METABOLIC REACTIONS OF PASTEURELLA PESTIS

I. Terminal Oxidation

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Received for publication June 15, 1953

Knowledge of the metabolism of *Pasteurella pestis* remains fragmentary and indirect, despite the fact that plague has long been the subject of considerable research. Information concerning the nutritional requirements of *P. pestis* can be found in the reports of Rao, 1940*b*; Doudoroff, 1943; Hills and Spurr, 1952; Rockenmacher *et al.*, 1952. Completely lacking are quantitative metabolic data on nonproliferating, resting cells.

It is known that resting cells of *P. pestis* will oxidize a variety of organic compounds including carbohydrates, amino acids, and several metabolically important organic acids (Rao, 1940*a*). Doudoroff (1943) established that growing cultures metabolize glucose via pyruvate and that *P. pestis* will produce lactic, acetic, formic, and succinic acids, ethanol, CO_2, and small amounts of pyruvate anaerobically in complex media containing glucose.

Certain other unrelated aspects of *P. pestis* metabolism have been described recently. The catalase content of this organism and its relation to virulence were studied in some detail by Rockenmacher (1949) and Avi-Dor and Yaniv (1952), and several aspects of its sulfur metabolism have been described by Englesberg (1952). The oxidative dissimilation of serine and pyruvate has been discussed by Levine *et al.* (1952) in a brief report. These authors found that cell-free extracts of *P. pestis* can convert pyruvate to acetic acid.

The purpose of the following is to describe certain aspects of the oxidative metabolism of *P. pestis* in whole resting cells and in cell-free extracts. The respiratory patterns have been followed by the use of intermediates labeled with C^{14} and by conventional spectrophotometric and manometric procedures. These experiments form a part of an investigation of the over-all metabolic pathways and toxin production of the plague bacillus.

MATERIALS AND METHODS

The avirulent Tjiwidej strain of *P. pestis* was used. To obtain large batches of cells twenty-four hour old slants were transferred to 500 ml of trypticase soy broth (BBL) and incubated overnight at room temperature. This culture was inoculated then into 16 liters of broth and incubated for 24 to 48 hours at room temperature with constant aeration. The bacteria were sedimented in the Sharples centrifuge, washed twice with distilled water, and either used immediately or stored in the ice box for 24 to 48 hours before use.

The *Escherichia coli* and *Corynebacterium creatinovorans* used in certain comparative studies were grown on acetate medium described by Ajl and Kamen (1951).

Radioactive *P. pestis* were used to determine the effect of exogenous substrate on their endogenous respiration. These were prepared in the same manner as normal cells except that radioactive glucose was added aseptically to the medium at 6 and again at 12 hours after the final transfer. The glucose had a specific activity of 50,000 disintegrations per minute per mg and was added in 20 mg amounts. In respirometer studies an aliquot of a labeled bacterial suspension was pipetted into each Warburg flask. Respiratory CO_2 was collected in the alkali contained in the center well; the last traces of CO_2 were driven over by tipping acid into the main compartment. After equilibration, the contents of the center well were collected; carrier Na_2CO_3 was added, CO_2 was liberated with acid and passed into a saturated solution of barium nitrate, and the resultant $BaCO_3$ was collected, washed twice with ethanol, dried, weighed, and counted.

For quantitative respiration studies, conventional Warburg methods were used. Respiratory quotients were obtained by the indirect method.

Experiments in which radioactive acetate was used as substrate were of two types. In procedure 1, methyl labeled acetate was incubated in the

presence of one or more unlabeled compounds to which the radioactive intermediate was thought to give rise. In procedure 2, methyl labeled acetate was metabolized in the absence of carriers by large batches of cells, and attempts were made to isolate the intracellular intermediates of acetate oxidation. This procedure was used originally by Saz and Krampitz (1951) and described in detail by Glover *et al.* (1952). Only a brief description will be given here. At the end of the incubation period, the reaction mixture was acidified with 6 N H_2SO_4 to a pH of 3.0 and transferred to three volumes of boiling 95 per cent ethanol. After an additional boiling period of 10–15 minutes, the contents were allowed to stand overnight in the refrigerator. The cells then were centrifuged, taken up in distilled water, and again added to boiling alcohol, after which both fractions were combined. The alcohol plus the residual acetate was removed by steam distillation and the residue ether-extracted for 78 hours. The ethereal layer was analyzed then for di- and tricarboxylic acids.

The isolation procedures for succinate, fumarate, malate, α-ketoglutarate, and citrate have been described by Ajl (1951*a*). α-Ketoglutarate was determined quantitatively by the method of Friedemann and Haugen (1943) and separated chromatographically by a modification of the method of Seligson and Shapiro (1952); citrate concentration was determined by the method of Natelson *et al.* (1948) and degraded with ceric sulfate (Ajl *et al.*, 1952). Succinate was estimated and degraded by the conventional succinoxidase method. In aerobic experiments, air was passed constantly through the reaction mixture in 100 ml test tubes in order to maintain adequate aerobiosis. In anaerobic experiments, O_2-free N_2 was passed constantly through the reaction mixture. In both instances the CO_2 evolved was caught in alkali.

Cell-free preparations were made by the Kalnitsky *et al.* (1945) grinding procedure. Isocitric dehydrogenase and aconitase were separated from the extracts with ammonium sulfate and their activities measured with the Beckman spectrophotometer as described by Barban and Ajl (1952).

Methods for the determination of carbon 14 were as previously described (Ajl, 1951*b*; Ajl and Kamen, 1951).

Adenosine triphosphate, triphosphopyridine nucleotide, diphosphopyridine nucleotide, and barium isocitrate (converted to the sodium salt immediately before use) were obtained from commercial sources.

EXPERIMENTAL RESULTS

Effect of substrate on the endogenous respiration of P. pestis. Preliminary experiments revealed that resting cells of *P. pestis* grown aerobically have a relatively high endogenous respiration; the oxygen uptake in the absence of substrate was 15 to 30 per cent of that in the presence of either glucose or succinate. With so high an endogenous rate it was important for interpretation of manometric data to determine whether or not endogenous values should be subtracted. Inasmuch as it is impossible to distinguish directly between the oxygen needed for endogenous and exogenous metabolism, some information on the course of the endogenous respiration was obtained directly by comparing the release of isotopically labeled CO_2 from labeled resting cells in the presence or absence of unlabeled substrate (Cochrane and Gibbs, 1951). Cells, therefore, usually are grown on a labeled substrate and the respiration studied in the Warburg apparatus. Endogenously produced CO_2 thus will be labeled. If on the addition of unlabeled substrate the total activity of the respired CO_2 is reduced, the extent of such reduction indicates the extent of suppression of the endogenous system by substrate. Further, if there is no change in the total activity of the respired CO_2, it can be interpreted that the two systems operate independently and that the actual measure of oxygen uptake or CO_2 evolution caused by added substrate is the total value minus the endogenous value of comparable cells.

Results of a typical experiment are exhibited in table 1. The data show that concomitant oxidation of glucose, succinate, or pyruvate has no suppressive effect on endogenous respiration, e.g., the total activity of evolved CO_2 is approximately the same for respiring cells irrespective of the presence or absence of substrate. From the O_2 uptake data it is clear that all three substrates were metabolized rapidly. Consequently, for *P. pestis*, it is permissible to correct respiratory gas exchange by subtracting endogenous values.

Oxidation of di- and tricarboxylic acids by resting cells. Resting cell suspensions of *P. pestis* oxidize all members of the tricarboxylic acid cycle. Representative respiratory data are plotted in

TABLE 1

Effect of substrate on endogenous respiration of Pasteurella pestis

SUBSTRATE	OXYGEN UPTAKE	TOTAL ACTIVITY OF EVOLVED CO_2
	μL	*cts/min*
Succinate	2,520	1,070
Pyruvate	1,460	1,190
Glucose	2,001	1,493
Endogenous	540	1,244

Total volume of reactants 30 ml. Each flask contained 1 ml of 0.2 M phosphate buffer, pH 7.0, 2 ml of a 20 per cent suspension of radioactive *P. pestis* (washed twice with distilled water), and 2 ml of 4 N NaOH in center well. Where indicated 100 μM of substrate were added. Temperature of bath 33 C.

the pH from 7.0 to 5.6 did not appreciably affect the rate of oxygen consumption with the possible exception of succinate and malate.

It was desirable to learn whether the fractions of the acids that were metabolized (as evidenced by O_2 uptake) were oxidized to completion. The respiratory quotients with glucose, acetate, citrate, succinate, and α-ketoglutarate, therefore, were determined, and in each instance the experimental R.Q.'s approached the theoretical indicating that *P. pestis* contains all enzymes necessary for the conversion of Krebs' cycle intermediates to carbon dioxide and water.

In an effort to determine the relative metabolic activities of these organisms, Q_{O_2}'s were determined simultaneously for *E. coli* and *P. pestis*. The data shown in table 2 indicate that the respiratory activity of *P. pestis* is much lower than

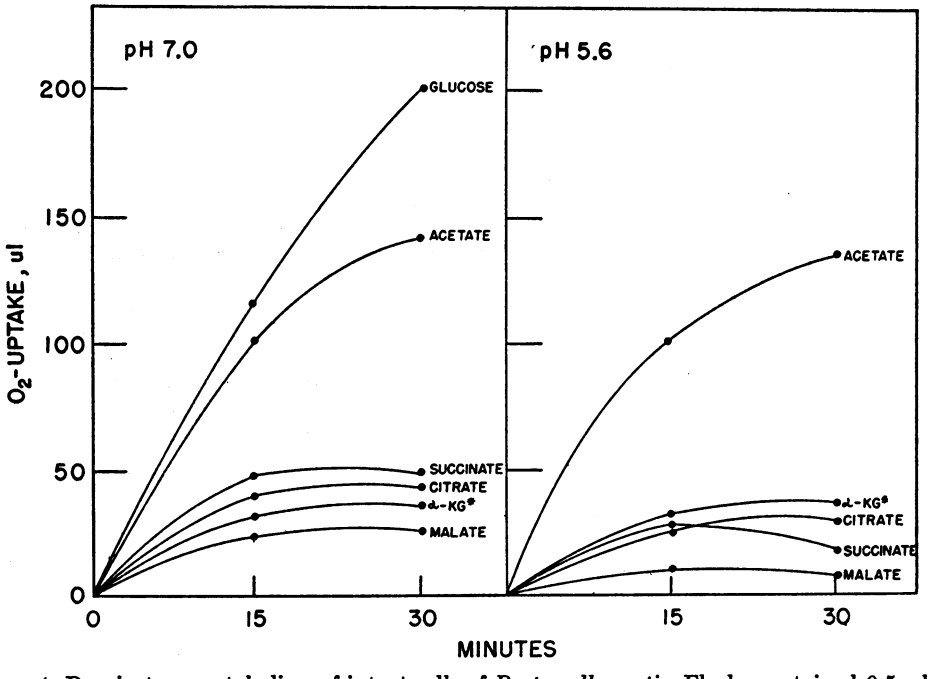

Figure 1. Respiratory metabolism of intact cells of *Pasteurella pestis*. Flasks contained 0.5 ml of 0.1 M phosphate buffer, pH 7.0 or pH 5.6, 50 μM of substrate, 1 ml of a 15 per cent suspension of washed organisms, 0.3 ml of NaOH in center well, and distilled water to volume (2.8 ml). Temperature 33 C. Endogenous values were subtracted.

* *alpha*-Ketoglutarate.

figure 1, with the purpose of giving a picture of the activity of intact bacteria. Citrate, α-ketoglutarate, succinate, and malate are all oxidized at approximately the same rate whereas acetate and glucose are metabolized much faster. Adjusting that of *E. coli*. This low metabolic behavior is reflected in the concentration of intracellular Krebs' cycle intermediates (table 3).

Oxidation of labeled acetate by whole cells of P. pestis in the presence of externally added carriers.

TABLE 2

Comparison between respiratory activities of Pasteurella pestis and Escherichia coli

SUBSTRATE	Q_{O_2} (μL O_2 UPTAKE/MG DRY WEIGHT/HOUR)	
	E. coli	*P. pestis*
Glucose	4.99	1.39
Acetate	4.23	1.25
Succinate	3.53	0.37
α-Ketoglutarate	1.09	0.32
Ribose	3.13	1.00

Total volume of reactants 2.0 ml. Each vessel contained 50 μM of substrate, 0.5 ml of 0.1 M phosphate buffer, pH 7.0, 0.3 ml of NaOH in center well, and distilled water to volume. Temperature 33 C.

TABLE 3

Intracellular concentration of Krebs' cycle intermediates in Pasteurella pestis, Escherichia coli, and Corynebacterium creatinovorans

ORGANISM	FRACTION	CONCENTRATION
		μM
P. pestis	Succinate	47.7
	Citrate	1.5
	α-Ketoglutarate	0.026
E. coli	Succinate	28.8
	Citrate	0.4
	α-Ketoglutarate	Not detected
C. creatinovorans	Succinate	19.3
	Citrate	0.25
	α-Ketoglutarate	Not detected

40 g of cells (wet weight) were extracted in each case.

In each of a series of experiments, labeled acetate was oxidized alone or in the presence of either unlabeled succinate, α-ketoglutarate, or citrate. It was found that when all substrates were metabolized by the bacteria as evidenced by both manometric data and substrate disappearance, acetate carbon was recovered primarily in succinate. The results of four experiments are summarized in table 4.

The salient qualitative results were: (1) a high C^{14} content in the evolved CO_2 when acetate was metabolized alone or in the presence of either α-ketoglutarate or citrate, (2) the low C^{14} content of evolved carbonate when acetate was oxidized in the presence of succinate, (3) the high C^{14} content in succinate, and (4) the absence of detectable C^{14} in α-ketoglutarate and citrate.

TABLE 4

Oxidation of labeled acetate with externally added carriers

EXPT NO.	FRACTION	O_2 UPTAKE	AMOUNT		FINAL SPECIFIC ACTIVITY
			Initial	Final	
		μL	μM	μM	cts/min/μM
1	Acetate		91.5	10	165
	Cells*	3,277			
	Carbonate		0	184	63
2	Acetate		91.5	25.1	129
	Succinate	4,058	100.0	53.7	1,309
	Carbonate		0	205	16.5
	Cells†				
3	Acetate		91.5	27.9	32
	α-Keto-glutarate	3,781	100.0	70	7.1
	Carbonate		0	177	105
	Cells‡				
4	Acetate		91.5	18.9	142
	Citrate	3,553	100.0	70	3.3
	Carbonate		0	189	74
	Cells§				

Total volume of reactants 15 ml. Each vessel contained 3 ml of a 20 per cent suspension of freshly harvested (twice washed) *P. pestis*, 1 ml of acetate-2-C^{14} containing 9×10^4 cts/min, 1 ml of phosphate buffer, 0.2 M, pH 7.0, substrates as indicated, and NaOH in center well. Aerobic. Temperature 33 C. Time of incubation 3 hours.

* Final activity, 16,328 cts/min.
† Final activity, 6,000 cts/min.
‡ Final activity, 10,000 cts/min.
§ Final activity, 11,650 cts/min.

The distribution of labeled carbon after oxidation of methyl labeled acetate by *P. pestis* is shown in experiment 1, table 4. The 5.9-fold dilution of acetate resulting from turnover with endogenous substances should be noted. Radioactive acetate was diluted likewise in the presence of externally added carriers. From experiments 2, 3, and 4 (table 4) it can be seen that oxidation of 91.5 μM of acetate in the presence of either 100 μM succinate, α-ketoglutarate, or citrate, the C_4-

dicarboxylic acid trapped 2.3 μM of acetate carbon, while 0.01 μM of acetate carbon was trapped in α-ketoglutarate and less than 0.006 μM of acetate carbon in citrate.[1]

Oxidation of labeled acetate by whole cells in the absence of added carriers. From the data thus far presented it would appear that resting cell suspensions of *P. pestis* metabolize acetate via succinate and not α-ketoglutarate or citrate. However, activities in externally added carriers may not necessarily reflect the true activities of these intermediates as they occur metabolically inside or outside the cells. Therefore, it was desirable to study the oxidation of labeled acetate in the presence of large amounts of cells (5–10 g of wet weight per experimental flask) without added carriers. Instead, the intracellular intermediates of acetate oxidation would be isolated and their activities studied directly.

Resting cells of *P. pestis* were allowed to metabolize methyl labeled acetate for varying periods of time. At the end of each interval, the cells were disrupted to isolate the intracellular Krebs' cycle intermediates. The data obtained are exhibited in table 5. Superficially, the results derived by this approach appeared to be analogous to those described with externally added carriers. Differences, however, became apparent when specific activities and *not* total activities were considered. From table 5 it is clear that after each time period the activities in the intracellular pools of both succinate and citrate vary within narrow limits. At the end of each incubation period, the cells were found to contain approximately 25 μM of succinate and only 0.171 μM of citrate. The deviations from these average figures were probably due to differences in extraction procedures and other experimental errors. It is reasonable to assume, therefore, that the intracellular pools of these acids remained essentially constant during the time the cells were oxidizing 200 μM of acetate. This is to be expected if the reactions that operate for the oxidative removal of acetic acid are cyclic. It is to be noted, however, that the intracellular pool of succinate is at least 70 times greater than that of citrate. Consequently, if (as was shown) the pools of these acids remain constant, it may be expected that the total activity of succinate will be about 70 times greater than that of citrate. Analysis of the data in table 5 reveals that at least for the first three time periods this is essentially the case. At the 30 minute period the ratio of activities of succinate to citrate is 22,500:365 or 62:1; at the 60 minute period the ratio is 48,800:730 or 62:1; and at the 120 minute period the ratio is 42,000:750 or 56:1. This no longer holds after 3 hours or longer, for the ratio of activities of succinate to citrate now becomes very large since the pools of these acids now largely consist of radioactive molecules only.

[1] For details of the method by which these values were obtained, refer to Ajl (1951*a*).

TABLE 5

Distribution of activity in intracellular Krebs' cycle intermediates during oxidation of labeled acetate

TIME	FRACTION	INTRACELLULAR CONCENTRATION	TOTAL C^{14} CONTENT	SPECIFIC ACTIVITY
min		*μM*	*cts/min*	*cts/min/μM*
30	Citrate	0.342	365	1,067
	Succinate	16.5	22,500	1,363
	α-Keto-glutarate	Could not be detected	100	—
60	Citrate	0.342	790	2,310
	Succinate	25.8	48,800	1,890
	α-Keto-glutarate	Could not be detected	~100	—
120	Citrate	0.342	750	2,190
	Succinate	14.6	42,000	2,870
	α-Keto-glutarate	Could not be detected	200	—
180	Citrate	0.085	435	5,117
	Succinate	25.4	124,050	4,980
	α-Keto-glutarate	Could not be detected	~100	—
270	Citrate	0.125	121	968
	Succinate	25.7	240,000	9,380
	α-Keto-glutarate	Could not be detected	200	—

Total volume of reactants 30 ml. Each vessel contained 10 grams (wet weight) of *P. pestis*, 1 ml of 0.2 M phosphate buffer, pH 7.2, 200 μM of radioactive acetic acid containing 1.8×10^6 cts/min, and distilled water to volume. Temperature 25 C. Aerobic.

TABLE 6

Equivalence of respiratory CO_2 and carboxyl carbon of succinate and citrate during the oxidation of methyl labeled acetate by Pasteurella pestis

FRACTION	INTRACELLULAR CONCENTRATION	SPECIFIC ACTIVITY	SPECIFIC ACTIVITY PER SINGLE CARBOXYL CARBON	SPECIFIC ACTIVITY OF RESPIRATORY CO_2
	μM	*cts/min/μM*	*cts/min/μM*	*cts/min/μM*
Citrate.....	0.28	29,000	106	125
Succinate....	22.80	26,500	139	

Total volume of reactants 50 ml. Reaction flask contained 30 grams (wet weight) of *P. pestis*, 2 ml of 0.2 M phosphate buffer, pH 7.2, 1 mM of radioactive acetic acid containing 17.5×10^6 cts/min, and distilled water to volume. Temperature 25 C. Aerobic.

TABLE 7

Oxidation of labeled acetate with externally added carriers by cell-free extracts of Pasteurella pestis

CONDITIONS	FRACTION	TOTAL ACTIVITY
		cts/min
Aerobic	Citrate	3,100
	Succinate	109,500
	α-Ketoglutarate	6,400
Anaerobic	Citrate	3,100
	Succinate	197,000
	α-Ketoglutarate	6,900

Total volume of reactants 15 ml. Each vessel contained 6 ml of cell-free extract, 50 μM each of succinate, α-ketoglutarate and citrate, 0.5 mg of $MgCl_2$, 10 mg of ATP, 7 units of CoA, 10 μM of cysteine, 1 ml of phosphate buffer, 0.1 M, pH 7.4, and 1 ml of methyl labeled acetate containing $\sim 1 \times 10^7$ cts/min. Time of incubation 60 minutes. Temperature 37 C. Aerobic conditions were maintained by bubbling through sterile air. Anaerobic conditions were maintained by bubbling through sterile O_2-free N_2.

In order to determine whether the labeled acetate in the course of its breakdown cycles through the Krebs' cycle intermediates, the specific activity of the respired CO_2 was compared with the specific activities of carboxyl carbon of succinate and citrate. The results are shown in table 6. It is clear from the data presented that even though methyl labeled acetate was used, the respiratory CO_2 becomes active, and its specific

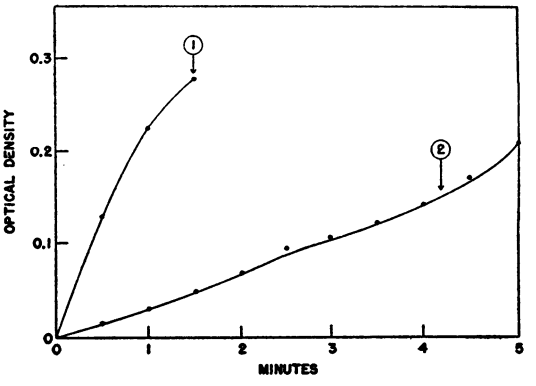

Figure 2. Spectrophotometric tests with isocitric dehydrogenase and aconitase. Total volume of reactants 3 ml. Each cuvette contained 0.25 ml of ammonium sulfate fraction containing isocitric dehydrogenase and aconitase, 0.40 mg of triphosphopyridine nucleotide, 0.5 ml of phosphate buffer, pH 7.4 (0.05 M), 0.05 mg $MnCl_2$ and either 0.5 mg of sodium isocitrate (curve 1) or 5 mg of citrate (curve 2).

activity is indeed equivalent to the specific activity of the individual carboxyls of those Krebs' cycle intermediates that were studied.

α-Ketoglutarate could not be detected in as much as 10 g (wet weight) of *P. pestis* (table 5). It is to be emphasized, however, that activity was always present in the band corresponding to the C_5-keto acid on a paper chromatogram. However, when 36 g (wet weight) of cells were analyzed for α-ketoglutarate, 5 μg of the free acid were detected and positively identified as α-ketoglutarate.

Oxidation of labeled acetate by cell-free extracts of P. pestis. Experiments were performed where labeled acetate was metabolized by crude cell-free extracts of *P. pestis* in the presence of unlabeled Krebs' cycle intermediates as carriers. Results are exhibited in table 7. It is evident that all carriers became active although the bulk of the activity was recovered in succinate. These experiments, however, indicate that not only succinate but citrate and α-ketoglutarate as well are involved in acetate oxidation.

Occurrence of isocitric dehydrogenase and aconitase. One of the key reactions involved in the Krebs' cycle is the enzymatic conversion of a tricarboxylic acid to α-ketoglutarate. *P. pestis* contains the enzymes necessary for this conversion. To obtain more information on this problem a purified preparation containing aconitase and a

triphosphopyridine nucleotide-dependent isocitric dehydrogenase was prepared from crude extracts of *P. pestis*. This enzymic preparation converts either citrate or isocitrate to α-ketoglutarate (figure 2). The formed α-ketoglutarate from this reaction was identified chromatographically and spectrophotometrically. On the addition of carbon dioxide the reaction was found to be reversible.

DISCUSSION

In establishing the quantitative interreaction of labeled components in an oxidative metabolic cycle, it is necessary to determine the isotope content and distribution in each postulated intermediate. It is essential furthermore that such data be obtained on intracellular intermediates and not on externally added carriers. It has been shown for *P. pestis* that whereas carrier α-ketoglutarate and citrate are almost completely ineffective as trapping agents for acetate carbon during the oxidation of labeled acetate, the *intracellular* specific activities (and not total activities) of these intermediates are such that they could be involved in the oxidative breakdown of this C_2-fatty acid. When such data are plotted (see figure 3), it becomes readily apparent that citrate, for example, precurses succinate during the initial stages of the experiment as the tricarboxylic acid cycle mandates. Further, when the specific activity of the respired CO_2 is compared with the specific activities of carboxyl carbon of the intracellular Krebs' cycle intermediates during the oxidation of methyl labeled acetate, one finds complete equivalence between the two. This is to be expected if acetate, during its oxidation, cycles and the respired CO_2 is coming off from successive decarboxylations of the tricarboxylic acid cycle intermediates.

In only some instances, was it possible to determine with any degree of accuracy the intracellular concentration of α-ketoglutarate. This required the use of as many as 36 g of cells. The evidence for the participation of this key keto acid in acetate oxidation is therefore largely indirect and includes the following observations: (1) Although α-ketoglutarate could not be isolated, the band corresponding to the C_5-keto acid on a paper chromatogram always contains some activity. Therefore, the specific activity of the acid is extremely high and it could be a precursor of succinate. (2) The occurrence of the enzymes aconitase and isocitric dehydrogenase in this organism, which when combined convert quantitatively citric acid to α-ketoglutarate.

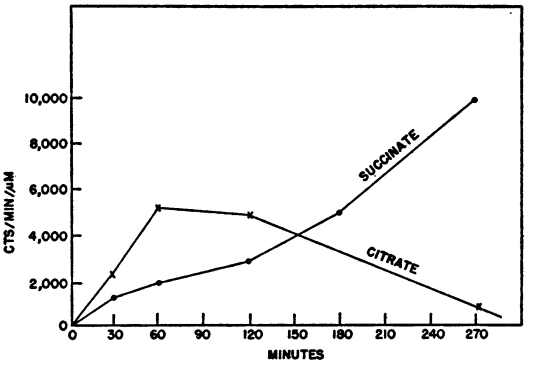

Figure 3. "Time-function" relating specific activity of precursor to product during oxidation of 2-C^{14}-acetate by nonproliferating *Pasteurella pestis*.

Each flask contained 10 grams (wet weight) of *P. pestis*, 1 ml of 0.1 M phosphate buffer, pH 7.0, and 200 μM of labeled acetate containing ~ 1.8 × 10^6 cts/min. Total volume 40 ml. Conditions completely aerobic.

The concentrations of succinate and citrate represent the intracellular pools of these acids.

The following explanations are offered for α-ketoglutarate not becoming significantly active during the metabolism of 2-C^{14}-acetate: (1) The C_5-keto acid is extremely reactive, and consequently there is essentially no significant intracellular pool of this acid at any time. This is substantiated by the finding that when α-ketoglutarate is exposed to crude cell-free extracts, as much as 10 μM of it will disappear in less than 30 minutes per two ml of extract. (2) Radioactive α-ketoglutarate (presumably formed during the oxidation of labeled acetate) does not dissociate to any appreciable extent from the enzyme surface, and consequently there is never enough of it inside the cell either to be detected chemically or to become exchanged with carrier.

One additional observation in connection with the over-all aerobic metabolism of *P. pestis* deserves comment. It will be recalled that the oxidative metabolism of this organism is sluggish when compared with other bacteria. It is interesting to note that this low metabolic behavior is reflected in the concentration of intracellular Krebs' cycle intermediates. Evidence has been presented which indicates that the intracellular

pools of succinate, citrate, and α-ketoglutarate are much greater in *P. pestis* than in *E. coli* or *Corynebacterium creatinovorans*, for example. Furthermore it is interesting to correlate these observations with the high endogenous respiration exhibited by *P. pestis*. With such large intracellular pools, it is expected that the endogenous metabolism of this organism would be rather high.

SUMMARY

Resting cells of *Pasteurella pestis* exhibit a high endogenous metabolism. This high endogenous respiration can be correlated with the large intracellular pools of Krebs' cycle intermediates. Using labeled cells it has been possible to show, however, that endogenous respiration does not interfere with the oxidation of exogenous substrates.

Whole, nonproliferating cells oxidize all members of the tricarboxylic acid cycle. The fraction of the acids attacked is oxidized to CO_2 and water.

Acetate oxidation proceeds via the tricarboxylic acid cycle. This conclusion was reached by studying the variation due to time in specific C^{14}-content of the intracellular intermediates during oxidation of labeled acetate.

The organism contains isocitric dehydrogenase and aconitase which when combined convert citrate to α-ketoglutarate. This reaction is reversible.

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