# SOME ENZYMATIC CHANGES ACCOMPANYING THE SHIFT FROM ANAEROBIOSIS TO AEROBIOSIS IN PASTEURELLA PESTIS[1]

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In previous papers (Englesberg *et al.*, 1953, 1954) it was shown that the terminal respiration of *Pasteurella pestis* is under adaptive control. The purpose of this study is to define some of the enzymes involved in the adaptive process and to present evidence concerning the operation of the tricarboxylic acid cycle in aerobically grown cells.

## MATERIALS AND METHODS

*Pasteurella pestis*, strain A1122 (avirulent), was employed in these experiments. The medium, cultural conditions, and procedures for harvesting and washing cells have been described previously (Englesberg *et al.*, 1954).

Enzymatically active cell extracts were prepared by grinding packed washed wet cells with alumina (Buehler no. 37052) for 3 to 5 minutes or until a noticeable change in consistency of the mix occurred (McIlwain, 1948). The mixture then was extracted with M/30 phosphate buffer (pH 7) or with distilled water (2 ml per gram wet weight of cells). The alumina and cell debris were spun down in a Spinco ultracentrifuge at 5,000 rpm for 30 minutes. The precipitate was extracted again with one ml of buffer or distilled water per gram wet weight. Some anaerobic cultures were divided into two parts. One part was extracted immediately while the remaining cells were adapted first to an oxidative metabolism by aeration in a casein hydrolyzate glucose medium (Englesberg *et al.*, 1954). Extracts were stored in the deep freeze. Comparisons of enzyme activity were made employing cell extracts of the same age and storage history. Anaerobically grown cells (anaerobic cells), although white initially, darken on harvesting, yielding a greyish extract, and the cell debris turns dark brown to black. This is in contrast to the white aerobically grown cells (aerobic cells) and the resulting amber-colored extract and cell debris.

The respiratory activity of the cell extracts was tested manometrically with the addition of co-factors, using the conventional manometric techniques (Umbreit *et al.*, 1949). Activity is expressed in terms of nitrogen determined by the micro-Kjeldahl method of Ma and Zuazaga (1942).

Isocitric dehydrogenase was determined by measuring triphosphopyridine nucleotide (TPN) reduction spectrophotometrically at 340 mμ by a method essentially as described by Grafflin and Ochoa (1950). The reaction mixture contained 0.1 ml of 0.01 M $MnCl_2$; 1.0 ml of 0.065 M glycyl-glycine buffer (pH 7.4); 0.2 ml of triphosphopyridine nucleotide (625 μg per ml); 0.05 to 0.2 ml of cell-free extract; 0.2 ml of 0.03 M DL-isocitrate; and $H_2O$ to yield a total volume of 3 ml. The blank contained all of the above except substrate.

Aconitase activity was determined by measuring the reduction of triphosphopyridine nucleotide spectrophotometrically at 340 mμ, as described above, with *cis*-aconitate as substrate in place of isocitrate, isocitric dehydrogenase activity not being rate limiting.

Fumarase activity was assayed by measuring fumarate production from malate spectrophotometrically at 240 mμ (Racker, 1950). The reaction mixture contained 0.1 ml of 0.05 M phosphate (pH 7.4); 0.2 ml of cell-free extract; 0.5 ml of 0.1 M L-malate; and $H_2O$ to yield a total volume of 3 ml. The blank contained all of the above except substrate.

All spectrophotometric measurements were carried out in a Beckman Model DU spectrophotometer using silica cells of 1.0 cm light path. The reactions were started by the addition of substrate. The rate of enzyme activity was de-

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termined on the basis of the change in optical density that occurred during the second 30 second period after the start of the reaction and is expressed in terms of the number of micromoles of substrate utilized per hour per mg of nitrogen. The molecular extinction coefficients employed are: triphosphopyridine nucleotide at 340 mμ = $6.24 \times 10^6$ ($cm^2 \times moles^{-1}$); sodium fumarate at 240 mμ = $2.11 \times 10^6$ ($cm^2 \times moles^{-1}$).

Heavy suspensions of intact cells, aerobically or anaerobically grown (1.5 g wet weight per 3 ml), and lyophilized cell debris obtained from 6 day old aerobic cultures (100 mg per 3 ml) (Englesberg and Levy, 1954) were examined for cytochrome bands using a Welch Replica Grating Bright-Line spectrometer. The cells and lyophilized debris were suspended in 20 to 70 per cent glycerin M/30 phosphate buffer at pH 7.0. A 500 watt projection lamp and a cell 1.3 cm in diameter were employed. The presence of free intracellular hematin was determined by a procedure described by Keilin (1933). Since anaerobic cells tend to blacken on standing, it was necessary to harvest and maintain the cells in the cold for spectroscopic examination.

Carbon monoxide inhibition of respiration was investigated in total darkness. To determine the effect of light on carbon monoxide poisoning of respiration, the Warburg vessels were illuminated by a 500 watt bulb placed over the water bath at a distance of about 10 cm from the vessels.

Cyanide inhibition of respiration was determined by using $Ca(CN)_2$ at 37.5 C (Robbie, 1946, 1948).

α-Ketoglutarate was obtained from Dr. I. Gunsalus, Nutritional Biochemicals, and General Biochemicals, sodium pyruvate C.P. from Nutritional Biochemicals, and *cis*-aconitic acid and DL-sodium isocitrate from H & M Chemical Co. Sodium adenosine triphosphate (ATP), chromatographically pure, was obtained from the Schwartz Laboratories, triphosphopyridine nucleotide (TPN) 80 per cent pure from Sigma Chemical Co., and diphosphopyridine nucleotide (Coenzyme I) 90 per cent pure, cocarboxylase, and cytochrome c from Nutritional Biochemicals. Coenzyme A (CoA) was obtained from Pabst Laboratories and from the laboratory of Dr. P. Stumpf.

## RESULTS

*Comparison of the oxidative activities of cell-free extracts of aerobic and anaerobic cells.* Cell-free extracts of aerobic and anaerobic *P. pestis*, supplemented with adenosine triphosphate, coenzyme I, and $MgSO_4$, were compared for ability to oxidize glucose, pyruvate, acetate, and some tricarboxylic acid cycle (TCA) compounds (table 1). Extracts from aerobic cells have a significantly higher oxidative activity than comparable extracts from anaerobic cells.

Although aerobic resting cells failed to oxidize α-ketoglutarate and citrate (Englesberg *et al.*, 1954), extracts from these cells oxidized these substrates at a high rate. The relationship between citrate, α-ketoglutarate, and succinate oxidation by an extract of aerobic cells is shown in figure 1. With citrate and α-ketoglutarate the initial rapid rates of oxidation fall with this particular extract after the uptake of 93 μL of oxygen (1.04 μM of oxygen per μM of substrate)

TABLE 1

*Comparison of the activity of cell-free extracts prepared from aerobically and anaerobically grown Pasteurella pestis*

| SUBSTRATE | OXYGEN UPTAKE ($Q_{O_2}N$) | |
|---|---|---|
| | Cell-free extract of aerobically grown cells | Cell-free extract of anaerobically grown cells |
| Glucose | 60 | 28 |
| Pyruvate | 16 | 8 |
| Acetate | 0 | 0 |
| Citrate | 58 | 1 |
| α-Ketoglutarate | 37 | 9 |
| Succinate | 27 | 4 |
| Fumarate | 33 | 16 |
| Malate | 33 | 15 |
| Oxalacetate | 20 | 8 |
| Ocalacetate + acetate | 20 | 8 |

The rates of oxygen uptake ($Q_{O_2}N$) were determined manometrically and are based upon the fastest ten minute period of oxygen utilization (usually the first ten minutes), with the corresponding endogenous respirations subtracted. The complete system contained 2 ml of cell-free extract in M/30 phosphate buffer (pH 7), 2 μM each of adenosine triphosphate, coenzyme I, and $MgSO_4$, and substrate in a total volume of 2.7 ml. The substrates were employed in the following concentrations: glucose, 2 μM; acetate, 6 μM; other substrates, 4 μM.

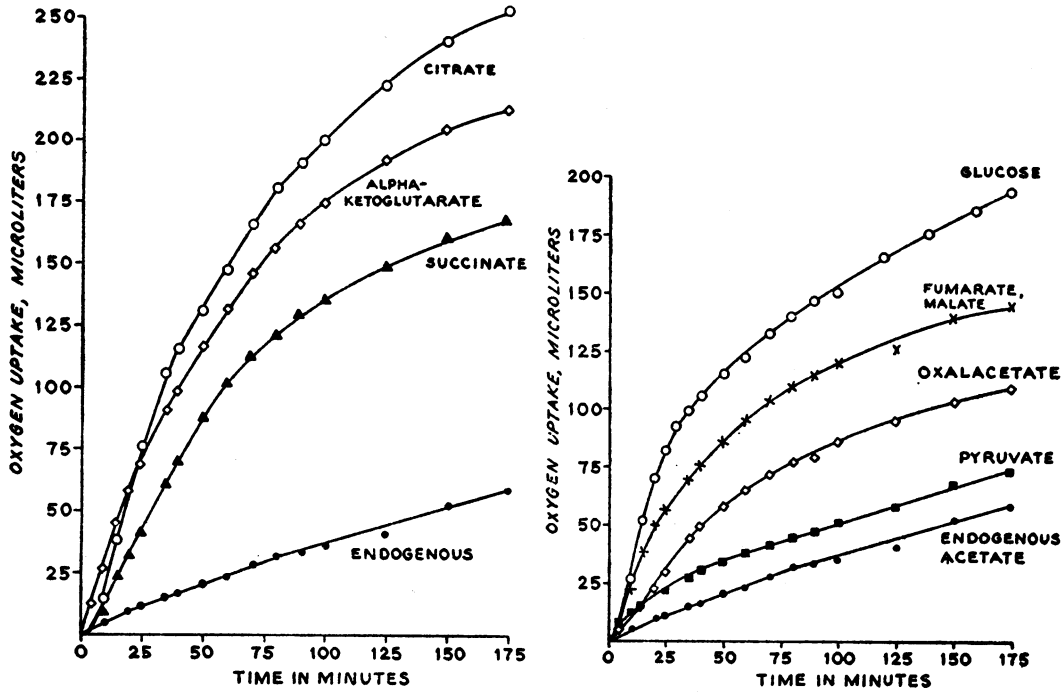


*Figure 1.* Oxidative metabolism of cell-free extract of aerobically grown *Pasteurella pestis*, strain A1122. Conditions are as described in table 1.

and 58 $\mu$L of oxygen (0.65 $\mu$M of oxygen per $\mu$M of substrate), respectively, the secondary rates being equal to that for the somewhat slower oxidation of succinate. The oxygen uptake at the change in rates is approximately equal to that required for the conversion of citrate and $\alpha$-ketoglutarate to succinate.

Sodium fluoride at a concentration of 0.016 M was found to produce a 93 per cent inhibition of succinate oxidation and causes a sharp break in the oxidation of citrate and $\alpha$-ketoglutarate at the point in oxygen uptake equivalent to the amount required for the conversion of these compounds to succinate. Succinic acid was recovered at this point by ether extraction of an acidified deproteinized supernatant and estimated manometrically using a succinic oxidase preparation (Umbreit *et al.*, 1949). Eighty-three per cent of the citrate and ninety-six per cent of the $\alpha$-ketoglutarate were accounted for as succinate.

The aerobic cell extracts are unable to oxidize acetate (figure 1), although aerobic resting cells oxidize this substrate readily (Englesberg *et al.*, 1954). Pyruvate is oxidized by aerobic extracts with the utilization of only 0.2 $\mu$M of oxygen per $\mu$M of pyruvate. Oxalacetate also is oxidized incompletely with the utilization of 0.55 $\mu$M of oxygen per $\mu$M of substrate. The oxygen uptake with the remaining substrates is approximately that required for their oxidation through oxalacetate or pyruvate. Evidence to be presented in greater detail elsewhere (Englesberg and Levy, unpublished data, 1954) indicates that the incomplete oxidation of oxalacetate and pyruvate (as well as the other tricarboxylic acid cycle compounds) is at least partly caused by: (1) a highly active oxalacetate decarboxylase which, in converting oxalacetate to pyruvate, prevents or inhibits the condensation reaction; and (2) the anaerobic dissimilation of pyruvate. The larger oxygen uptake with oxalacetate as compared to that with pyruvate can be interpreted most reasonably in the light of our present knowledge as indicating condensation of a portion of the oxalacetate to citrate, followed by oxidation of the citrate. This interpretation is in harmony with the observation that extracts of anaerobic cells, which barely oxidize citrate, oxidize oxalacetate slowly and consume approximately the same amounts of oxygen per mole of

pyruvate or oxalacetate (0.2 to 0.3 μM of oxygen per μM of substrate).

The addition of oxalacetate and other C4 dicarboxylic acids, cocarboxylase, and coenzyme A failed to stimulate pyruvate oxidation by either extract.

The rates of oxidation of the tricarboxylic acid cycle compounds by the aerobic extract, the total oxygen consumption for each compound, and the isolation of succinate as a product of citrate and α-ketoglutarate oxidation are all compatible with the operation of the tricarboxylic acid cycle in aerobically grown *P. pestis*, the functioning of which has been interfered with in the preparation of cell extracts at the stage of the condensation reaction. Although the poor activity of extracts of anaerobic cells for oxidation of the tricarboxylic acid cycle compounds (especially citrate) suggests the nonoperation of the tricarboxylic acid cycle in these cells, the possibility also exists that these results are caused by deficiencies in electron carrier.

*The adaptive synthesis of isocitric dehydrogenase, aconitase, and fumarase.* To determine the site or sites of enzymatic differences between aerobic and anaerobic cells, cell-free extracts were tested for isocitric dehydrogenase, aconitase, and fumarase activity. Isocitric dehydrogenase activity was determined with cell extracts which had lost the ability to utilize oxygen either as a result of repeated freezing and thawing or as a result of dialysis for 5 hours against distilled water at 0 C. The data indicate that aerobic cells possess a very active triphosphopyridine nucleotide-linked isocitric dehydrogenase, whereas anaerobic cells possess little of this enzyme (table 2). Diphosphopyridine nucleotide cannot substitute for triphosphopyridine nucleotide. Extracts made from anaerobic cells after aeration for 10 hours in a casein hydrolyzate glucose medium, in which negligible cell division occurred (Englesberg *et al.*, 1954), are almost equal in isocitric dehydrogenase activity to that of extracts of aerobic cells, thus demonstrating an induced synthesis of this enzyme. Similar results were found with aconitase and fumarase (tables 3 and 4). The low activity of aconitase, as compared to that of the other two enzymes, is probably the result of partial inactivation of this enzyme during dialysis (Racker, 1950).

TABLE 2

*Triphosphopyridine nucleotide-linked isocitric dehydrogenase activity of cell-free extracts of aerobically and anaerobically grown Pasteurella pestis*

| EXPERIMENT | μM OF ISOCITRATE CONSUMED PER HR PER MG N | | |
|---|---|---|---|
| | Aerobic cells | Anaerobic cells | Anaerobic cells after aerobic adaptation |
| 1 | 73.6 | 1.2 | |
| 2 | | 3.5 | 66.4 |

Isocitric dehydrogenase was determined by measuring triphosphopyridine nucleotide reduction spectrophotometrically at 340 mμ (see Materials and Methods). The activity of the cell extracts toward oxygen was destroyed in experiment 1 by repeated freezing and thawing and in experiment 2 by dialysis.

TABLE 3

*Aconitase activity of cell-free extracts of aerobically and anaerobically grown Pasteurella pestis*

| EXPERIMENT | μM OF *cis*-ACONITATE CONSUMED PER HR PER MG N | | |
|---|---|---|---|
| | Aerobic cells | Anaerobic cells | Anaerobic cells after aerobic adaptation |
| 1 | 2.4 | 0.3 | |
| 2 | | 0.1 | 1.2 |

Experiments were performed by measuring triphosphopyridine nucleotide reduction spectrophotometrically with *cis*-aconitate as substrate using dialyzed cell extracts in which isocitric dehydrogenase activity was not rate limiting (see Materials and Methods).

*Electron transport.* Spectroscopic examination of aerobic cells showed a broad absorption band centering at 560 mμ and a fine, almost imperceptible band at 530 mμ. To test for free intracellular hematin, a suspension of cells was heated for 10 minutes at 100 C to destroy the existing cytochrome, and pyridine and hydrosulfite then were added. Spectroscopic examination showed a band at 548 to 556 mμ of the pyridine hemochromogen which was more intense than the original 560 mμ band, thus demonstrating the presence in aerobic cells of large quantities of free intracellular hematin (Keilin, 1933). No cytochrome bands were detectable in anaerobic cells, and only traces of free intracellular hematin were found.

After aeration in a casein hydrolyzate mineral glucose medium for 10 hours, anaerobic cells show the cytochrome absorption band at 560

TABLE 4

*Fumarase activity of cell-free extracts of aerobically and anaerobically grown Pasteurella pestis*

| EXPERIMENT | μM OF FUMARATE FORMED PER HR PER MG N | | |
|---|---|---|---|
| | Aerobic cells | Anaerobic cells | Anaerobic cells after aerobic adaptation |
| 1 | 50.3 | 11.1 | |
| 2 | | 4.3 | 46.9 |

Fumarase activity was assayed by measuring fumarate production from malate spectrophotometrically at 240 mμ. The activity of the cell extracts toward oxygen was destroyed by dialysis (see Materials and Methods).

mμ, large increases in free intracellular hematin are demonstrable, and the tendency of cells to blacken on standing, a process so characteristic of anaerobic cells, no longer occurs.

This evidence therefore shows that in addition to displaying deficiencies in enzymes acting directly on compounds in the tricarboxylic acid cycle, anaerobic cells of *P. pestis* differ from aerobic cells in the nature of their respiratory carriers.

In aerobic cells or in anaerobically grown but aerobically adapted cells, the 560 mμ absorption band was demonstrated without the addition of reducing agents or substrate, probably as a result of endogenous reduction, and hydrosulfite failed to intensify the band. Shaking such cell suspensions in air resulted in the complete disappearance of the 560 mμ absorption band which reappeared rapidly upon standing. The thermolability of this cytochrome was demonstrated by the permanent disappearance of the 560 mμ band after heating a thick cell suspension for 10 minutes at 100 C. Its oxidation appears to be sensitive to cyanide, and urethane has no noticeable effect on the intensity of its absorption band. In contrast to resting cells, cell debris harvested after lysis during six days of aerobic growth in a casein hydrolyzate mineral glucose medium incubated at 37 C (Englesberg and Levy, 1954) showed an absorption band at 560 mμ only after the addition of hydrosulfite.

The rapid endogenous reduction of cytochrome in resting cells made it impossible to determine the relationship of this hemochromogen to substrate oxidation. Cell debris, although containing considerable quantities of cytochrome, apparently is devoid of any dehydrogenase activity and therefore could not be used for this purpose. Various attempts to reduce the endogenous respiration in resting cells by continuous aeration in phosphate buffer, using cultures of different ages or by several washings, were unsuccessful. By combining the cell-free extracts, which contain highly active substrate activating enzymes including dehydrogenases but not enough cytochrome to be detected spectroscopically, with the cytochrome concentrated cell debris and coenzyme I, adenosine triphosphate, and $MgSO_4$ we were able to demonstrate by visual spectroscopy the stimulatory effect of glucose on cytochrome reduction (see table 5). With the omission of the cell-free extract or the cofactors no cytochrome reduction occurred, and the endogenous reduction (that is, cytochrome reduction by the complete system less glucose) was negligible.

Cytochrome c oxidase activity could not be detected manometrically in aerobic or anaerobic cells or in oxygen utilizing cell extracts using ascorbic acid, *p*-phenylene diamine, di-methyl-*p*-phenylene diamine, or hydroquinone in the

TABLE 5

*Glucose stimulation of cytochrome reduction*

| | TIME OF APPEARANCE OF REDUCED CYTOCHROME BAND (MINUTES) | | | |
|---|---|---|---|---|
| | 0 | 10 | 20 | 30 |
| Complete system | − | ++ | ++++ | ++++ |
| Glucose omitted | − | − | ± | ± |
| Cell-free extract omitted | − | − | − | − |
| Adenosine triphosphate, coenzyme I, $MgSO_4$ omitted | − | − | − | − |

The complete system consisted of 1 ml of aerobic cell-free extract; 2 ml of cell debris (100 mg) in 70 per cent glycerol M/30 phosphate buffer, pH 7; 2 μM each of adenosine triphosphate, coenzyme I, $MgSO_4$; 4 μM of glucose; in a total volume of 3.5 ml. The reaction mixture was incubated at 30 C in stoppered tubes 1.3 cm in diameter and examined spectroscopically for reduced cytochrome band (560 mμ) at zero time and at ten minute intervals after addition of substrate. Intensities of reduced cytochrome absorption band observed: − = no band; ± = faint hardly perceptible band; ++ = dark distinct band; ++++ = very dark distinct band.

TABLE 6

*The effect of respiratory inhibitors on glucose oxidation by Pasteurella pestis*

| INHIBITORS | AEROBICALLY GROWN CELLS | ANAEROBICALLY GROWN CELLS |
|---|---|---|
| | *per cent inhibition* | |
| KCN | | |
| $1 \times 10^{-3}$ M | 96 | 95 |
| $1 \times 10^{-4}$ M | 90 | 85 |
| $1 \times 10^{-5}$ M | 33 | 45 |
| CO | | |
| 90 CO/10 $O_2$ | 24 | 67 |
| 80 CO/20 $O_2$ | 15 | 20 |
| $NaN_3$ | | |
| pH 7 | | |
| $2 \times 10^{-2}$ M | +134* | 0 |
| $1 \times 10^{-2}$ M | +157* | +90* |
| $1 \times 10^{-3}$ M | +74* | +51* |
| pH 5.6 | | |
| $2 \times 10^{-2}$ M | 73 | 79 |
| $1 \times 10^{-2}$ M | 33 | 82 |
| $1 \times 10^{-3}$ M | +88* | — |
| pH 6.2 | | |
| $2 \times 10^{-2}$ M | | 70 |
| $1 \times 10^{-2}$ M | | 63 |
| $1 \times 10^{-3}$ M | | 0 |

* Indicates the per cent stimulation of glucose oxidation.

presence of cytochrome c. Cytochrome c showed no stimulatory effect on the rate of succinate oxidation by aerobic or anaerobic cell extracts.

*The effect of respiratory inhibitors on glucose oxidation by resting cells.* In order to gain some further insight as to the type of carrier systems involved in glucose oxidation by both aerobic and anaerobic cells, the effects of various inhibitors of glucose oxidation by resting cell suspensions were tested (table 6).

Glucose oxidation by both aerobic and anaerobic cells is extremely sensitive to cyanide (table 6). There does not appear to be any significant difference in the pattern of inhibition of glucose oxidation by cyanide with these two different types of cells.

On the other hand, anaerobic cells are much more sensitive to carbon monoxide poisoning. The carbon monoxide inhibition of respiration of either type of cell was not reversed by light.

While azide employed at pH 7 has a stimulatory effect on the rate of oxygen uptake in the oxidation of glucose by both types of cells, aerobic cells are much more sensitive to this effect. When the pH is lowered to 5.6 or 6.2, 0.02 M azide becomes inhibitory to glucose oxidation by *P. pestis*. Keilin (1933) previously has shown that the inhibitory action of azide can best be shown at acid pH values. Gary and Bard (1952) noticed a similar effect of pH with *B. subtilis*. However, even at pH 5.6, $10^{-3}$ M azide remained stimulatory to glucose oxidation by aerobic cells and failed to inhibit glucose oxidation by anaerobic cells. Anaerobic cells appear to be more sensitive to azide inhibition and less sensitive to the stimulatory effect of this chemical at acid pH values.

At pH 7, with concentrations of azide of $1 \times 10^{-3}$ to $1 \times 10^{-2}$, it is possible that larger increases in rate of oxygen uptake observed with aerobic cells, as compared to anaerobic cells, may be the result of the greater stimulation of total oxygen uptake possible with the former. Anaerobic cells oxidize glucose incompletely as a result of oxidative assimilation and the inability to oxidize intermediates of glucose oxidation. With these cells, therefore, even though azide inhibits assimilation, the resulting stimulation in oxygen uptake is slight as compared to that possible with aerobic cells. At acid pH values it appears that the resulting rate of glucose oxidation by *P. pestis* in the presence of azide is a composite of inhibition of oxidation of glucose and stimulation of oxygen uptake. Because of these effects it is possible that the apparent greater sensitivity of anaerobic cells to azide inhibition may be just a result of the small degree of stimulation of oxygen uptake possible with these cells.

## DISCUSSION

In experiments with resting cells previously reported (Englesberg *et al.*, 1954), it was demonstrated that growth in the presence or absence of oxygen has a profound effect on the physiology of *P. pestis*. Anaerobic cells fail to oxidize acetate and oxidize pyruvate, succinate, fumarate, and malate at low rates, and glucose is oxidized incompletely with the accumulation of pyruvate and other organic end products. Aerobic cells, on the other hand, oxidize acetate, pyruvate, and the C4 compounds at a rapid rate, and glucose is

oxidized rapidly and "completely". Anaerobic cells, adapted to the complete oxidation of glucose, adapt simultaneously to the oxidation of pyruvate, acetate, and the C4 compounds. Neither citrate nor $\alpha$-ketoglutarate is oxidized by either type of cells. Although these experiments indicated that terminal respiration of *P. pestis* is under adaptive control, it was not possible to indicate with any certainty the enzymatic basis for the different oxidative behavior of these cells. As a result of the experiments with cell extracts we are now in a position to explain, at least partly, this phenomenon.

First, evidence suggests that the tricarboxylic acid cycle is probably functioning as the terminal respiratory mechanism in aerobic cells. Although aerobic resting cells fail to oxidize $\alpha$-ketoglutarate and citrate, these substances are oxidized by cell extracts at faster rates than other tricarboxylic acid cycle compounds. This ability of cell extracts to oxidize compounds not attacked by intact cells has been shown previously by several investigators (Campbell and Stokes, 1951; Stone and Wilson, 1952; Repaske and Wilson, 1953; Barrett *et al.*, 1953). These findings demonstrate that one cannot exclude a compound as an intermediate in terminal respiration on the basis of its unoxidizability by intact cells. The fact that many microorganisms can oxidize tricarboxylic acid cycle compounds, irrespective of the aerobic growth substrate employed, is no indication that the enzymes involved are "constitutive" but probably indicates that the tricarboxylic acid cycle is a common terminal respiratory pathway.

In the case of *P. pestis*, adaptation to an anaerobic existence results in a loss in ability to oxidize tricarboxylic acid cycle compounds. Enzymatic analysis indicates that anaerobic cells are deficient in, at least, isocitric dehydrogenase, aconitase, fumarase, and cytochrome. In particular, the feeble activity toward citrate is positive evidence that the tricarboxylic acid cycle is not operating in anaerobic cells. Aeration in a casein hydrolyzate glucose medium results in the induced synthesis of these enzymes. It seems reasonable to assume that the other enzymes involved in this metabolic pathway also respond in a similar fashion.

The cytochrome present in aerobic cells has an alpha band at 560 m$\mu$, is heat labile, cyanide sensitive, and is closely associated with the insoluble portion of the cell. It is evident from the stimulatory effect of glucose on the reduction of this cytochrome and the cyanide sensitivity of both glucose oxidation and cytochrome reduction that this hemochromogen functions as an electron carrier in glucose oxidation. Cytochrome c oxidase is not present as a component of the electron transport system of *P. pestis*, as evidenced by the irreversibility of carbon monoxide inhibition by light, the failure of intact cells as well as oxidatively active cell-free extracts to oxidize ascorbic acid, hydroquinone, di-methyl-*p*-phenylene diamine, and *p*-phenylene diamine in the presence of cytochrome c.

The absence of any detectable cytochrome and the presence of only traces of intracellular hematin in anaerobic cells, which are capable of oxidizing glucose, together with the apparent greater sensitivity to carbon monoxide inhibition and perhaps to azide and equal sensitivity to cyanide inhibition, indicate that the hydrogen transport system involved in the primary phase of glucose oxidation in these cells involves a metalloprotein quite different from that in aerobic cells.

The general adaptive response of *P. pestis* to oxygen is similar to that observed in *Saccharomyces cerevisiae* by Ephrussi and Slonimski (1950) and Hirsch (1952). There are certain major differences, however. In *P. pestis* a portion of the initial glucose oxidation (prior to pyruvate) appears to be constitutive (Englesberg *et al.*, 1954), indicating that this organism may have two mechanisms for glucose dissimilation. In *S. cerevisiae* adaptation to anaerobic conditions results in a complete loss in ability to oxidize glucose. Both organisms form cytochromes adaptively in response to the presence of oxygen, but in *S. cerevisiae* cytochromes a, b, c, and oxidase present in aerobic cells disappear during growth under anaerobic conditions and two new compounds, cytochromes $a_1$ and $b_1$, appear (Ephrussi and Slonimski, 1950), while in *P. pestis* only a single cytochrome is seen in aerobic cells and no cytochrome at all and little intracellular hematin are detectable when these cells are grown anaerobically.

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SUMMARY

Growth in the presence or absence of oxygen has a pronounced effect on the enzymatic constitution of *Pasteurella pestis*. Although the tricarboxylic acid cycle appears functional in aerobically grown cells, it is not operating in anaerobically grown cells as evidenced by deficiencies in isocitric dehydrogenase, aconitase, and fumarase. Differences in carrier system are also evident. Aerobically grown cells possess free intracellular hematin and a cytochrome with an alpha absorption band at 560 mμ. No such cytochrome and little free hematin were detected in anaerobically grown cells. Aeration in the presence of a casein hydrolyzate glucose medium, which converts anaerobic cells to the physiological characteristics of aerobic cells, induces the synthesis of isocitric dehydrogenase, aconitase, fumarase, cytochrome, and free hematin, and causes the disappearance of the characteristic blackening process of anaerobic cells.

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