JOURNAL OF BACTERIOLOGY, Oct., 1965 Pp. 1013-1016
Vol. 90, No. 4

Adenosine Triphosphate Pool During the Growth Cycle in *Streptococcus faecalis*

W. W. FORREST

Division of Biochemistry and General Nutrition, Commonwealth Scientific and Industrial Research Organiatzion, University of Adelaide, Adelaide, South Australia

Received for publication 19 June 1965

ABSTRACT

FORREST, W. W. (University of Adelaide, Adelaide, South Australia). Adenosine triphosphate pool during the growth cycle in *Streptococcus faecalis*. J. Bacteriol. **90**:1013-1018. 1965.—The adenosine triphosphate (ATP) pool has been studied throughout the growth cycle of *Streptococcus faecalis*. Normally, the pool is quantitatively related to the concentration of organisms and the growth rate, but deviations from these relationships can occur without affecting the growth rate of the organisms. A critical concentration of ATP seems necessary for exponential growth, and, at lower levels, only linear growth can occur. If no growth can take place, catabolism of added energy source gives rise to a large increase in the pool level. The pool level represents the balance between the demands of the organisms for energy and the supply of energy derived from catabolism of the substrate.

It is well established (Bauchop and Elsden, 1960) that the amount of cellular material synthesized during growth of microorganisms is related to the amount of adenosine triphosphate (ATP) produced by catabolism of the energy source; the growth-yield coefficient Y_{ATP} is 10 mg (dry weight) of cellular material per mmole of ATP. This coefficient has usually been determined by growing organisms in batch culture with growth limited by the energy source, so that a single determination is made of the equilibrium condition after growth has ceased, or under steady-state conditions in continuous culture. Kinetic experiments in batch culture (Forrest and Walker, 1962, 1964) also indicate that this same proportionality between ATP and cellular material synthesized applies throughout the period of growth. The relationship, of course, is based on calculation of the amount of ATP produced during catabolism of a substrate by a specified biochemical pathway.

It would appear then that measurements of the ATP pool in an organism would exhibit regularities related to the rate of production of ATP. It would also be expected that the pool level would be related to the energy requirements of the organism (Krebs 1962), so that comparisons of the ATP pool with the synthetic activity of an organism would give an indication of its energetic behavior. However, studies reported in the literature are not easily interpreted on this basis.

From measurements of the ATP pool in *Escherichia coli* (Franzen and Binkley, 1961) it has been calculated that the concentration of ATP is not affected by the growth rate of the organisms, whereas in *Aerobacter aerogenes* it has been shown (Strange, Wade, and Dark, 1963) that the main factor affecting the ATP pool in resting cells of this organism is the oxygen tension in the suspending medium, and that the pool level is not necessarily related to the energy requirements of the organisms. Thus, in *A. aerogenes*, the pool level seems to depend on whether oxidative metabolism can take place. Similarly, in washed suspensions of *Streptococcus faecalis*, addition of exogenous substrate causes a rise in the ATP pool level; this level can be modified by the synthetic activities of the organism, but the calculated rate of ATP production from catabolism of the energy source is not affected by this change in the pool level, so that ATP production appears unaffected by change in the energy requirements of the organism (Forrest and Walker, 1965*a*).

It seems that the behavior of the ATP pool may be more complex than would be suggested by a simple steady-state balance between well-regulated (Krebs, 1962) rates of production and utilization for synthetic reactions. Accordingly, a more detailed examination of the pool during the growth cycle of *S. faecalis* has been made; the results reveal regularities which are apparently not so obvious in organisms with a more complex aerobic metabolism.

MATERIALS AND METHODS

Organism. S. faecalis (ATCC 4083) was used throughout this work.

Chemicals. ATP and dried firefly lanterns were obtained from Sigma Chemical Co., St. Louis, Mo. Dried yeast, peptone, and Tryptone for bacterial growth media were obtained from Difco Laboratories.

Bacterial growth media. Medium A consisted of 2% each of yeast extract, peptone, glucose, and sodium citrate, all in distilled water. Medium B consisted of: 1% Tryptone, 0.5% yeast extract, 0.5% stock salts solution (Bauchop and Elsden, 1960), 0.75% each of KH_2PO_4 and K_2HPO_4, 0.1% glucose, and 0.5% ethylenediaminetetraacetic acid (EDTA) disodium salt. Medium C was the same as medium B, but glucose was replaced by 0.6% sodium pyruvate. Subcultures of the organism were maintained on medium A.

Washed suspensions of bacteria. The bacteria were grown on medium A at 37 C, harvested by centrifugation 17 hr after inoculation, washed twice with deaerated 0.2 M potassium phosphate buffer (*p*H 6.2), and suspended in the same buffer at 37 C under a gas phase of nitrogen.

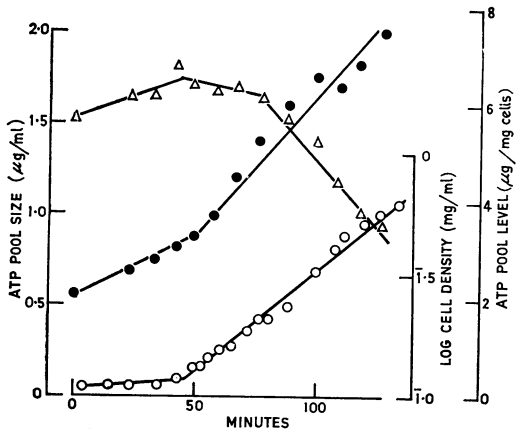

FIG. 1. *Pool of ATP during the lag phase and at the beginning of exponential growth on complex medium A. Symbols:* ○, *cell density;* ●, *pool size;* △, *pool level.*

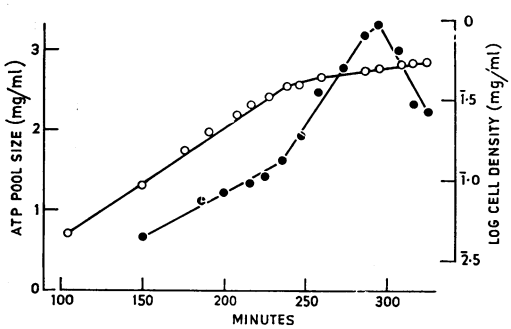

FIG. 2. *Pool of ATP during exponential and linear growth on complex medium A (medium containing excess glucose). Symbols:* ○, *cell density;* ●, *pool size.*

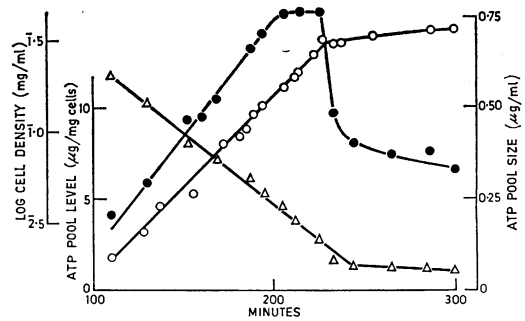

FIG. 3. *Pool of ATP during exponential growth on semidefined medium B. Growth was limited by the energy source (glucose). Symbols:* ○, *cell density;* ●, *pool size;* △, *pool level.*

Bacterial growth yields. The progress of growth was followed by nephelometric measurements. Bacterial dry weights were determined by measurement of the optical density at 650 mμ and referring to a standard curve.

Luciferase assay for ATP. A volume of bacterial suspension sufficient to contain about 10 μg of ATP was centrifuged; the cell paste so obtained was extracted for 30 min at room temperature with 0.5 ml of 0.3 M H_2SO_4, neutralized with NaOH, and the volume was made up to 10 ml. This suspension was centrifuged, and 1 ml of the supernatant fluid was assayed for ATP by the method of Forrest and Walker (1965*b*).

The quantity measured in the assay was the ATP pool size—the amount of ATP per unit volume of bacterial suspension. The pool level in the organisms—the amount of ATP per unit mass of bacteria—was calculated from the pool size.

RESULTS

The behavior of the ATP pool during the lag phase is shown in Fig. 1. A comparatively large inoculum of bacteria was used so that there was only a short lag period. The level of the ATP pool rose throughout the lag phase to a maximum at the point where exponential growth began, then fell steadily as growth proceeded. The size of the ATP pool also increased slowly during the lag phase, then increased rapidly when growth began. Measurements of the rate of glycolysis, which gives an index of the rate of production of ATP, showed no corresponding inflection; the rate increased steadily throughout the lag phase and beginning of exponential growth (Forrest and Walker, 1964).

Figure 2 shows the behavior of the pool during the succeeding phase of growth. During exponential growth, the size of the pool increased

at a constant rate. When excess energy source was present, a later phase of linear growth occurred. At this inflection in the growth curve, there was a corresponding change in the rate of increase in the size of the ATP pool. As the rate of growth decreased, the size of the ATP pool increased more rapidly till it reached a maximal level and declined, even though the remaining energy source was still being metabolized by the organisms.

When the amount of growth was limited by energy source (Fig. 3), there was again a constant rate of increase in the size of the ATP pool during exponential growth, but when the energy source had all been degraded, the size of the pool decreased rapidly and growth stopped. The maximal level of the pool at the beginning of growth was over 12 μg of ATP per mg (dry weight) of cells; this level then fell steadily till the end of growth.

In the previous experiments, the energy source was glucose. When pyruvate was used as the energy source, there was again a maximal level of

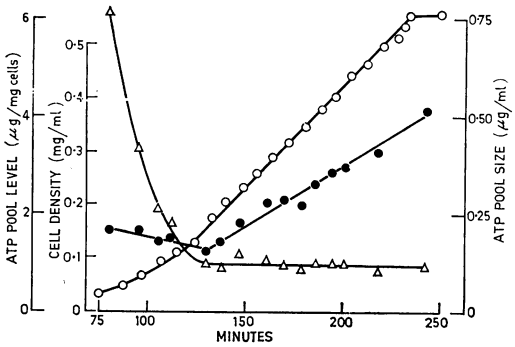

FIG. 4. *Pool of ATP during growth on semidefined medium C. Growth was limited by the energy source (sodium pyruvate). Symbols: ○, cell density; ●, pool size; △, pool level.*

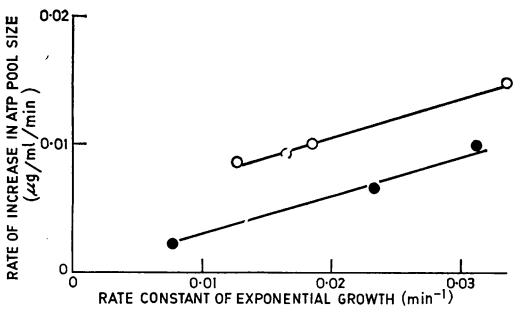

FIG. 5. *Relationship between rate of exponential growth and rate of increase in the pool of ATP. Symbols: ○, medium A (containing citrate buffer); ●, medium B (containing phosphate buffer).*

TABLE 1. *Level of ATP pool during exponential growth**

Medium	Mean pool level	Rate constant k_1 (min⁻¹)	ATP
	μg/mg		μg/ml
A	6.75	0.0128	1.05
		0.0156	1.02
		0.0184	1.00
		0.0336	0.98
B	5.1	0.0077	0.75
		0.0233	0.80
		0.0312	0.75
C	0.9	0.0322	0.14

* The pool level was calculated from best-fit curves to data from growth experiments shown in Fig. 1 to 5. Comparison is made at a cell density of 0.15 mg/ml of medium.

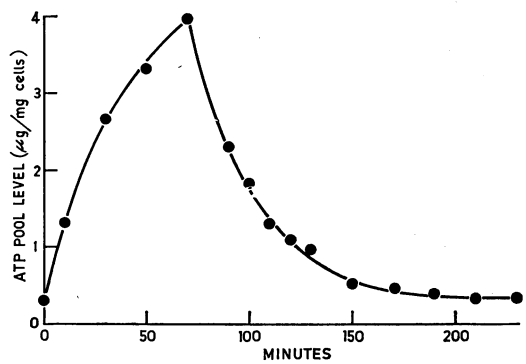

FIG. 6. *Level of the ATP pool during glycolysis by a washed suspension. Cell density, 1.34 mg/ml; glucose added at zero-time, 8 μmoles/ml.*

ATP at the beginning of exponential growth, but the pool level fell very rapidly to a low and constant value (Fig. 4). This period of rapid fall in the level was correlated with the very short period of exponential growth, and the constant low level of ATP was maintained during linear growth.

The first-order rate constants k_1 for exponential growth of the organisms were calculated for a number of experiments of the type shown in Fig. 1 to 3 with medium A and B (energy source, glucose). The rate of increase of the size of the ATP pool was correlated closely with the rate constant k_1 (Fig. 5). The results fell into two sets for the different media.

It was found also that the level of the ATP pool was correlated with the concentration of organisms independently of the rate of growth. The common range of cell densities where exponential growth occurred on different media

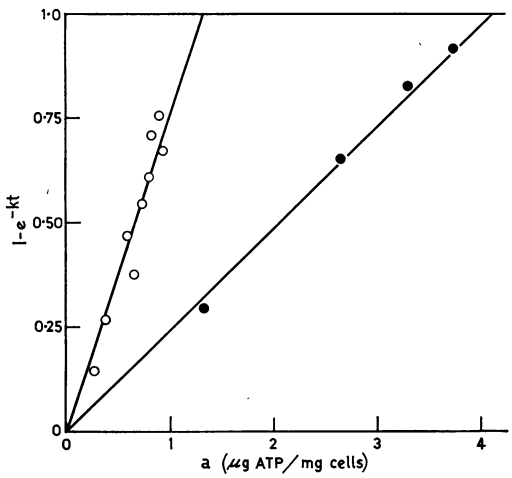

FIG. 7. *Plot of equation 2 (see text), showing ATP pool levels in washed suspensions catabolizing energy sources as a combination of constant rate of input to the pool opposed by exponential decay. Energy source:* ○, *arginine;* ●, *glucose (experiment shown in Fig. 6).*

was restricted, but a cell density of 0.15 mg/ml was common to all media. The comparison between the ATP pool and the rate of growth at this cell density is given in Table 1.

A washed suspension of cells grown on medium A exhibits a substantial endogenous metabolism, characterized among other properties of the organisms by a constant level (and size, since the bacterial population is constant) of ATP in the pool (Forrest and Walker, 1965*a*).

When an exogenous energy source was added to such a suspension (Fig. 6), it was degraded at a constant rate, and catabolism of the energy source gave rise to ATP which could not be used for growth, since no materials were available for synthesis. The ATP pool level rose, but the constant input of ATP to the pool was balanced by an exponential decay process, so that the pool level rose toward an upper limit. When all the added energy source had been used, the decay process alone operated, so that the level of the pool fell back to the base level characteristic of cells carrying on endogenous metabolism in the absence of exogenous energy sources.

The curve in Fig. 6 can be resolved into these components of linear increase and exponential decay:

If a is the level of ATP above the base level, then the rate of increase da/dt of a with time t is

$$\frac{da}{dt} = -ka + b \qquad (1)$$

where b is the constant rate of input of ATP to the pool and k is the first-order rate constant for the decay process.

Integrating this function

$$a_t = \frac{b}{k}(1 - e^{-kt}) \qquad (2)$$

where e is the base of the natural system of logarithms.

From the decay curve of Fig. 6, $k = 0.0350$ min^{-1}; Fig. 7 shows the initial observations of Fig. 6 plotted by use of this value in equation (2). The intercept at $1 - e^{-kt} = 1$ corresponds to the asymptotic upper limit of the pool at infinite time, and the slope of the plot gives the rate of input to the pool.

A similar experiment with arginine as substrate is also plotted in Fig. 7. Here the decay constant $k = 0.0156$ min^{-1}. The slopes of the two plots are correlated with the rates of generation of ATP estimated from the catabolism of the substrates: 75 μg per mg of cells per min from glycolysis, and 42 μg per mg of cells per min from the degradation of arginine.

DISCUSSION

S. faecalis has a comparatively simple anaerobic metabolism; in washed suspension, glucose is degraded by the Embden-Meyerhof pathway to give 2 moles of lactic acid and 2 moles of ATP per mole of glucose degraded. During growth, the products of catabolism may include steam-volatile fatty acids, with production of up to 3 moles of ATP per mole of glucose (Forrest and Walker, 1965*b*). Arginine and pyruvate each produce 1 mole of ATP per mole of energy source. However, even in this simple system the pool of ATP is influenced by opposing factors.

One of these factors is the rate of generation of ATP by the metabolic processes of the organisms. In washed suspension, catabolism of glucose produces ATP much more rapidly than the degradation of arginine does, so that the difference in the rates of generation of ATP is reflected in different rates of input of ATP to the pool, and different limiting values to which the level in the pool can rise. In both cases, the amount of ATP in the pool is small compared with the amount generated by catabolism of the substrate, so that the calculated turnover time of the ATP in the pool, assuming full coupling, is a few seconds.

A second factor is the rate of use by the organisms. In a nongrowing suspension metabolizing glucose, the pool level may be markedly lowered if the organisms increase their demand for

energy, though the rate of glycolysis is unaffected (Forrest and Walker, 1965*a*).

Both these processes of generation and use vary during the lag phase and at the beginning of exponential growth. Measurements of the rate of glycolysis show a rate per unit mass of bacterial cells which increases steadily throughout the lag phase and at the beginning of exponential growth (Forrest and Walker, 1964); thus, the rate of generation of ATP increases continuously, and the pool level also rises during the lag phase. However, when growth begins, the synthetic reactions which occur make heavy demands on the ATP pool, so that there is now a decrease in pool level, and this level continues to fall during exponential growth. As growth proceeds, the rate of glycolysis per unit mass of cells remains constant (Forrest and Walker, 1964), and the rate of input of ATP to the pool increases exponentially. However, the pool size increases only linearly.

Growth continues until it is checked by some change in environmental conditions; if the substrate is exhausted, there is no input of ATP to the pool, and synthesis cannot proceed. However, the pool size does not fall immediately but exhibits a decay curve similar to that found in washed suspensions. If growth is limited by a factor other than energy source, a period of linear growth may succeed the exponential growth. Here, ATP is still being generated by glycolysis, though the demands of the organism decrease (Forrest and Walker, 1965*b*). Consequently, there is a sharp rise in the rate of increase in pool size; this rise continues till a maximal pool size is reached, but eventually the pool size falls again even though excess substrate is still present.

From the experiments with pyruvate as energy source, it seems that yet another cause of transition to linear growth may operate. It has been suggested that a critical level of ATP is necessary for growth (Hess, 1963), and it has been shown that in this organism endogenous metabolism cannot be coupled to biologically useful energy of maintenance unless a sufficient pool of ATP is present (Forrest and Walker, 1965*a*). In growth with pyruvate as energy source, the rate of input of ATP to the pool is low, so that the pool level falls very rapidly during exponential growth. It appears then to fall below the critical level necessary to sustain exponential growth, so that linear growth takes place, apparently limited by the availability of ATP for synthesis. In this strain of *S. faecalis*, growth will not occur with arginine alone as energy source; glucose is also necessary in small amounts (Bauchop and Elsden, 1960), and it may be that arginine alone will not produce the critical level of ATP necessary for growth to begin.

The level of the ATP pool is also affected by the medium. The complex medium A used in these experiments contains only the phosphate naturally present in the medium constituents, about 0.01%, whereas medium B is made up in phosphate buffer. It has been demonstrated that the ATP pool of resting cells carrying on endogenous metabolism is markedly affected by the replacement of phosphate by citrate buffer in the suspending medium (Forrest and Walker, 1965*a*), so that the effect of the growth medium on the pool level is probably due also to the differing phosphate concentration.

The level of the ATP pool is apparently independent of growth rate over a wide range of rates of exponential growth. A similar finding was reported with *E. coli* by Franzen and Binkley (1961). When pyruvate is the energy source, the rate of exponential growth (Table 1) is comparable with that found when glucose is the substrate; thus, the rate of withdrawal of ATP from the pool for synthetic reactions must also be comparable. However, the rate at which the pool falls during growth is much greater with pyruvate as substrate, and the rate of input of ATP to the pool during catabolism of pyruvate must therefore be correspondingly lower than with glucose as substrate. Similarly, towards the end of exponential growth on medium B with glucose as energy source (Fig. 2), the size of the ATP pool reaches a limiting value, without apparently affecting the rate of exponential growth. This, of course, is not the usual situation during exponential growth, where the quantitative relationships shown in Fig. 6 and Table 1 normally hold between the ATP pool, growth rate, and cell density.

It seems, therefore, that the level of the pool of ATP has only an indirect effect on the growth rate of the organism. The level in the pool is normally subject to steady-state conditions, but the conditions can vary widely from these without there being any effect on the rate of growth. Conversely, the rate of growth can vary widely without affecting the steady-state level in the pool. The main requirement for exponential growth, so far as the ATP pool is concerned, appears to be that it should be above a critical value necessary to provide energy for all the synthetic processes which occur; if for any reason the pool level falls below the critical value, growth cannot proceed exponentially.

These results are important in their relationship to the hypothesis of feed-back control of glycolysis (Krebs, 1962). ATP, adenosine diphos-

phate (ADP) (Hommes and Schuurmans Stekhoven, 1964), and related compounds each have been proposed as the controlling intermediate compound. It is postulated that the organism tends to maintain a steady level of ATP, and, if the demands of the organism for energy deplete the ATP pool, the feed-back mechanism opposes the change by increasing the rate of catabolism of energy source. Krebs' (1964) calculations indicate that, under physiological conditions, small changes in ATP concentration reflect larger changes in ADP and adenosine monophosphate (AMP). The present measurements, indicating that during growth wide variations can occur in the ATP pool level, and presumably also in the ADP and AMP pools, without affecting the growth rate, are not in accordance with this hypothesis. A similar situation occurs in nongrowing suspensions where the pool-level can change 10-fold without affecting the rate of glycolysis; in this situation it has been proposed that direct feed-back control of glycolysis mediated by the pools of adenine nucleotides does not occur (Forrest and Walker, 1965*a*).

In mixed populations of microorganisms degrading complex substrates, such as occur in natural systems, there is considerable difficulty in measurement of the processes taking place. The size of the ATP pool in such a population may be a useful index of the efficiency of the system in coupling energy to biologically useful processes, particularly since the overall pattern of metabolism can be determined calorimetrically (Walker and Forrest, 1964). It is planned to carry out such studies in mixed cultures of microorganisms.

ACKNOWLEDGMENT

The technical assistance of P.R. Monk is gratefully acknowledged.

LITERATURE CITED

BAUCHOP, T., AND S. R. ELSDEN. 1960. Growth of micro-organisms in relation to their energy supply. J. Gen. Microbiol. **23**:457–469.

FORREST, W. W., AND D. J. WALKER. 1962. Thermodynamics of biological growth. Nature **196**:990–991.

FORREST, W. W., AND D. J. WALKER. 1964. Change in entropy during bacterial metabolism. Nature **201**:49–52.

FORREST, W. W., AND D. J. WALKER. 1965*a*. Control of glycolysis in washed suspensions of *Streptococcus faecalis*. Nature **207**:46–48.

FORREST, W. W., AND D. J. WALKER. 1965*b*. Synthesis of reserve materials for endogenous metabolism in *Streptococcus faecalis*. J. Bacteriol. **89**:1448–1452.

FRANZEN, J. S., AND S. B. BINKLEY. 1961. Comparison of the acid-soluble nucleotides in *Escherichia coli* at different growth rates. J. Biol. Chem. **236**:515–519.

HESS, B. 1963. Control of metabolic rates, p. 347. *In* B. Wright [ed.], Control mechanisms in respiration and fermentation. Ronald Press, New York.

HOMMES, F. A., AND F. M. A. H. SCHUURMANS STEKHOVEN. 1964. Aperiodic changes of reduced nicotinamide-adenine dinucleotide during anaerobic glycolysis in brewers' yeast. Biochim. Biophys. Acta **86**:427–428.

KREBS, H. 1962. Control of cellular metabolism, p. 279. *In* J. M. Allen [ed.], The molecular control of cellular activity. McGraw-Hill Book Co., Inc., New York.

KREBS, H. 1964. Gluconeogenis. Proc. Roy. Soc. (London) Ser. B **159**:545–563.

STRANGE, R. E., H. E. WADE, AND F. A. DARK. 1963. Effect of starvation on adenosine triphosphate concentration in *Aerobacter aerogenes*. Nature **199**:55–57.

WALKER, D. J., AND W. W. FORREST. 1964. Application of calorimetry to the study of the ruminal fermentation *in vitro*. Australian J. Agr. Res. **15**:299–315.