Effect of Growth Rate and Nutrient Limitation on the Adenine Nucleotide Content, Energy Charge and Enzymes of Adenylate Metabolism in *Azotobacter beijerinckii*

By IAN D. MARRIOTT,[†] EDWIN A. DAWES*
AND THE LATE BERNARD I. ROWLEY

Department of Biochemistry, University of Hull, Hull HU6 7RX, U.K.

(Received 20 October 1980; revised 22 January 1981)

The effects of dilution rate and glucose, nitrogen or oxygen limitation on the intracellular and extracellular concentrations of adenine nucleotides, on the adenylate energy charge and on the specific activities of four enzymes of adenylate metabolism, have been investigated with chemostat cultures of *Azotobacter beijerinckii*. Under glucose limitation both ATP and ADP contents and the total adenylate pool increased with an increase in dilution rate while the energy charge remained constant at 0.85; the concentrations of extracellular ADP and AMP rose. With nitrogen limitation all the intracellular adenylates decreased with an increase in dilution rate while the concentration of extracellular ADP and AMP decreased markedly; the energy charge rose from 0.72 to 0.79. Under oxygen limitation the ATP content and total adenylate pool increased at higher dilution rates and the energy charge increased from 0.71 at $D = 0.1 \text{ h}^{-1}$ to 0.81 at $D = 0.15 \text{ h}^{-1}$ and then remained fairly constant; the concentration of extracellular adenylates decreased.

The specific activity of adenylate kinase was relatively unaffected by dilution rate under nitrogen or oxygen limitation but was inversely related to it in organisms grown under glucose limitation. AMP nucleosidase activity was inversely related to dilution rate under glucose-, nitrogen- and oxygen-limited conditions, while adenosine deaminase was relatively unaffected by dilution rate except for a decrease in organisms from oxygen-limited cultures at lower growth rates. The specific activity of adenine deaminase was inversely related to dilution rate in organisms grown under glucose and nitrogen limitation but showed little change under oxygen limitation. The principal route of AMP degradation in *A. beijerinckii* is mediated by AMP nucleosidase and adenine deaminase.

INTRODUCTION

The important role of adenine nucleotides in the regulation of cellular activities is now well established and the unifying concept of the adenylate energy charge (Atkinson, 1968), while not without its critics, has stimulated considerable research effort (reviewed by Knowles, 1977). Our previous studies on the regulation of glucose metabolism and poly- β -hydroxybutyrate biosynthesis in *Azotobacter beijerinckii* (Senior & Dawes, 1971, 1973; Senior *et al.*, 1972) and on the effects of dissolved oxygen concentration (Jackson & Dawes, 1976; Ward *et al.* 1977; Carter & Dawes, 1979) led us to investigate intracellular adenine nucleotide concentrations under oxygen, nitrogen and carbon limitation in the chemostat. Relatively little work has been published on the effect of the growth rate of the population on the adenine nucleotide content of bacteria (Chapman & Atkinson, 1977) and it was of interest to examine this parameter, which influences poly- β -hydroxybutyrate deposition in *A.*

[†] Present address: Department of Plant Biology, University of Hull, Hull HU6 7RX, U.K.

beijerinckii (Senior *et al.*, 1972). Poly- β -hydroxybutyrate is unique among bacterial reserve polymers in that its synthesis does not directly involve the participation of ATP; further, it is synthesized by *A. beijerinckii* in substantial amounts only under conditions of oxygen limitation, when its formation serves as an electron acceptor alternative to oxygen.

Studies with *Azotobacter vinelandii* (Haaker & Veeger, 1976; Jones *et al.*, 1973) demonstrated a dependence of the intracellular adenine nucleotide concentration of washed suspensions on the oxygen input, while batch culture experiments have indicated an important role for AMP nucleosidase in the regulation of adenylyate pool size and energy charge (Schramm, 1974; Leung & Schramm, 1978). We have therefore also examined the effect of growth rate on several enzymes associated with adenylyate metabolism and on the excretion of adenine nucleotides by *A. beijerinckii*.

METHODS

Growth of the organism. *Azotobacter beijerinckii* NCIB 11292 was grown at 30 °C in chemostat culture in a glucose/minimal salts medium as described by Senior *et al.* (1972). Under nitrogen-limited conditions the dissolved oxygen tension was maintained at 3.9% of air saturation (0.8 kPa) and for oxygen limitation (undetectable dissolved oxygen tension) the O₂ supply was 30 ml min⁻¹ with an N₂ flow rate of 800 ml min⁻¹. For nitrogen limitation the gas was supplied at 20 ml min⁻¹ and the O₂/N₂ mixture was diluted with 591 ml argon min⁻¹. For carbon limitation, 69.4 mM-glucose was provided; under all other conditions the glucose concentration was 90 mM.

Sampling procedure. Samples were taken directly from the chemostat via a three-way lever tap attached to the culture vessel (Jackson & Dawes, 1976) into a syringe containing H₂SO₄ (to give a final concentration of 300 mM). Immediately before sampling, a syringe was attached to the tap and sufficient culture was removed from the chemostat to clear the sampling system. The syringe containing H₂SO₄ was then substituted, its plunger withdrawn to secure a vacuum, and the tap opened to collect the sample. Extremely rapid mixing of the culture and acid was achieved. When comparisons were made visually using dye and water, this technique proved superior in our hands to other methods described by Knowles (1977). The sample was immediately divided; one half was mixed with 0.05 M-Na/K phosphate buffer, pH 7.3, and the remainder with the same buffer containing known concentrations of ATP, ADP and AMP. After 10 min the pH of both samples was adjusted to 7.3 with 1 M-NaOH. Denatured material was removed by centrifuging for 15 min at 3000 g at 4 °C and the supernatant was stored at 0 °C. The extracts were assayed for adenine nucleotides within 5 h.

Measurement of ATP, ADP and AMP. ATP in bacterial extracts was measured by the firefly luminescence method using a high-gain photomultiplier tube as described by Dawes & Large (1970) except that the output of the photomultiplier tube was recorded by a Smith 'Servoscribe' linear chart recorder (Smith Industries, Wembley, Middlesex). ADP and AMP in extracts were determined as ATP after enzymic conversion of each to ATP (Johnson *et al.*, 1970). An increase in the period prior to neutralization of the acidified samples, variation of the concentration of H₂SO₄, substitution of HClO₄ for H₂SO₄, or centrifuging the samples prior to neutralization, had no effect on the ATP measured in these assays.

Protein. Protein was measured by the method of Lowry using bovine serum albumin standards with each estimation.

Harvesting of bacteria and preparation of cell-free extracts for enzyme assays. These operations were carried out as described by Senior & Dawes (1973) using a French press for disruption of the bacteria and 167 mM-triethanolamine/HCl buffer, pH 8.0, for washing and resuspending the organisms.

Enzyme assays. AMP nucleosidase (EC 3.2.2.4), adenine deaminase (EC 3.5.4.2), adenosine deaminase (EC 3.5.4.4) and AMP deaminase (EC 3.5.4.6) were assayed essentially as described by Schramm & Lazorik (1975) after optimizing the assay conditions for *A. beijerinckii*. Extracts dialysed for either 3 h or overnight against 50 to 100 vol. triethanolamine/HCl buffer gave higher activities than undialysed extracts in each of these assays except that for AMP deaminase, significant activity of which was not detected under any of the conditions used in this work. Because of the report by Yoshino *et al.* (1979) on the effects of monovalent cations on AMP nucleosidase, tests were carried out with the standard assay system to ensure that optimum conditions were being used. Adenylyate kinase (EC 2.7.4.3) was assayed by the method of Sottocasa *et al.* (1967).

Analysis of culture supernatants for adenine and hypoxanthine. Portions of culture supernatant containing about 1 μ mol of A₂₆₀-absorbing material (assuming a molar absorption coefficient, ϵ , of 13×10^3 l mol⁻¹ cm⁻¹ for adenine) were evaporated to dryness under reduced pressure and then taken up in 100 μ l water. Portions (10 μ l), together with known amounts of adenine and hypoxanthine, were applied to thin-layer cellulose sheets containing a fluorescent indicator (Eastman, cat. no. 13254) and developed with 2-propanol/NH₄OH/water

(70:10:20, by vol.) (Schramm & Lazarik, 1975). After drying the plates, spots were visualized under u.v. light. The cellulose of each spot was carefully scraped off and added to 2 ml 0.1 M-HCl. Cellulose was removed from the resulting suspensions by centrifuging and adenine ($\epsilon_{262} = 13 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$) and hypoxanthine ($\epsilon_{248} = 10.8 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$) in the supernatant were assayed spectrophotometrically against the standards. The limit of sensitivity was 0.05 μmol .

Poly- β -hydroxybutyrate. This was determined by the method of Carter & Dawes (1979).

Viability measurements. The viability of chemostat cultures was measured by a slide culture technique (Ward *et al.*, 1977) and by plate counts.

RESULTS AND DISCUSSION

Effect of culture dilution rate and nutrient limitation on the intracellular adenylate pool, energy charge and extracellular adenylate concentration

The effect of culture dilution rate on the intracellular adenine nucleotide content and adenylate energy charge of *A. beijerinckii* was studied under three different nutrient limitations, and the extracellular concentrations of adenylates were also examined under these conditions (Table 1). In Table 1, the intracellular adenylate concentrations are expressed on the basis of both intracellular protein and bacterial dry weight. We have made this comparison because protein content varies according to nutrient limitation and growth rate, e.g. under oxygen limitation organisms contain poly- β -hydroxybutyrate in amounts inversely related to their growth rate (Senior *et al.*, 1972; and Table 1).

Glucose limitation. Both ATP and ADP contents and the total adenylate pool increased with an increase in dilution rate, while the energy charge remained unchanged at 0.85. The intracellular concentration of AMP was fairly constant but the extracellular ADP and AMP concentrations were higher at the faster dilution rates.

Nitrogen limitation. At the higher dilution rate all the intracellular adenine nucleotides showed some decrease in concentration, while the concentration of extracellular ADP and AMP decreased by about 89% and 96%, respectively, and the energy charge increased from 0.72 to 0.79.

Oxygen limitation. Although the total adenylate pool showed little change on a protein basis it increased when referred to dry bacterial weight. The ATP content increased at higher dilution rates and the energy charge increased from 0.71 at $D = 0.1 \text{ h}^{-1}$ to 0.81 at $D = 0.15 \text{ h}^{-1}$ and then remained fairly constant. At the two lowest dilution rates, organisms from oxygen-limited cultures displayed the highest concentrations of extracellular adenylates of the three different nutrient limitations investigated, AMP accounting for the major proportion. With an increase in dilution rate the extracellular AMP and ADP concentrations decreased while ATP remained at a constant low concentration.

Comparison of nitrogen-limited growth (very low rate of poly- β -hydroxybutyrate formation) with oxygen-limited growth (high rate of polymer synthesis) revealed no significant difference in the energy charge of these organisms which have access to unlimited glucose. But at $D = 0.1 \text{ h}^{-1}$ organisms from both oxygen- and nitrogen-limited cultures displayed similar, lower energy charges than at higher dilution rates.

Jackson & Dawes (1976) found that the intracellular nicotinamide nucleotide concentrations in oxygen- and nitrogen-limited steady state cultures of *A. beijerinckii* were also very similar, although a marked increase in the NADH/NAD ratio occurred in the transient state following the imposition of an oxygen limitation on a nitrogen-limited culture, re-adjustment taking place within 1 h as poly- β -hydroxybutyrate synthesis commenced and served as an alternative electron acceptor.

Extracellular adenylates could clearly arise both by excretion and by lysis of dead cells. The former process can have a physiological role by serving to stabilize the energy charge of the organism, e.g. excretion of AMP will buffer the energy charge against a decrease. The problems of assessing the specific lysis rate in continuous cultures of *Methylococcus* sp.

Table 1. *Effect of dilution rate and nutrient limitation on adenine nucleotide content and energy charge of A. beijerinckii*

For nitrogen limitation the dissolved oxygen tension was maintained at 0.8 kPa and for oxygen limitation (undetectable dissolved oxygen tension) the O₂ supply was 3.6% of the inflowing gas (N₂ flow rate 800 ml min⁻¹). For nitrogen limitation the N₂ supply rate was 20 ml min⁻¹ and the O₂/N₂ mixture was diluted with 591 ml argon min⁻¹. The nitrogen source was N₂ in all experiments. For each steady state assays were carried out in triplicate on two or more successive days. The values recorded are means of assays which did not differ by more than 5%.

Nutrient limitation	Dilution rate (h ⁻¹)	Adenine nucleotide concn [nmol (mg protein) ⁻¹]*										Energy charge	Adenylyate kinase <i>K</i> _{app} [†]	PHB [‡] content (% dry wt)
		Intracellular				Extracellular								
		ATP	ADP	AMP	Total	ATP	ADP	AMP	Total					
Glucose	0.05	7.4 (4.6)	1.4 (0.9)	1.1 (0.7)	9.9 (6.2)	0.6 (0.4)	0.2 (0.1)	0.1 (0.05)	0.9 (0.55)	0.82	4.2	2.0		
	0.10	9.4 (5.4)	1.7 (1.0)	0.9 (0.5)	12.0 (6.9)	0.5 (0.3)	0.5 (0.3)	1.2 (0.7)	2.2 (1.3)	0.85	2.9	1.5		
	0.20	11.3 (5.5)	2.6 (1.3)	1.0 (0.5)	14.9 (7.3)	0.5 (0.2)	1.4 (0.7)	2.8 (1.4)	4.7 (2.3)	0.85	1.7	ND		
Oxygen	0.10	8.8 (1.5)	4.8 (0.8)	2.2 (0.4)	15.8 (2.7)	0.3 (0.05)	2.5 (0.4)	11.0 (1.9)	13.8 (2.35)	0.71	0.8	40.1		
	0.15	8.9 (1.6)	3.0 (0.5)	0.9 (0.2)	12.8 (2.3)	0.3 (0.05)	1.6 (0.3)	4.0 (0.7)	5.9 (1.05)	0.81	0.9	28.6		
	0.20	11.2 (2.7)	3.1 (0.7)	1.1 (0.3)	15.4 (3.7)	0.3 (0.1)	0.7 (0.2)	2.4 (0.6)	3.4 (0.9)	0.83	1.3	21.9		
Nitrogen	0.25	11.0 (3.8)	3.0 (1.0)	1.3 (0.5)	15.3 (5.3)	0.3 (0.1)	0.8 (0.3)	0.8 (0.3)	1.9 (0.7)	0.79	1.6	12.1		
	0.10	8.0 (4.5)	4.1 (2.3)	1.9 (1.1)	14.0 (7.9)	0.3 (0.2)	1.8 (1.0)	5.5 (3.1)	7.6 (4.3)	0.72	0.9	2.3		
	0.20	7.7 (3.1)	2.3 (0.9)	1.2 (0.5)	11.2 (4.5)	0.5 (0.2)	0.2 (0.1)	0.2 (0.1)	0.9 (0.4)	0.79	1.7	ND		

ND, Not detected.

* The values in parentheses are the adenylyate concentrations expressed as nmol (mg dry bacterial wt)⁻¹.

† $K_{app} = [ATP][AMP]/[ADP]^2$.

‡ PHB, Poly-β-hydroxybutyrate.

have been considered by Drozd *et al.* (1978) who found the specific lysis rate of this organism increased with dilution rate under three different growth limitations. We have viability data only for the oxygen-limited cultures but these organisms, assessed by either slide culture or plate count, remained about 90% viable under the conditions investigated. Consequently, it would seem reasonable to attribute the changes in extracellular adenylate concentration observed with oxygen-limited cultures principally to excretion of adenylates and not to a change in the specific lysis rate. While the possible influence of lysis on the extracellular adenylate concentrations in carbon- and nitrogen-limited cultures cannot be positively decided, it should be noted that dilution rate would have to affect the specific lysis rates of these two different cultures in an opposite manner to account for the observed changes in extracellular concentrations. Thus, in view of the findings of Drozd *et al.* (1978), it seems more likely that excretion of adenylates is the principal factor involved in these changes and we therefore adopt this premise in the following discussion.

The proportion of the total adenine nucleotide consumption involved in the synthesis of RNA is directly related to the growth rate, as is the turnover time of the adenylate pool. The adenylates of fast-growing populations of bacteria are committed principally for the biosynthesis of nucleic acids, proteins and adenine cofactors and, in the present experiments, also for nitrogen fixation. When the energy and carbon source was not limiting the excretion of adenylates was inversely related to dilution rate (Table 1). With glucose-limited cultures adenylate excretion was not only much lower but displayed a different pattern by increasing slightly at higher dilution rates, possibly reflecting less stringent control of AMP formation under these conditions. With all three limitations AMP excretion underwent the most marked changes and, by this means, possibly served to buffer the energy charge. In contrast, excretion of AMP does not occur with either *Escherichia coli* or *Beneckea natriegens* as a means of regulating the energy charge under conditions of carbon or nitrogen limitation (Knowles, 1979).

Effect of culture dilution rate and nutrient limitation on enzymes of adenylate metabolism

The adenylate pool is maintained by the action of various enzymes. Adenylate kinase brings the three nucleotides into equilibrium ($2\text{ADP} = \text{AMP} + \text{ATP}$) and while its specific activity was largely unaffected by dilution rate under nitrogen and oxygen limitation, it was inversely related to dilution rate in organisms from glucose-limited cultures (Fig. 1). In Table 1, the K_{app} for the reaction has been calculated from the intracellular adenine nucleotide concentrations.

The energy charge can be stabilized under conditions where ATP regeneration is restricted (e.g. by oxygen limitation of an obligate aerobe) by decreasing the intracellular concentration of AMP either by excretion or by its metabolism. The major pathway of adenylate degradation in *Azotobacter vinelandii* involves two enzymes – AMP nucleosidase, which converts AMP to adenine and ribose 5-phosphate, and adenine deaminase, which yields hypoxanthine and NH_3 from adenine (Schramm & Lazorik, 1975). Since the latter enzyme has a greater activity than the former under physiological conditions in this organism, adenine does not accumulate and is a transient intermediate in adenylate breakdown (Schramm & Leung, 1973). Evidence has been adduced that AMP nucleosidase is involved in the control of the adenylate pool size and is allosterically modulated by MgATP^{2-} and P_i (Hurwitz *et al.*, 1957; Schramm, 1974). When batch cultures of *A. vinelandii* were perturbed by anaerobiosis, growth ceased and the adenylate pool either increased slightly or remained constant while the energy charge decreased to about 0.3, although the viable count was unchanged for at least 5 h; RNA degradation occurred but AMP breakdown was inhibited, so that the lowered energy charge was attributable to the organism's inability to produce ATP under anaerobic conditions (Leung & Schramm, 1978).

We found that AMP nucleosidase was present in *A. beijerinckii* and its specific activity was inversely related to dilution rate under nitrogen-, glucose- and oxygen-limited conditions;

Fig. 1

Fig. 2

Fig. 1. Effect of dilution rate on the activity of adenylate kinase in *A. beijerinckii* under glucose (O), nitrogen (●) or oxygen (Δ) limitation. Results are shown for two independent experiments.

Fig. 2. Effect of dilution rate on the activity of AMP nucleosidase in *A. beijerinckii* under glucose (O), nitrogen (●) or oxygen (Δ) limitation.

Fig. 3

Fig. 3. Effect of dilution rate on the activity of adenosine deaminase in *A. beijerinckii* under glucose (O), nitrogen (●) or oxygen (Δ) limitation.

Fig. 4

Fig. 4. Effect of dilution rate on the activity of adenine deaminase in *A. beijerinckii* under glucose (O), nitrogen (●) or oxygen (Δ) limitation.

at a dilution rate of 0.25 h^{-1} activity was very low under oxygen limitation (Fig. 2).

AMP deaminase, which converts AMP to inosine 5'-monophosphate (IMP) and NH_3 in eukaryotes, has not yet been detected in prokaryotes although a non-specific adenine nucleotide deaminase is present in *Desulfovibrio desulfuricans* (Yates, 1969). However, adenosine deaminase, yielding inosine and NH_3 , is present in *A. beijerinckii* and its activity was relatively unaffected by dilution rate (Fig. 3) except for a decrease at lower dilution rates in the case of bacteria grown under oxygen-limited conditions. The activity of adenine deaminase, which produces hypoxanthine and NH_3 , was inversely related to dilution rate in organisms from both glucose- and nitrogen-limited cultures but showed little change under oxygen-limited conditions (Fig. 4).

At low dilution rates the activity of AMP nucleosidase exceeded that of adenine deaminase, so it seemed likely that some adenine might accumulate and be excreted by the cells. Analysis of supernatants from two glucose-limited steady states ($D = 0.1$ and 0.2 h^{-1}) revealed very low concentrations of about 1 and $3 \mu\text{M}$ -adenine, respectively, but none could be detected in supernatants under oxygen-limited conditions.

It thus seems that the principal route of AMP degradation in *A. beijerinckii* resembles that in *A. vinelandii* and is mediated by AMP nucleosidase and adenine deaminase. As the regulatory properties of the former suggest that it is active only under conditions of metabolic stress, such as imposition of anaerobiosis (Schramm & Lazarik, 1975; Leung & Schramm, 1978), it is interesting to note that the specific activity of this enzyme increased at low growth rates under all three nutrient limitations studied. Under these conditions therefore AMP nucleosidase activity would complement AMP excretion as a means of stabilizing the energy charge against a decrease.

We are grateful to Dr I. S. Carter who carried out some preliminary experiments, to Dr C. J. Knowles who loaned a spring-loaded syringe for comparative tests of rapid sampling methods and to Professor D. Lloyd for helpful discussions.

REFERENCES

- ATKINSON, D. E. (1968). The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. *Biochemistry* **7**, 4030–4034.
- CARTER, I. S. & DAWES, E. A. (1979). Effect of oxygen concentration and growth rate on glucose metabolism, poly- β -hydroxybutyrate biosynthesis and respiration of *Azotobacter beijerinckii*. *Journal of General Microbiology* **110**, 393–400.
- CHAPMAN, A. G. & ATKINSON, D. E. (1977). Adenine nucleotide concentrations and turnover rates. Their correlation with biological activity in bacteria and yeast. *Advances in Microbial Physiology* **15**, 253–306.
- DAWES, E. A. & LARGE, P. J. (1970). Effect of starvation on the viability and cellular constituents of *Zymomonas anaerobia* and *Zymomonas mobilis*. *Journal of General Microbiology* **60**, 31–42.
- DROZD, J. W., LINTON, J. D., DOWNS, J. & STEPHENSON, R. J. (1978). An *in situ* assessment of the specific lysis rate in continuous cultures of *Methylococcus* sp. (NCIB 11083) grown on methane. *FEMS Microbiology Letters* **4**, 311–314.
- HAAKER, H. & VEEGER, C. (1976). Regulation of respiration and nitrogen fixation in different types of *Azotobacter vinelandii*. *European Journal of Biochemistry* **63**, 499–507.
- HURWITZ, J., HEPPEL, L. A. & HORECKER, B. L. (1957). The enzymatic cleavage of adenylic acid to adenine and ribose 5-phosphate. *Journal of Biological Chemistry* **226**, 525–540.
- JACKSON, F. A. & DAWES, E. A. (1976). Regulation of the tricarboxylic acid cycle and poly- β -hydroxybutyrate metabolism in *Azotobacter beijerinckii* grown under nitrogen or oxygen limitation. *Journal of General Microbiology* **97**, 303–312.
- JOHNSON, R. A., HARDMAN, J. G., BROADUS, A. E. & SUTHERLAND, E. W. (1970). Analysis of adenosine 3',5'-monophosphate with luciferase luminescence. *Analytical Biochemistry* **35**, 91–97.
- JONES, C. W., BRICE, J. M., WRIGHT, V. & ACKRELL, B. A. C. (1973). Respiratory protection of nitrogenase in *Azotobacter vinelandii*. *FEBS Letters* **29**, 77–81.
- KNOWLES, C. J. (1977). Microbial metabolic regulation by adenine nucleotide pools. *Symposia of the Society for General Microbiology* **27**, 241–283.
- KNOWLES, C. J. (1979). Adenine nucleotide pool maintenance during bacterial growth and starvation. In *Covalent and Non-Covalent Modulation of Protein Function*, pp. 13–26. Edited by D. E. Atkinson & C. F. Fox. New York: Academic Press.
- LEUNG, H. B. & SCHRAMM, V. L. (1978). The role of adenosine monophosphate nucleosidase in the regulation of adenine nucleotide levels in *Azotobacter vinelandii* during aerobic–anaerobic transitions. *Archives of Biochemistry and Biophysics* **190**, 46–56.
- SCHRAMM, V. L. (1974). Kinetic properties of allosteric adenosine monophosphate nucleosidase from *Azotobacter vinelandii*. *Journal of Biological Chemistry* **249**, 1729–1736.
- SCHRAMM, V. L. & LAZARIK, F. C. (1975). The pathway of adenylate catabolism in *Azotobacter vinelandii*. Evidence for adenosine monophosphate nucleosidase as the regulatory enzyme. *Journal of Biological Chemistry* **250**, 1801–1808.
- SCHRAMM, V. L. & LEUNG, H. (1973). Regulation of adenosine monophosphate levels as a function of adenosine triphosphate and inorganic phosphate. A proposed metabolic role for adenosine monophosphate nucleosidase from *Azotobacter vinelandii*. *Journal of Biological Chemistry* **248**, 8313–8315.
- SENIOR, P. J. & DAWES, E. A. (1971). Poly- β -hydroxybutyrate biosynthesis and the regulation of glucose metabolism in *Azotobacter beijerinckii*. *Biochemical Journal* **125**, 55–56.
- SENIOR, P. J. & DAWES, E. A. (1973). The regulation of poly- β -hydroxybutyrate metabolism in *Azotobacter beijerinckii*. *Biochemical Journal* **134**, 225–238.

- SENIOR, P. J., BEECH, G. A., RITCHIE, G. A. F. & DAWES, E. A. (1972). The role of oxygen limitation in the formation of poly- β -hydroxybutyrate during batch and continuous culture of *Azotobacter beijerinckii*. *Biochemical Journal* **128**, 1193–1201.
- SOTTOCASA, G. L., KUYLENSTIERNA, B., ERNSTER, L. & BERGSTRAND, A. (1967). Separation and some enzymatic properties of the inner and outer membranes of rat liver mitochondria. *Methods in Enzymology* **10**, 448–463.
- WARD, A. C., ROWLEY, B. I. & DAWES, E. A. (1977). Effect of oxygen and nitrogen limitation on poly- β -hydroxybutyrate biosynthesis in ammonium-grown *Azotobacter beijerinckii*. *Journal of General Microbiology* **102**, 61–68.
- YATES, M. G. (1969). A non-specific adenine nucleotide deaminase from *Desulfovibrio desulfuricans*. *Biochimica et biophysica acta* **171**, 299–310.
- YOSHINO, M., MURAKAMI, K. & TSUSHIMA, K. (1979). Effect of monovalent cations on AMP nucleosidase from *Azotobacter vinelandii*. *Biochimica et biophysica acta* **570**, 118–123.