

# Effect of Oxygen on Several Enzymes Involved in the Aerobic and Anaerobic Utilization of Glucose in *Escherichia coli*

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By using the continuous culture technique, the transition from aerobiosis to anaerobiosis and its effect on a number of enzymes has been investigated in *Escherichia coli* K-12. A decrease in the oxygen partial pressure below 28.0 mm of Hg resulted firstly in an increase of the respiratory enzymes (reduced nicotinamide adenine dinucleotide [NADH] oxidase, 2.53-fold; succinic dehydrogenase, 1.4-fold; cytochrome  $b_1$ , 3.91-fold; and cytochrome  $a_2$ , 2.45-fold) before the electron transport system gradually collapsed as cytochrome  $a_2$ , followed by cytochrome  $b_1$ , succinic dehydrogenase, and finally NADH oxidase decreased in activity. The change from respiration to fermentation was initiated well before the oxygen tension reached zero by the increase in levels of fructose diphosphate-aldolase, glucose 6-phosphate, and 6-phosphogluconate dehydrogenases and a decrease in 2-oxoglutarate dehydrogenase. When the dissolved oxygen tension reached zero, dry weight and  $\text{CO}_2$  formation together with isocitrate dehydrogenase decreased, whereas acid production and phosphofructokinase synthesis started to increase. Enzymatic investigations revealed that the kinetics of the enzyme phosphofructokinase from strict aerobic cultures (6.9 ppm oxygen in solution) was adenosine triphosphate (ATP)-insensitive, whereas the same enzyme from anaerobic cultures was ATP-sensitive. A mechanism is proposed for the change from aerobiosis to anaerobiosis together with the occurring change in glucose regulation.

In a previous publication (20), it was proposed that oxygenation of a facultative anaerobe causes a transient inhibition of phosphofructokinase (PFK) activity and a reduction in the rate of synthesis of PFK. It was observed that, when oxygen became limiting in a chemostat culture, the steady-state level of PFK is inversely proportional to the degree of oxygenation. During this transitional period, aerobic respiration changes to fermentation, which is reflected also in changes of activity of the respiratory enzymes (10, 16, 17, 26, 27) and the change from a fully operative tricarboxylic acid cycle to a branched, noncyclic tricarboxylic acid pathway (1). These changes were attributed to the effect of oxygen (10) or the redox potential changes (27) occurring during the transitional period.

To elucidate further the nature and kinetics of the transition from aerobiosis to anaerobiosis, further evidence will be given that PFK activity does not regulate aerobic glucose me-

tabolism. The allosteric control occurs only when oxygen becomes limiting. In including a number of enzymes of the Embden-Meyerhof-Parnas (EMP), hexosemonophosphate (HMP) and tricarboxylic acid cycle pathways together with cytochrome measurements, a control mechanism is being proposed for the transitional change from aerobiosis to anaerobiosis in chemostat cultures of *Escherichia coli* K-12.

## MATERIALS AND METHODS

Culture conditions of *E. coli* K-12, as well as the arrangements for the continuous culture, were identical to those conditions described by Reichelt and Doelle (20).

**Preparation of cell-free extracts.** Samples were taken after steady states had been obtained. After centrifugation at 4°C and  $8,000 \times g$  for 20 min, separate cell suspensions were prepared for sonic treatment, by using distilled water for fructose diphosphate aldolase (FDP-aldolase) (6), 0.1 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.0), 3 mM mercaptoethanol, and 0.45 mM fructose 6-

P (F 6-P) for PFK determinations and 0.1 M Tris-hydrochloride (pH 7.0) plus 3 mM mercaptoethanol for assaying glucose 6-P dehydrogenase, 6-phosphogluconate dehydrogenase, isocitrate and 2-oxoglutarate dehydrogenases. For the determination of reduced nicotinamide adenine dinucleotide (NADH)-oxidase, succinate dehydrogenase, cytochrome  $a_2$  and  $b_1$ , the cells were resuspended in the type of buffer required for the particular assay. The cell suspensions were sonically treated for 3 min by using an ultrasonic oscillator (Kerry's Ltd.) at 100 w and maintaining the temperature of the suspension at 4 to 8 C throughout. The supernatant fraction after centrifugation at 4 C for 30 min at  $18,000 \times g$  was used for the soluble enzyme assays. The different suspending fluids used for sonic treatment were necessary to obtain enzyme stability.

For the kinetic studies as well as the investigations of the effect of nucleotides and intermediates on the specific activity of PFK, the enzymes were partially purified. Solid ammonium sulfate was added to the crude, cell-free extract to give 40% saturation. After 15 min of stirring, the precipitate was removed by centrifugation at  $40,000 \times g$ . Further addition of ammonium sulfate to the resulting supernatant fluid brought saturation to 80%. The resulting pellet was obtained by centrifugation at  $40,000 \times g$ . The pellet containing the enzyme was dissolved in 10 mM Tris-hydrochloride buffer (pH 7.2) containing 3 mM 2-mercaptoethanol and 0.45 mM F 6-P. After dilution to 5 mg of protein per ml, the pH was adjusted to 5.9 with 3 N acetic acid, and the nucleic acids were precipitated at 0.4% protamine sulfate by the addition of a 2% solution (pH 4.0). The supernatant fluid obtained by centrifugation at  $80,000 \times g$  was neutralized with NaOH and 2.1 mg of bovine albumin per ml was added. Dialysis was carried out for a minimum of 5 hr against 10 volumes of 10 mM Tris-hydrochloride buffer (pH 7.2) at 4 C. For the investigations on the effect of nucleotides, the auxiliary enzymes were diluted in 10 mM Tris-hydrochloride buffer (pH 7.2) containing 2.1 mg of bovine albumin per ml and dialyzed for a minimum of 3 hr against 10 volumes of the same buffer at 4 C.

**Enzyme assays.** NADH-oxidase was determined as described by Green and Ziegler (11).

Succinate dehydrogenase was estimated by using the method of Arrigoni and Singer (2).

Cytochrome  $b_1$  and  $a_2$ . Cytochromes in the cell wall membranes and soluble preparations were estimated with the Beckman DK-2A recording spectrophotometer with a 0 to 0.1 slide wire attachment, connected to a second recorder for greater ease in discerning the spectrum peak heights. Difference spectra (air oxidized versus dithionite reduced) were recorded and the peak heights were taken. The spectra were recorded from two cuvettes, each containing sonically disrupted but noncentrifuged cell extracts. Specific activity for the cytochromes are expressed as micromoles of substrate transformed per minute per milligram of protein.

PFK was determined as described by Reichelt and Doelle (20).

Glucose 6-P and 6-phosphogluconate dehydrogen-

ases were determined as described by Sly and Doelle (23, 24).

FDP-aldolase was assayed by the chromogen method by using a modification of Sibley and Lehninger (22) as described by Doelle and Manderson (6).

2-Oxoglutarate dehydrogenase was assayed spectrophotometrically. The assay mixture contained in 3.0 ml:  $5.55 \times 10^{-2}$  M Tris-hydrochloride buffer (pH 8.2),  $10^{-3}$  M cysteine,  $10^{-3}$  M  $MgCl_2$ ,  $10^{-4}$  M thiamine pyrophosphate,  $4 \times 10^{-5}$  M coenzyme A,  $5.2 \times 10^{-4}$  M oxidized nicotinamide adenine dinucleotide,  $5.6 \times 10^{-4}$  M 2-oxoglutarate, and extract protein in the range 0.4 to 1.5 mg. The reaction was started by the addition of the extract.

Isocitrate dehydrogenase was determined spectrophotometrically. The assay mixture contained in 3.0 ml:  $5.5 \times 10^{-2}$  M potassium phosphate buffer (pH 8.0),  $6.7 \times 10^{-3}$  M  $MgCl_2$ ,  $1.1 \times 10^{-4}$  M oxidized nicotinamide adenine dinucleotide phosphate,  $2.2 \times 10^{-4}$  M isocitrate, and up to 0.6 mg of extract protein. The reaction was started by the addition of the substrate. All spectrophotometric methods were carried out with a Beckman DK-2A recording spectrophotometer. Unless otherwise stated, the rate of absorbance was followed at 340 nm and the enzyme activity was expressed in specific activity ( $\mu$ moles/mg of protein).

Protein was estimated according to the method of Lowry et al. (15).

Glucose was determined by the mixed enzyme dye reaction of Huggett and Nixon (12).

Ammonium sulfate remaining in the medium was determined in the supernatant fluid spectrophotometrically by using the assay method of Ecker and Lockhart (7).

Dry weights of the cells were determined in crucibles, weighed to a constant weight before and after addition of cells and drying at 105 C.

Determination of the acid production rate was carried out as described by Reichelt and Doelle (20).

$CO_2$  was estimated with the Radiometer gas analyzer AA1.

The relative amount of internal plus cell membrane in the *E. coli* cell was determined by using a system devised by Gibbs et al. (9) for determining the change in chromatophore material in *Rhodospirillum molischianum* with varying light intensity. The enlarged micrographs necessary for this work were of *E. coli* cells fixed and embedded after the method of Kellenberger and Ryter (14) and modified slightly by Pontrefact and co-workers (19). Each figure represents the mean of 10 determinations.

## RESULTS

The results of the investigations are summarized in Table 1 and Fig. 1 and 2, which demonstrate that the oxygen partial pressure in the medium has an effect on all of the enzymes studied.

With a reduction of the input partial pressure of oxygen ( $pO_2$ ) below 30 mm of Hg, the dissolved oxygen tension (DOT) in the me-

TABLE 1. Effect of oxygen partial pressure on acid production, dry weight, and CO<sub>2</sub> production in chemostat cultures of *E. coli* K-12

Input pO <sub>2</sub> (mm Hg)	Specific acid production rate (ml N NaOH/hr/g dry wt)	Dry wt (mg/ml)	CO <sub>2</sub> production (%)
100	0.0	ND <sup>a</sup>	ND
50	0.0	0.34	1.33
30	0.0	0.34	1.33
20	7.1	0.27	0.67
10	11.9	0.18	0.13
6	15.2	ND	ND
0	54.5	0.11	0.0

<sup>a</sup> ND, not determined.

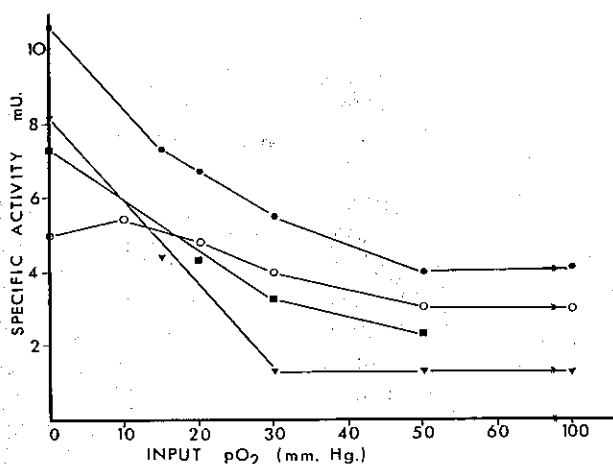


FIG. 1. Effect of input pO<sub>2</sub> on the activities of the glucose metabolizing enzymes of *E. coli* K-12. Symbols: ●, glucose 6-P dehydrogenase; ○, 6-phosphogluconate dehydrogenase; ■, FDP-aldolase; ▼, PFK.

dium drops to zero; that is, in this limited oxygen state, the oxygen demand exceeds the oxygen being supplied. Before this happens, the enzyme activities of FDP-aldolase, glucose 6-P dehydrogenase, and 6-phosphogluconate dehydrogenase begin to increase from the normal levels formed under strictly aerobic growth conditions. FDP-aldolase and 6-phosphogluconate dehydrogenase rise in a linear fashion, whereas glucose 6-P dehydrogenase increases much more steeply. At the same time, 2-oxoglutarate dehydrogenase and isocitrate dehydrogenase decrease markedly, indicating a severe reduction of tricarboxylic acid cycle activity. When the DOT reaches zero, 2-oxoglutarate dehydrogenase activity is zero and the full tricarboxylic acid cycle activity is lost. At this time, PFK synthesis starts and, together with FDP-aldolase, continues its linear increase, while glucose 6-P dehydro-

genase exhibits an increased rise in activity. All three enzymes reached their maxima during anaerobiosis (pO<sub>2</sub> = 0 mm of Hg).

Considering the respiratory enzymes (Fig. 2), it is the cytochromes (*b*<sub>1</sub> and *a*<sub>2</sub>) that first show an increase to maximal value, which would be expected since these are the terminal respiratory enzymes. At the point of highest cytochrome activity, both NADH-oxidase and succinic dehydrogenase are still increasing in their activity. As the pO<sub>2</sub> is reduced further, the NADH-oxidase and succinic dehydrogenase reach their peaks (at 14.2 mm of Hg), while the cytochrome levels are rapidly decreasing toward the anaerobic level. The maximal peaks of the pyridinoprotein at a lower pO<sub>2</sub> than those of the cytochromes is probably due to their position at the start of the respiratory chain.

As the input pO<sub>2</sub> approaches zero, all respiratory enzymes decrease from their peak values, each obtaining a lower value than the corresponding aerobic level, except for cytochrome *b*<sub>1</sub> which is 1.5 times higher. It is also of interest to note that the ratio of cytochrome *b*<sub>1</sub> to cytochrome *a*<sub>2</sub> remains relatively constant throughout the steady states at a value of 65.55.

As the respiratory enzymes are attached to the cell membrane, actual amounts of cell membrane in the cell were measured and recorded as percentage of the total volume in order to obtain a relationship between enzyme activity and the amount of membrane being formed. In the fully aerobic state, there was a volume of 6.6 to 6.7%; at anaerobiosis it was

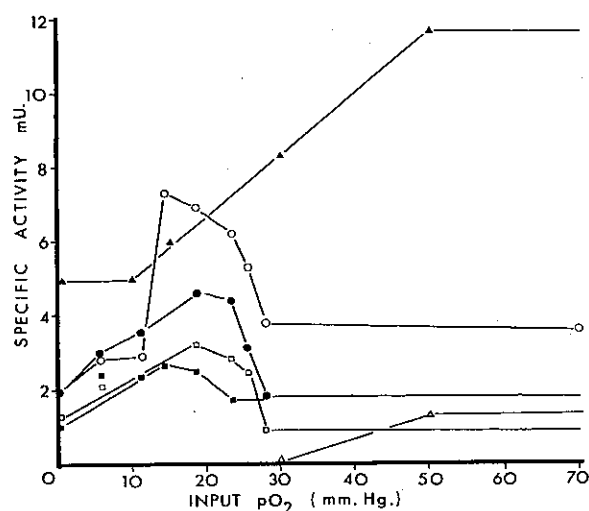


FIG. 2. Effect of input pO<sub>2</sub> on the respiratory activity of *E. coli* K-12. Symbols: ○, NADH oxidase; ●, cytochrome *a*<sub>2</sub>; □, cytochrome *b*<sub>1</sub>; ■, succinate dehydrogenase; ▲, isocitrate dehydrogenase; Δ, 2-oxoglutarate dehydrogenase.

6.0%. During the limited oxygen state, the volume reached a value of 7.3%. Oxygen thus appears to have an effect on the amount of cell membrane as well as cytochrome activity. The cell length at  $pO_2$  0 mm of Hg was greater than that under aerobic conditions. The length varied from 1.5  $\mu$ m to 2.1  $\mu$ m, whereas the width remained constant at 0.36  $\mu$ m.

To determine whether the increase in the respiratory enzyme activity was due to increased production of the actual enzyme, the activities of the four enzymes per unit cell membrane were compared at the various  $pO_2$  levels (Fig. 3). In each case, a maximal peak was obtained at 18.8 mm of Hg.

The drastic cut in tricarboxylic acid cycle activity at an input  $pO_2$  of 30 mm of Hg was also reflected in the growth and  $CO_2$  formation. The growth measured in dry weight decreased linearly as did the  $CO_2$  production. The switchover to fermentation could also be demonstrated by the start of acid production.

The confirmation that the aerobically grown cell contains only approximately 30% of the PFK activity compared with the anaerobically grown cell, and the important role this enzyme plays in the Pasteur effect were the reasons for further kinetic investigations on partially purified cell preparations.

The familiar allosteric effect of adenosine triphosphate (ATP) from cells grown under anaerobic conditions (input  $pO_2 = 0$  mm of Hg) is illustrated in Fig. 4A. In contrast, the same enzyme from cells grown under strict aerobic conditions (input  $pO_2 = 201$  mm of Hg

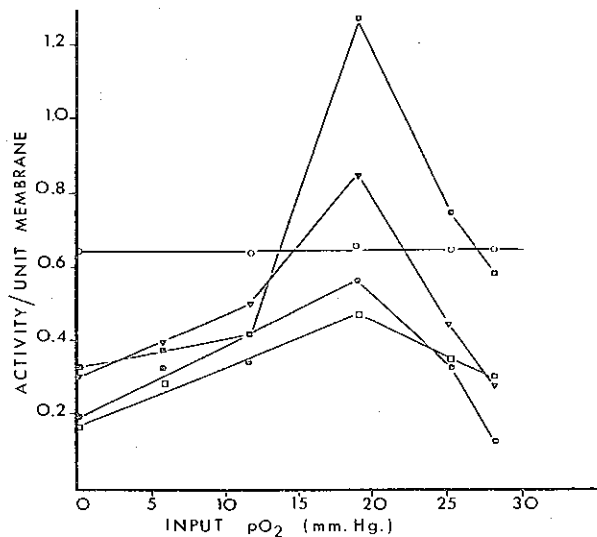


FIG. 3. Effect of input  $pO_2$  on the cytochrome and associated enzyme activity per unit cell membrane of *E. coli* K-12. Symbols:  $\square$ , NADH oxidase;  $\nabla$ , cytochrome  $a_2$ ;  $\odot$ , cytochrome  $b_1$ ;  $\square$ , succinate dehydrogenase;  $\circ$ , cytochrome  $b_1$ /cytochrome  $a_2$ .

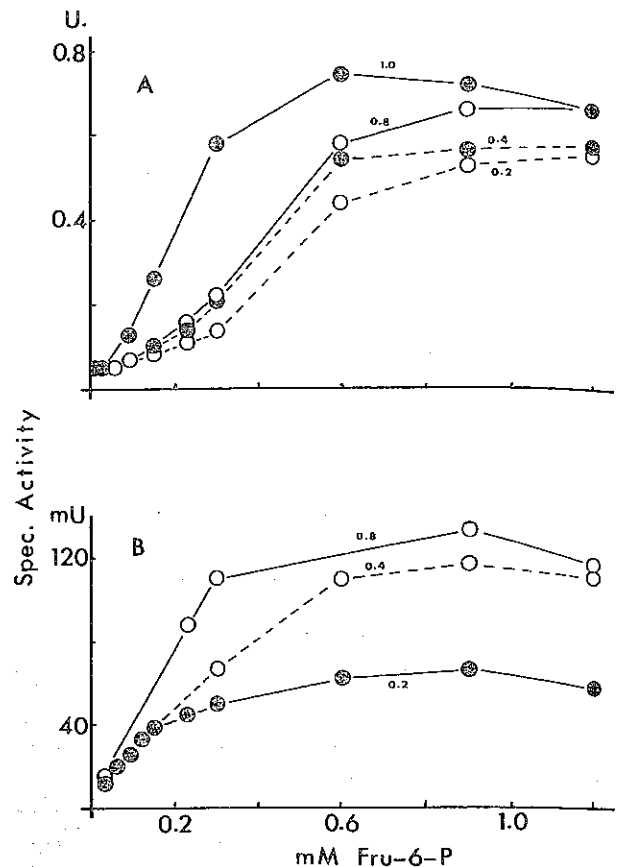


FIG. 4. PFK activity from anaerobically (A) and aerobically (B) grown *E. coli* K-12 as a function of the concentration of F 6-P in the presence of varying amounts of ATP. Each assay contained 0.18 or 0.36 mg of protein, respectively. A:  $\circ$ - $\circ$ , 0.2 mM ATP;  $\odot$ - $\odot$ , 0.4 mM ATP;  $\circ$ - $\circ$ , 0.8 mM ATP;  $\odot$ - $\odot$ , 1.0 mM ATP. B:  $\odot$ - $\odot$ , 0.2 mM ATP;  $\circ$ - $\circ$ , 0.4 mM ATP;  $\circ$ - $\circ$ , 0.8 mM ATP.

= 6.9 ppm oxygen in solution), i.e., when no acid is being produced and aerobic respiration prevails, does not exhibit the same ATP inhibition (Fig. 4B). The PFK is ATP-insensitive under aerobic conditions. The apparent Michaelis constants ( $K_m$ ) for F 6-P and ATP were estimated by using linear plots of the initial rate data as described by Lineweaver and Burk (Fig. 5). Due to the allosteric character of the anaerobic PFK, the  $K_m$  values for F 6-P and ATP were determined only with 0.9 mM concentrations, as all other concentrations resulted in parallel lines. The values were  $5.5 \times 10^{-4}$  M for F 6-P and  $5.0 \times 10^{-4}$  M for ATP. With the aerobic PFK, the  $K_m$  values of  $8.3 \times 10^{-4}$  M for F 6-P and  $2.0 \times 10^{-4}$  M for ATP were in the same order of magnitude although a higher affinity for ATP and a lower affinity for F 6-P, compared with the anaerobic enzyme, appear to emerge.

The addition of the nucleotides ADP, 5'-adenosine monophosphate (AMP) and cyclic 3',5'-AMP did not affect the enzyme activity

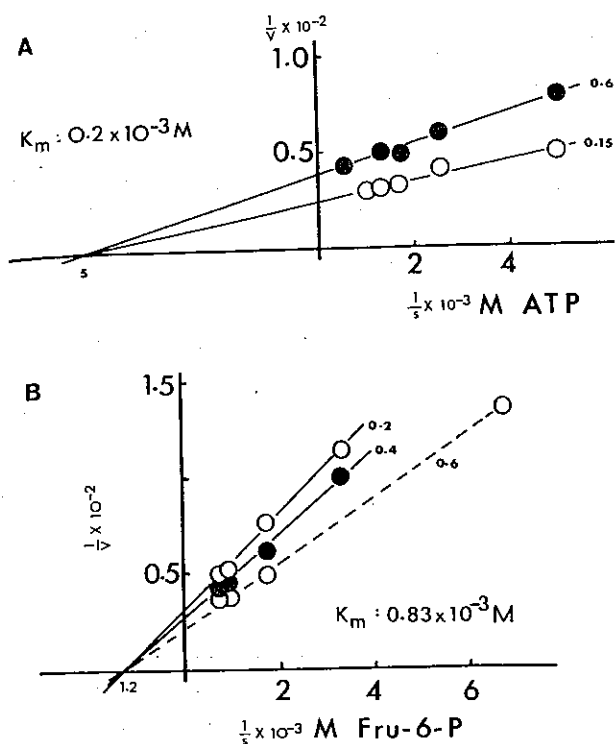


FIG. 5. Lineweaver-Burk plots of the aerobic PFK activity using partially purified and dialyzed enzyme solutions (0.36 mg of protein). A, Effect of ATP at 0.6 (●) and 0.15 mM (○) Fru-6-P. B, Effect of F 6-P at 0.2 (○-○), 0.4 (●-●), and 0.6 (○- -○) mM ATP.

of the aerobic PFK (Fig. 6B). The investigations into the anaerobic PFK give the expected results. The addition of 10 mM 5'-AMP to the assay mixture converted the sigmoidal kinetics to conventional Michaelis-Menten kinetics (Fig. 6A) and also reduced the level of activity itself. It was of interest to note that the velocity curve with the addition of 10 mM 5'-AMP was almost identical to the aerobic PFK velocity curve.

DISCUSSION

The results of our investigations revealed that the switch from aerobiosis to anaerobiosis commences well before the dissolved oxygen tension in the fermenter reaches zero.

Under aerobic conditions, *E. coli* utilizes a higher proportion of glucose via the HMP pathway, which is indicated by the low activities of FDP-aldolase and PFK. However, the low activities indicate that at least PFK is functional under aerobic conditions, although the activity represents only 30% of the level obtainable under anaerobic conditions. Under these conditions PFK is ATP-insensitive and therefore not under ATP control, while at the same time FDP-aldolase activity is low. In this situation, FDP accumulation is facilitated and, although some of the FDP is recirculated via

the HMP pathway, it can also function as an inhibitor of 6-phosphogluconate dehydrogenase (4) and, in this respect, could be an important control step in glucose utilization via the HMP pathway. A regulatory mechanism of this type may be essential in that uncontrolled use of the pathway could lead to an overproduction of intermediate compounds, e.g., pentose phosphate and reduced nicotinamide adenine dinucleotide phosphate plus H<sup>+</sup>, and place an undue strain on the available energy (ATP) required for driving the endergonic biosynthetic reactions in which these compounds are involved.

Our results suggest that PFK is not a limiting factor in the control of aerobic glucose

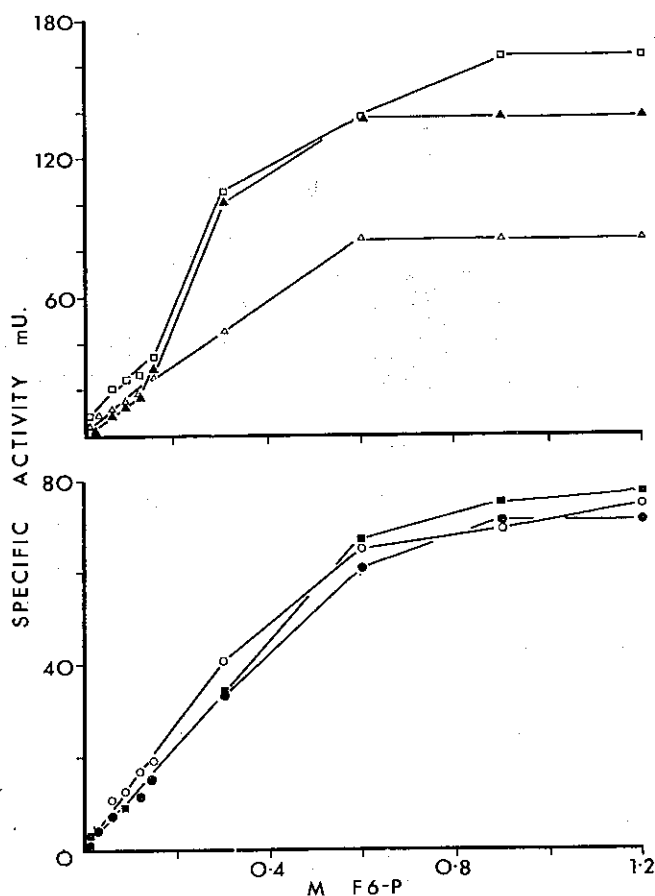


FIG. 6. The effect of various modulators on the PFK activity from anaerobically (A) and aerobically (B) grown *E. coli* K-12.A, Each assay cuvette (see Materials and Methods) contained 0.1 mg of protein of the partially purified and dialyzed enzyme, 0.2 mM ATP, and dialyzed auxiliary enzymes. Symbols: Δ, 0.2 mM ATP + 10 mM 5'-AMP; ▲, 0.2 mM ATP, 0.2 mM ATP + 1 mM ADP; □, 0.2 mM ATP + 1 mM cyclic 3'5'-AMP. B, Each assay cuvette contained 0.3 mg of protein of the partially purified and dialyzed enzyme, 1.0 mM ATP, and dialyzed auxiliary enzymes. Symbols: ●, 1 mM ATP + 1 mM cyclic 3'5'-AMP; ○, 1 mM ATP + 5 mM 5'-AMP; ■, 1 mM ATP; 1 mM ATP + 1 mM ADP.

utilization, and it is possible that FDP-aldolase and 6-phosphogluconate dehydrogenase are the major enzymes involved. The implication of FDP-aldolase as an aerobic control point of glucose utilization via the EMP pathway is new, and mechanisms for this control have yet to be investigated.

As the dissolved oxygen tension approaches zero level, the respiratory system is the first to be affected. Both 2-oxoglutarate and isocitrate dehydrogenases respond with a decrease in activity. At the same time, the increase in FDP-aldolase activity indicates a "stepping-up" of anaerobic glucose utilization via the EMP pathway. A similar increase in FDP-aldolase has been observed in *Aspergillus nidulans* when grown under comparable conditions (5). In lowering the oxygen input further, PFK synthesis begins to increase at the same time 2-oxoglutarate dehydrogenase activity falls to zero, while other respiratory enzymes retain some anaerobic activity (which is in accordance with the suggestion by Amarasingham and Davis [1] that a branched, noncyclic tricarboxylic acid pathway operates in *E. coli* grown under anaerobic conditions). The rapid fall of isocitrate dehydrogenase activity as acid production increased is a further indication that the cycle is degenerating and glucose utilization is becoming fermentative. Simultaneously, certain respiratory enzymes (succinic dehydrogenase, NADH-oxidase and cytochrome  $a_2$ ) acquire maximal activity, which is in agreement with earlier work (10, 16, 17, 26, 27). During this decrease in oxygen partial pressure, it was possible to observe the gradual collapse of the respiratory chain. Wimpenny and Necklen (27) have suggested that this graded collapse could be a result of redox potential changes, and it has been shown to correlate with the sudden appearance of lactate as a metabolic product (20).

During anaerobic growth, the gradual increase of PFK and FDP-aldolase results in an increase in glucose metabolism via the EMP pathway. Since the synthesis of PFK is more rapid than that of FDP-aldolase under these conditions, FDP can still accumulate and inhibit 6-phosphogluconate dehydrogenase (Westwood and Doelle, unpublished data), which is reflected in the almost constant levels of this enzyme. The greater participation of the EMP pathway in anaerobic glucose utilization also increases the formation of NADH, which is known to be an allosteric effector of glucose 6-P dehydrogenase (21). With the decrease in FDP concentration due to increased FDP-aldolase activity and an increase in NADH, the control switches from 6-phospho-

gluconate to the glucose 6-P dehydrogenase. Such an arrangement would make it possible for 6-phosphogluconate dehydrogenase to have sufficient substrate to utilize 20 to 30% of the glucose through the HMP pathway under anaerobic conditions (8, 13). The latter could also be the reason for the increase in glucose 6-P dehydrogenase activity. A general scheme for the regulatory mechanism of aerobic and anaerobic glucose metabolism in *E. coli* is given in Fig. 7.

With this gradual switch to fermentation, the increasing amount of PFK takes over its regulatory control. The additionally formed enzyme is ATP-sensitive and shows all characteristics of an allosteric enzyme. Such an existence of PFK as two different proteins would support the hypothesis (18, 20) that the rate of the reaction will depend not only on the activity of the PFK as controlled by ATP, adenosine diphosphate, and F 6-P concentrations, but also on the concentration of PFK protein in the cell. The theory for control of the Pasteur effect by the control of the PFK activity appears to assume a constant concentration of PFK in the cell and hence a constant rate of PFK synthesis. The high efficiency of aerobic energy production leads to high ATP production rates and low levels of intermediates, so that ATP, although a substrate for PFK, becomes inhibitory. Under strict aerobic growth conditions, there would exist a constant PFK inhibition. By reducing the synthesis by over

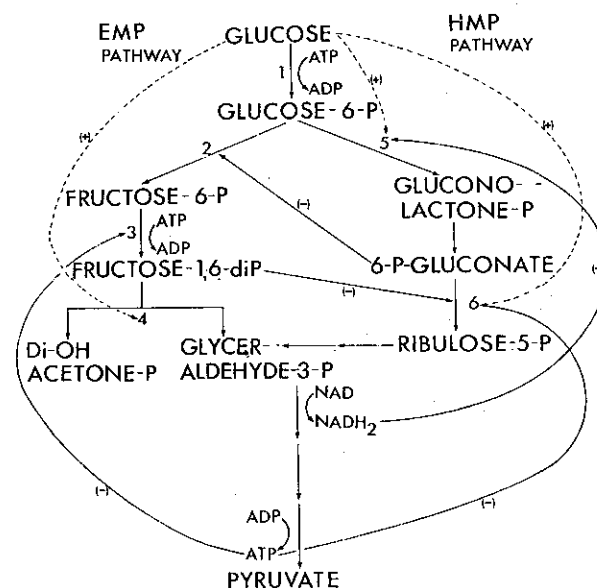


FIG. 7. Proposed schematic representation of the regulatory mechanisms of aerobic and anaerobic glucose metabolism in *E. coli* K-12. Symbols: +, activation; -, inhibition. 1, Glucokinase; 2, phosphoglucose isomerase; 3, PFK; 4, fructose 1,6-diphosphate aldolase; 5, glucose 6-P dehydrogenase; 6, 6-phosphogluconate dehydrogenase.

70% and changing its protein to ATP insensitivity, such a constant inhibition would be unnecessary. The organism would have a more suitable enzymic composition. It is therefore proposed that, under all growth conditions, a certain proportion of the PFK protein remains ATP-insensitive. Under fully aerobic conditions, this proportion of PFK would be the only one synthesized. Under fully anaerobic conditions, a ratio of 70:30 for allosteric to nonallosteric PFK protein exists, the variable being the 70% allosteric PFK protein. During transition from aerobiosis to anaerobiosis, synthesis of the allosteric PFK protein would start at a certain critical level of oxygen tension. This induction point has been shown earlier (20) and is being confirmed here as being below an input  $pO_2$  of 30 mm of Hg or at a DOT of 0 mm of Hg.

Enzymatic and end product determinations of the effect of oxygen on glucose metabolism of *E. coli* K-12 indicate, therefore, that the Pasteur effect appears not to be a single enzyme effect. There is no doubt that PFK becomes, under anaerobic conditions, the energy regulator (3, 25), but FDP-aldolase seems to play an important role as far as the functioning of the EMP pathway is concerned. Preliminary experiments revealed that increased glucose concentrations did not affect the synthesis of PFK under anaerobic conditions, but markedly affected FDP-aldolase synthesis, and that high ATP concentrations inhibited 6-phosphogluconate dehydrogenase activity.

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#### LITERATURE CITED

1. Amarasingham, C. R., and B. D. Davis. 1965. Regulation of  $\alpha$ -ketoglutarate dehydrogenase formation in *Escherichia coli*. *J. Biol. Chem.* **240**:3664-3668.
2. Arrigoni, O., and T. P. Singer. 1962. Limitations of the phenazine methosulfate assay for succinic and related dehydrogenases. *Nature (London)* **193**:1256-1258.
3. Bray, H. G., and K. White. 1966. Kinetics and thermodynamics in biochemistry, p. 366. J. & A. Churchill Ltd., London.
4. Brown, A. T., and C. L. Wittenberger. 1971. Mechanism for regulating the distribution of glucose carbon between the Embden-Meyerhof and hexose-monophosphate pathways in *Streptococcus faecalis*. *J. Bacteriol.* **106**:456-467.
5. Carter, B. L. A., and A. T. Bull. 1969. Studies of fungal growth and intermediary carbon metabolism under steady and nonsteady state conditions. *Biotechnol. Bioeng.* **11**:785-804.
6. Doelle, H. W., and G. J. Manderson. 1971. Comparative studies of fructose 1,6-diphosphate aldolase from *Escherichia coli* 518 and *Lactobacillus casei* var. rhamnosus ATCC 7469. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **37**:21-31.
7. Ecker, R. E., and W. R. Lockhart. 1961. Specific effect of limiting nutrient on physiological events during culture growth. *J. Bacteriol.* **82**:511-516.
8. Fraenkel, D. G., and B. L. Horecker. 1965. Fructose 1,6-diphosphatase and acid hexose phosphatase of *Escherichia coli*. *J. Bacteriol.* **90**:837-842.
9. Gibbs, S. P., W. R. Sistrom, and P. B. Worden. 1965. The photosynthetic apparatus of *Rhodospirillum molischianum*. *J. Cell Biol.* **26**:395-412.
10. Gray, C. T., J. W. T. Wimpenny, and M. R. Mossman. 1966. Regulation of metabolism in facultative bacteria. II. Effects of aerobiosis, anaerobiosis and nutrition on the function of Krebs cycle enzymes in *Escherichia coli* K-12. *Biochim. Biophys. Acta* **117**:33-41.
11. Green, D. E., and D. M. Ziegler. 1963. Electron transport particles, p. 416-424. In S. P. Colowick and N. O. Kaplan (ed.), *Methods in enzymology*, vol. 9. Academic Press Inc., New York.
12. Huggett, A. St. G., and D. A. Nixon. 1957. Enzymic determination of blood glucose. *Biochem. J.* **66**: 12P.
13. Katz, J., and H. G. Wood. 1960. The use of glucose-C<sup>14</sup> for the evaluation of the pathways of glucose metabolism. *J. Biol. Chem.* **235**:2165-2177.
14. Kellenberger, E., and A. Ryter. 1968. Cell wall and cell membrane of *E. coli*. *J. Biophys. Biochem. Cytol.* **4**: 323-326.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
16. Moss, F. 1952. The influence of oxygen tension on respiration and cytochrome  $a_2$  formation of *Escherichia coli*. *Aust. J. Exp. Biol. Med. Sci.* **30**:531-540.
17. Moss, F. 1956. Adaptation of the cytochromes of *Aerobacter aerogenes* in response to environmental oxygen tension. *Aust. J. Exp. Biol. Med. Sci.* **34**:395-406.
18. Pardee, A. B. 1959. Mechanisms for control of enzyme synthesis and enzyme activity in bacteria. p. 295. In G. E. W. Wolstenholme and C. M. O'Connor (ed.), *Regulation of cell metabolism*. J. & A. Churchill Ltd., London.
19. Pontrefract, R. D., G. Bergeren, and F. S. Thatcher. 1969. Mesosomes in *Escherichia coli*. *J. Bacteriol.* **97**: 367-375.
20. Reichelt, J. L., and H. W. Doelle. 1971. The influence of dissolved oxygen concentration on phosphofructokinase and the glucose metabolism of *Escherichia coli* K-12. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **37**:497-506.
21. Sanwal, B. D. 1970. Regulatory mechanisms involving nicotinamide adenine nucleotides as allosteric effectors. III. Control of glucose 6-phosphate dehydrogenase. *J. Biol. Chem.* **245**:1626-1631.
22. Sibley, J. A., and A. L. Lehninger. 1949. Determination of aldolase in animal tissues. *J. Biol. Chem.* **177**:859-872.
23. Sly, L. I., and H. W. Doelle. 1968. Glucose 6-phosphate dehydrogenase in cell free extracts of *Zymomonas mobilis*. *Arch. Mikrobiol.* **63**:197-213.
24. Sly, L. I., and H. W. Doelle. 1968. 6-Phosphogluconate dehydrogenase in cell free extracts of *Escherichia coli* K-12. *Arch. Mikrobiol.* **63**:214-223.
25. Stadtman, E. R. 1966. Allosteric regulation of enzyme activity. *Advan. Enzymol.* **28**:41-154.
26. Wimpenny, J. W. T. 1969. Oxygen and carbon dioxide as regulators of microbial metabolism. *Symp. Soc. Gen. Microbiol.* **19**:161-197.
27. Wimpenny, J. W. T., and D. K. Necklen. 1971. The redox environment and microbial physiology. I. The transition from anaerobiosis to aerobiosis in continuous cultures of facultative anaerobes. *Biochim. Biophys. Acta* **253**:352-359.