{14}C in a larger quantity than that of glucose-1-^{14}C or glucose-2-^{14}C indicate that the C-1～C-3 moiety of glucose and the C-4～C-6 moiety do not behave in equimolecular ratio. Thus, the C-4～C-6 moiety transfers more predominantly to lactate than the C-1～C-3 moiety, as indicated in Table 2. However, such an unhomogeneity is not so large. The quantity of the glucose catabolized along the PP pathway is roughly 0.13 *m* moles per *m* mole glucose consumed, as shown in Table 1. The resultant pentose, being 0.13 *m* moles, gives rise to the unhomogeneity. If the pentose is to be catabolized according to the metabolism of ribose-1-^{14}C in Table 3, 0.11(=0.13×0.86) *m* moles lactate and 0.01 (=0.13×0.07) *m* moles fumarate are to be produced from the C-3～C-5 portion and the C-1～C-2 portion of the pentose, respectively.

That is, the extra lactate, 0.11 *m* moles, is to be added to the lactate produced from the C-4～C-6 moiety of the common pool of triose derived via the EM pathway from glucose. This might account for the larger radioactivity of the lactate derived from glucose-6-^{14}C, contrary to the lesser radioactivity of the lactate derived from glucose-1-^{14}C or -2-^{14}C. Since the pentose produced through the PP pathway from glucose forms only a minute quantity of fumarate, the bulk of fumarate might be produced by other pathways. With regard to fumarate formation two pathways have been proposed. The one is the Wood-Werkman reaction which includes CO_2 fixation.[12,14] The other is some condensation reaction of 2 moles of a 2-carbon compound, the Thunberg-Wieland reaction or the glyoxylate pathway[15,16] being the case.

The result in Table 2 that the radioactivities of the fumarate derived from the differentially labeled glucose were concentrated in the methin carbons of the acid, irrespective of the ^{14}C position of the labeled glucose, is consistent with the assumption that the fumarate was produced via the Wood-Werkman reaction from the C-1～C-3 and C-4～C-6 moieties of glucose, excluding the possibility of a condensation reaction of a 2-carbon compound, such as $\overset{1\ 2}{C\text{-}C}$ or $\overset{6\ 5}{C\text{-}C}$ derived from $\overset{1\ 2\ 3\ 4\ 5\ 6}{C\text{-}C\text{-}C\text{-}C\text{-}C\text{-}C}$. However, the experimental data that one third of ^{14}C of the fumarate derived from glucose-2-^{14}C was found in the carboxyl carbons, might result from the condensation reaction, in which 2 moles of

$\overset{1\ 2}{C\text{-}C}$ may be condensed to $\overset{2\ 1\ 1\ 2}{C\text{-}C\text{-}C\text{-}C}$, containing 2 atoms of ^{14}C in one molecule. Formation of such a 2-carbon compound might be borne out by the most active evolution of CO_2 from the C-3 or C-4 position, as shown in Table 1. According to the assumption, molar ratio of the fumarate formed by the condensation reaction to the fumarate formed by the Wood-Werkman reaction is 1 : 6, this corresponding 1 : 3 in the ratio of radioactivity.

^{14}C in the carboxyl carbon of the lactate derived from the labeled glucose might originate from the incorporation of metabolic CO_2, as demonstrated by the experiment in which unlabeled glucose was provided in the presence of $^{14}CO_2$. Since only a small quantity of CO_2 was picked up into lactate contrary to fumarate as shown in Table 4, it appears reasonable to assume that the incorporation of CO_2 into lactate is presumably due to the pyruvate-CO_2 exchange as in *Clostridium thermoaceticum*.[17,18] CARSON *et al.*[19] observed that lactate-2,3-^{14}C was obtained from ethanol-2-^{14}C or fumarate-2,3-^{14}C in the presence of unlabeled glucose. The observation suggests the existence of the pathway of lactate formation from a 4-carbon compound. However, the possibility appears to be least reliable from the present experiment.

SUMMARY

1. With washed cells of *Rhizopus oryzae* aerobic catabolisms of the ^{14}C-labeled glucose and ribose-1-^{14}C were studied.

In the experiment of CO_2 evolution from the labeled glucose, about 13 % of CO_2 was observed to evolve by the PP pathway, although the most active evolution of CO_2 was brought from the positions of C-3 and/or C-4.

2. Although the major route of lactate formation from glucose was confirmed to be the EM pathway, the C-1～C-3 moiety and the C-4～C-6 moiety of glucose was not necessarily transferred to lactate in equimolecular ratio. Since about 13 % of glucose is cleaved via the PP pathway without recycling and thereafter only the C-4 ～C-6 portion of the original glucose transfers to lactate, some predominant formation of lactate is assumed to take place out of the C-4～C-6 moiety of glucose.

3. Fumarate was demonstrated to be produced by two pathways, the Wood-Werkman reaction and a condensation reaction of a 2-carbon compound. The fumarate from glucose is principally formed by 1) the Wood-Werkman reaction of CO_2 fixation into the C-1～C-3 and C-4～C-6 moieties and 2) a condensation reaction taking place in a way such as $\overset{1\ 2}{C\text{-}C} \longrightarrow \overset{2\ 1\ 1\ 2}{C\text{-}C\text{-}C\text{-}C}$, the ratio being 3 : 1 in radioactivity. The fumarate formation from ribose was brought about only via a condensation reaction of 2 moles of 2-carbon compound derived from C-1～C-2 portion of ribose.

4. ^{14}C in the carboxyl of the lactate derived from the differentially labeled glucose or from unlabeled glucose in the presence of $^{14}CO_2$ was suggested to be a pyruvate-CO_2 exchange reaction.

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