Environment Canada
Environnement Canada

SCIENTIFIC SERIES NO. 63
(Résumé en français)

INLAND WATERS DIRECTORATE,
CANADA CENTRE FOR INLAND WATERS,
BURLINGTON, ONTARIO, 1976.

Environment Canada
Environnement Canada

Adenosine Triphosphate (ATP) Levels in Microbial Cultures and a Review of the ATP Biomass Estimation Technique

Ansar A. Qureshi * and Jayantkumar Patel †

* Present address: Microbiology Section, Ministry of the Environment, P.O. Box 213, Rexdale, Ontario M9W 5L1.

† Present address: Faculty of Medicine, University of Toronto, Toronto, Ontario M5S 1A4.

SCIENTIFIC SERIES NO. 63
(Résumé en français)

INLAND WATERS DIRECTORATE,
CANADA CENTRE FOR INLAND WATERS,
BURLINGTON, ONTARIO, 1976.

Cat. No.: En 36-502/63

ISBN 0-662-00375-6

Contract No. 07KX.KL210-6-4526
THORN PRESS LIMITED

Contents

Tables

Tables (cont.)

Illustrations

Abstract

In laboratory-grown cultures of a lake bacterium and three lake fungi, the concentration of cellular ATP was found to vary appreciably during the various stages of growth.

Generally, the highest level of ATP was observed during the lag and early log growth phases. Thereafter, the ATP concentration rapidly decreased and stabilized at a relatively constant level during the stationary phases of the growth cycle.

With respect to the use of the DuPont Biometer for ATP determinations, it was observed that after calibration and strict adherence to procedure during analysis, relatively consistent results could be obtained with both standard ATP solutions and test solutions. Of the various solvents examined for determining the stability of the standard ATP, boiling Tris-HCl buffer (0.02 *M*, pH 7.75) was found to be the best solvent. This solvent also proved to be highly satisfactory for the extraction of ATP from bacterial and fungal cultures used in this investigation.

The report also provides background information on the ATP biomass estimation technique, general remarks on the technique, and an extensive bibliography of its use and application in diverse systems (Appendix A).

Résumé

On a observé que la concentration en ATP dans les cellules d'une bactérie et de trois champignons lacustres mis en culture en laboratoire variait de façon notable au cours des diverses étapes de la croissance.

En général, les plus fortes concentrations ont été observées au cours de la phase de latence et au début de la phase de croissance logarithmique. Elles ont par la suite rapidement diminué et se sont relativement stabilisées au cours des phases stationnaires du cycle de croissance.

En ce qui concerne l'utilisation du biomètre DuPont on a remarqué que, lors du dosage de l'ATP, l'analyse des solutions étalons d'ATP et des solutions à examiner donnait des résultats relativement cohérents, si l'on avait étalonné l'appareil et si l'on appliquait strictement les méthodes recommandées. Parmi les divers solvants examinés en vue de la détermination de la stabilité de la solution étalon d'ATP, la solution tampon de Tris-HCl en ébullition (0.02 *M*, pH 7.75) s'est révélée comme étant le meilleur. Ce dernier a aussi donné des résultats très satisfaisants dans l'extraction de l'ATP des cultures de bactéries et de champignons utilisées dans la présente étude.

Le rapport fournit en outre des renseignements de base sur la technique d'évaluation de la teneur en ATP, des remarques générales sur la technique, ainsi qu'une vaste bibliographie qui a trait à son utilisation et à son application dans divers systèmes (annexe A).

Acknowledgments

The authors wish to express their sincere appreciation to B.J. Dutka and B.K. Burnison for their personal interest in this study, their advice and valuable suggestions in the preparation of the manuscript.

We would also like to thank D. Bolton for her assistance in the bacteriological aspects of this project; the library staff, particularly Barbara Davis, for their continued and generous help in the preparation of the bibliography; and M. Jurkovic and S. Austin for their endless patience in typing the manuscript.

CHAPTER 1

Introduction

Adenosine triphosphate (ATP) assay for biomass estimation has been used extensively in various systems. As a result, a voluminous literature is available on the ATP biomass technique (see Appendix A—Bibliography). However, as pointed out by Cheer *et al.* (1974), "there has been a paucity of comprehensive information published on the techniques required for routine ATP analysis," and a variety of problems have been encountered by several investigators. Although some improvements and modifications have been made for routine ATP analysis, answers to some fundamental questions remain unresolved. For example, one area of confusion and controversy is that of ATP concentration during various stages of growth of an organism. There have been conflicting reports regarding the level of ATP pools in various microorganisms grown under variable cultural conditions and across their lag, log and stationary phases of growth when grown under similar conditions (Strange *et al.* 1963; Cole *et al.* 1967; Forrest 1965; Hamilton and Holm-Hansen 1967; D'Eustachio and Johnson 1968b; D'Eustachio and Levin 1967, 1968; Lee *et al.* 1971b; Ausmus 1973; Chiu *et al.* 1973; Kao *et al.* 1973; Slayman 1973; Gadkari and Stolp 1975; and Johansson and Hägerby 1974). More critical studies of the methodology involved are required before the ATP biomass estimation technique can be used on a routine basis both in natural and artificial (laboratory) environments. The present investigation was designed to provide answers to some of these basic questions.

The main objectives of this study were:

(i) To evaluate the stability and calibration of the DuPont Biometer, using standard solutions of ATP;
(ii) To determine the stability of ATP standards in various extracting solvent systems;
(iii) To examine the concentration of ATP during the various stages of growth in bacteria and fungi;
(iv) To furnish background information on the ATP biomass estimation technique; and
(v) To prepare a comprehensive bibliography of the use and application of this technique.

CHAPTER 2

Materials and Methods

EQUIPMENT

ATP analyses were made using the DuPont 760 Luminescence Biometer (E.I. DuPont De Nemours & Co., Inc., Wilmington, Delaware 19898). The biometer possesses a fixed wavelength, from 350 nm (near-ultraviolet) to 600 nm (visible light), and is specifically designed for the measurement of light emitted during bioluminescent or chemiluminescent reactions. Using the ATP firefly reaction, the biometer can assay directly for ATP, or for a substance that can be coupled to a reaction involving ATP. The instrument can detect 10^{-13} g ATP/0.01 ml (10^{-8} mg ATP/ml) of injected sample using specially purified reagents. The biometer also has an automatic five-decade linear response capability which allows most samples to be measured directly without any predilutions. The instrument's readout includes a direct digital display of either ATP or cell concentrations (Anon. 1970; Allen 1973; Daly 1974; Wise 1974). Unlike certain other ATP analyzers (e.g. the JRB Photometer), the DuPont Biometer measures only the peak height of a luminescent reaction rather than the amount of light emitted over a sustained time period. To use the instrument for ATP measurements, the luciferin-luciferase reaction mixture is dispensed into a cuvette, which is then placed into the biometer and rotated 180° to bring it under a light-tight injection port in the dark facing the photomultiplier tube. Using a 50- or 100-μl Hamilton syringe (adapted to a Shandon reprojector), a 10- or 20-μl aliquot of ATP or cellular extract containing ATP is injected into the reaction mixture. Upon mixing of the ATP with the luciferin-luciferase reaction mixture, a flash of light is emitted. The peak intensity of the light produced is directly proportional to the amount of ATP injected. The biometer is calibrated, with a standard solution of ATP, for each reaction mixture so that the concentration of ATP is read out directly (Anon. 1970; Allen 1973; Daly 1974).

GLASSWARE

The glassware was washed first with a hot soap solution and then with 10% (2 *N*) HCl, and finally rinsed 3 or 4 times in fresh double glass-distilled deionized water.

WATER

All buffers, ATP stock solutions and reaction mixtures were prepared using fresh double glass-distilled deionized water (DGDDW). This water was found to be of excellent quality (comparable to the Low Response Water recommended by DuPont) and reagents prepared in it showed no ATP activity (Qureshi and Spry, unpublished results).

BUFFERS

(i) 0.02 *M* Tris-HCl (pH 7.75): prepared using Tris (hydroxymethyl) methylamine crystals (BDH Chemicals Ltd.).
(ii) 0.1 *M* $NaHCO_3$ (pH 8.0): prepared using sodium bicarbonate crystals (BDH Chemicals Ltd.).
(iii) 0.0.1 *M* MOPS (pH 7.4): prepared using morpholinopropane sulphonic acid powder (E.I. DuPont De Nemours & Co., Inc.).

EXTRACTION SOLVENTS

Tris-HCl and sodium bicarbonate buffers alone and in combination with 5% ethanol (V/V) were used to check the stability and recovery of standard ATP solutions in these solvents. For the extraction of ATP from bacterial and fungal cultures, only Tris-HCl buffer was used.

ATP STANDARDS

A standard ATP solution was prepared by dissolving 119.3 mg of crystalline adenosine 5'-triphosphate-disodium salt (ATP-Na_2·$3H_2O$, Sigma Chemical Co., St. Louis, Mo.) in 100 ml of 0.01 *M* MOPS buffer (pH 7.4). This primary stock solution was serially diluted (with 0.01 *M* MOPS buffer) to furnish ATP concentrations of 100, 10, 1 and 0.1 μg ATP/ml. Aliquots of 1 ml of ATP standard solutions were dispensed in screw-cap vials and stored at -20°C until required for ATP assay.

LUCIFERIN-LUCIFERASE REACTION MIXTURE

The DuPont Biometer reagent kit was used to prepare the reaction mixture. The kit includes 20 buffer-salt tablets in a vial and 20 vials of lyophilized, purified and stabilized luciferin-luciferase powder. Each buffer-salt tablet (which contains 0.01 *M*, pH 7.4 MOPS buffer and 0.01 *M* $MgSO_4$) was dissolved in 3 ml fresh DGDDW and was mixed gently with the entire contents of one vial of enzyme-substrate powder. It is important that the luciferin-luciferase powder be added to the buffer solution. Aliquots of 0.1 ml of the reaction mixture were dispensed, with the help of the DuPont automatic pipettor, into each reaction cuvette. The mixture was maintained at room temperature for 30 min to allow dissipation of inherent light before placing the cuvette in the biometer for calibration and assay of ATP.

CALIBRATION AND ASSAY PROCEDURES

The procedures employed for checking the stability of the biometer, its calibration and normal operation for ATP measurements are given in the DuPont Instruction Manual (Anon. 1970).

PREPARATION OF BACTERIAL AND FUNGAL CULTURES FOR GROWTH MEASUREMENTS AND ATP EXTRACTION

The bacterial isolate (#2788) used in this investigation was previously isolated from Lake Ontario waters. The stock cultures of this isolate were maintained on nutrient agar (Oxoid). For growth measurements and ATP extraction, the bacterial cultures were initiated by transferring a 1-ml aliquot from a 24-hr nutrient broth culture into 500-ml Erlenmeyer flasks containing 200 ml of a balanced synthetic liquid medium. The medium, which was sterilized by autoclaving at 115°C for 10 min, contained KH_2PO_4, 0.165 g; K_2HPO_4, 0.265 g; $FeCl_3$, 0.005 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; proteose peptone #3 (Oxoid), 2.5 g; yeast extract (Oxoid), 0.5 g; glucose, 3.0 g per litre of distilled water. The cultures were incubated at 20°C on a shaker at a speed of 100 rpm and were harvested at intervals for growth and ATP measurements. Bacterial growth was determined either by measuring turbidity in Klett units using a Photoelectric Colorimeter (Klett-Summerson Co., Inc., N.Y.) or by conventional plate count technique using the same balanced medium modified by the addition of 1.5 % agar (Oxoid).

Three fungi viz. *Aspergillus niger* (6-8), *Trichoderma viride* (14-7) and *Penicillium* sp. (4-8), previously isolated from Lake Ontario waters, were used in the present investigation. The stock cultures of these fungi were maintained on potato dextrose agar (Oxoid). To determine the growth patterns of these fungi, they were cultivated on the following three media*:

(i) Synthetic Liquid Medium (Balanced)-SLM-1

NH_4NO_3	— 2.0 g
KH_2PO_4	— 0.5 g
$MgSO_4 \cdot 7H_2O$	— 0.2 g
Glucose	— 18.0 g

Distilled water to 1000 ml

(ii) Synthetic Liquid Medium (C-limiting)-SLM-2

NH_4NO_3	— 2.0 g
KH_2PO_4	— 0.5 g
$MgSO_4 \cdot 7H_2O$	— 0.2 g
Glucose	— 4.5 g

Distilled water to 1000 ml

(iii) Synthetic Liquid Medium (N-limiting)-SLM-3

NH_4NO_3	— 0.5 g
KH_2PO_4	— 0.5 g
$MgSO_4 \cdot 7H_2O$	— 0.2 g
Glucose	— 18.0 g

Distilled water to 1000 ml

For preliminary growth studies, cultures of these fungi were initiated by transferring a small agar block (from stock cultures) onto freshly prepared malt extract agar-MEA (Oxoid) plates which were incubated at 20°C for 5 days. Agar discs (approximately 5 mm) were cut with a sterile cork borer from the margin of 5-day-old colonies and were transferred to dilution bottles containing 50 ml of one of the three sterilized media. These were incubated at 20°C on a shaker at a speed of 100 rpm and were harvested at 2-day intervals for 18 days for the determination of fungal growth.

Fungal growth was measured by determining mycelial dry weight. At the end of each designated incubation period, cultures of each isolate were filtered using pre-weighed 47-mm, 0.45-μ membrane filters (Millipore HAWP 04700, Lot. No. 29891-1). Prior to the filtration of the mycelial suspension, 30 ml of APHA (American Public Health Association) Standard Methods Buffer was passed through each membrane, which was then dried at 60°C for 24 hr and weighed (in a trial experiment, it was

* All media were sterilized by autoclaving at 115°C for 10 min.

observed that membranes treated in such a fashion showed little variation in weight compared to untreated and undried membrane filters). After concentrating the mycelial suspension, it was washed with about 15-20 ml APHA Standard Methods Buffer. The membranes containing the mycelial mat were placed in 50-mm glass petri plates and dried at 60°C to constant dry weight.

Fungi were grown on SLM-1 for determination of growth and ATP concentration as described below. The 5-day cultures (on MEA) of each isolate were flooded with 5-7 ml SLM-1 and a fungal suspension was prepared by scrubbing the surface of the colony with a sterile inoculating needle. One-ml aliquots of appropriately diluted fungal suspension were then transferred to dilution bottles containing 50 ml of SLM-1, which were then incubated at 20°C for 5 days on a shaker at a speed of 100 rpm. These 5-day cultures served as the source of inoculum (1 ml/dilution bottle containing 50 ml SLM-1) for starting cultures used for ATP extraction and growth measurements, which were carried out after 1, 2, 4, 6, 8, 9 and 14 days of incubation under conditions identical with those described above.

ATP EXTRACTION PROCEDURE

The method of ATP extraction, in general, was similar to the procedure described by several workers (Holm-Hansen and Booth 1966; Browne 1971; Sorokin and Kadota 1972; Holm-Hansen 1973a, 1973b). A few modifications were made, however, and the methodology used in this study is described below.

Bacterial or fungal suspensions were filtered through Millipore membrane filters (47 mm, 0.45 μ) previously washed with 10 ml of ice-cold 0.02 *M* Tris-HCl buffer (pH 7.75). As soon as no liquid remained above the membrane, the filter was washed with 20 ml of ice-cold Tris-HCl. The filter was immediately removed and transferred to a 100-ml beaker and boiling Tris-HCl buffer (0.02 *M*, pH 7.75) was pipetted (10.0 or 12.0 ml and 10.0 or 20.0 ml for bacterial and fungal cultures, respectively) onto the filter. The beaker, sealed with aluminum foil, was placed in a boiling water bath and was held, with occasional shaking, at 100°C for 6 min. The beaker was then quickly placed in an ice bath (0-4°C), and the filter was transferred to the wall of the beaker. With a Pasteur pipette, the extracting solvent (Tris) was directed (5-6 times) onto the filter surface. The filter was inverted and the reverse side was treated in similar fashion. The filter was then pressed with a clean spatula to drain as much liquid as possible. For fungal cultures only, the cellular extract was transferred to clean pre-chilled centrifuge tubes and was centrifuged to settle cell debris at 3000 rpm for 10 min.

Two- to three-ml aliquots of the supernatant were gently transferred to clean test tubes, which were then frozen and stored at -20°C until required for ATP analyses. The frozen samples were thawed in an ice bath for about 30 min before being analyzed.

Results and Discussion

EVALUATION OF THE ELECTRONIC STABILITY OF THE BIOMETER

The instruction manual for the DuPont 760 Luminescence Biometer recommends that for proper functioning of the instrument, the electronic stability should be checked daily. It is also suggested that, "should the coarse sensitivity dial setting vary more than 0.50 over a period of 24 hr, electronic defects may be indicated." In a preliminary experiment, the electronic stability of the biometer was monitored twice a day (in the morning and the afternoon) for five consecutive days. After recording the coarse sensitivity dial setting, the instrument was calibrated by injecting 10 μl of a standard ATP solution (0.1 μg ATP/ml 0.01 *M* MOPS, pH 7.4) into a cuvette containing 100 μl of the reaction mixture. The instrument was calibrated in terms of femtograms per millilitre (10^{-15} g ATP/ml) and the calibration was rechecked using the same standard ATP.

The results of this experiment are shown in Table 1. The data indicate that within a period of a few hours on the same day, the coarse sensitivity dial setting showed a variation of greater than 0.50. Over a period of 24 hr, however, the variation was less than 0.50 units. It was also observed that if the analysis of ATP samples could be completed within 1 hr (after allowing a 1-hr warm-up time), the instrument drift was minimal; otherwise reproducible results could not be obtained with standard ATP solutions because of considerable instrument drift. A considerable variation was occasionally observed in the electronic stability of the instrument, but after careful calibration fairly consistent results could be obtained with standard ATP solutions (see Table 1, Section III), especially if the operator developed and followed a particular routine for analysis.

ANALYSIS OF STANDARD ATP

In the next experiment, the range of values for a standard ATP solution (0.1 μg/ml) was determined in order to obtain some additional information about the stability and reproducibility of the biometer. Aliquots of 10 μl of standard ATP were mixed with 100 μl of the reaction mixture and assayed (20 times) on two consecutive days. The values obtained are recorded in Table 2. The values ranged from 0.94×10^8 to 1.02×10^8 fg/ml (i.e. 0.094-0.102 μg ATP/ml). The mean value was 0.98×10^8 fg/ml (0.098 μg ATP/ml) with a standard deviation of 0.02.

In a separate experiment, a standard curve for ATP was prepared by individually mixing 10 μl of a series of known ATP concentrations (0.025-1.00 μg/ml) with 100 μl of enzyme-substrate mixture and determining the biometer readout. The biometer readings for different concentrations of standard ATP solutions are given in Table 3, and the ATP standard curve is shown in Figure 1.

It is apparent from these results, as also discussed above, that after proper calibration and careful operation of the biometer relatively constant and comparable results can be obtained by using standard ATP solutions.

STABILITY AND RECOVERY OF ATP IN EXTRACTING SOLVENTS

In a series of experiments, the stability and recovery of a standard ATP solution were examined in Tris-HCl, Tris-HCl + 5% ethanol (V/V), $NaHCO_3$ and $NaHCO_3$ + 5% ethanol (V/V). Aliquots of 50 μl of a standard ATP solution (0.1 mg/ml 0.01 *M* MOPS, pH 7.4) were added to 5 ml of the solvent either previously heated to boiling point or held at 0-4°C. All mixtures were prepared in screw-capped 12-ml centrifuge tubes to avoid evaporation. The mixture prepared with boiling solvent was heated at 100°C for 6 min and then transferred to an ice bath where it was held for 15 min. The ATP solutions added to ice-cold solvents were held at 0-4°C for 21 min. All ATP solutions were kept frozen at -20°C until the time of analysis.

The results of these experiments are given in Table 4. In general, the nucleoside triphosphate did not appear to degrade appreciably, upon heating, in all solvent systems. Compared to the standard ATP values (Table 2), the ethanolic Tris-HCl and sodium bicarbonate systems yielded lower values. It is probable that ethanol inhibits the luciferin-luciferase reaction. Holmsen *et al.* (1966) have made similar observations and found that ethanol and

Table 1. Evaluation of the Electronic Stability of the DuPont Biometer over a 5-day Period.

		Day 1		Day 2		Day 3		Day 4		Day 5	
		M	A	M	A	M	A	M	A	M	A
I.	Electronic Stability* Coarse sensitivity	5.85	5.08	5.62	5.04	5.41	5.42	5.59	5.05	5.56	5.70
II.	Calibration Mean light response	$1.74x10^8$	$1.86x10^8$	$1.86x10^8$	$1.42x10^8$	$1.31x10^8$	$1.30x10^8$	$1.54x10^8$	$1.33x10^8$	$1.27x10^8$	$1.34x10^8$
	Lamp intensity	$1.73x10^8$	$1.87x10^8$	$1.86x10^8$	$1.42x10^8$	$1.32x10^8$	$1.30x10^8$	$1.54x10^8$	$1.34x10^8$	$1.27x10^8$	$1.37x10^8$
	Lamp intensity dial turns	7.75	7	7	7.75	7.75	7.75	7.75	7.75	7.5	7.75
	Conversion factor (femtograms ± 0.02)	$1.00x10^8$	$1.00x10^8$	$1.00x10^8$	$1.00x10^8$	$1.00x10^8$	$1.00x10^8$	$1.00x10^8$	$1.00x10^8$	$1.00x10^8$	$1.00x10^8$
	Coarse sensitivity dial reading	3.50	3.40	3.42	3.66	3.76	3.74	3.58	3.74	3.78	3.71
III.	Analysis of a Standard ATP Solution† after Calibration	$0.97x10^8$	$0.96x10^8$	$1.03x10^8$	$1.00x10^8$	$0.89x10^8$	$0.93x10^8$	$0.98x10^8$	$0.98x10^8$	$1.04x10^8$	$1.00x10^8$
		$1.02x10^8$	$0.98x10^8$	$0.99x10^8$	$0.97x10^8$	$0.98x10^8$	$1.00x10^8$	$1.01x10^8$	$1.01x10^8$	$1.01x10^8$	$0.98x10^8$
		$0.95x10^8$	$0.93x10^8$	$1.01x10^8$	$0.98x10^8$	$0.98x10^8$	$0.95x10^8$	$0.98x10^8$	$0.97x10^8$	$0.98x10^8$	$0.95x10^8$
		$0.97x10^8$	$0.97x10^8$	$1.03x10^8$	$0.98x10^8$	$1.00x10^8$	$0.93x10^8$	$0.99x10^8$	$1.00x10^8$	$1.01x10^8$	$0.98x10^8$

M = Morning; A = Afternoon.

*Electronic stability was checked with the DuPont standard Radioactive Light Source (RLS).

†0.1 μg /ml 0.01 *M* MOPS, pH 7.4 = $1.00x10^8$ femtograms/ml; injection volume 10μl.

Table 2. Analysis of a Standard ATP Solution* Using the DuPont Biometer.

Sample No.	Biometer Reading† (femtograms/ml)x10^8	Calculated ATP (μg/ml)	Sample No.	Biometer Reading† (femtograms/ml)x10^8	Calculated ATP (μg/ml)
1	0.98	0.098	21	0.99	0.099
2	0.98	0.098	22	0.96	0.096
3	0.97	0.097	23	0.98	0.098
4	0.95	0.095	24	0.99	0.099
5	1.01	0.101	25	0.99	0.099
6	0.99	0.099	26	0.95	0.095
7	1.00	0.100	27	0.97	0.097
8	0.95	0.095	28	0.96	0.096
9	0.98	0.098	29	0.98	0.098
10	0.94	0.094	30	0.98	0.098
11	0.97	0.097	31	0.98	0.098
12	0.98	0.098	32	0.94	0.094
13	0.96	0.096	33	0.99	0.099
14	0.99	0.099	34	0.97	0.097
15	0.99	0.099	35	0.98	0.098
16	1.01	0.101	36	1.02	0.102
17	1.00	0.100	37	0.97	0.097
18	1.02	0.102	38	0.99	0.099
19	0.95	0.095	39	0.96	0.096
20	0.99	0.099	40	0.99	0.099

*0.1 μg/ml 0.01 *M* MOPS, pH 7.4 = 1.00x10^8 femtograms/ml; injection volume 10 μl.
†The first twenty readings were obtained on the first day and the last twenty on the following day.

Table 3. Analysis of Standard ATP Solutions Using the DuPont Biometer.

Standard ATP (μg/ml)	Biometer Reading* (femtograms/ml)	Mean	Calculated ATP (μg/ml)
0.025	$2.53x10^7$ $2.51x10^7$ $2.49x10^7$	$0.25x10^8$	0.025
0.05	$4.47x10^7$ $4.49x10^7$ $4.49x10^7$	$0.45x10^8$	0.045
0.10	$0.98x10^8$ $1.01x10^8$ $0.98x10^8$	$0.99x10^8$	0.099
0.20	$1.98x10^8$ $1.97x10^8$ $2.01x10^8$	$1.99x10^8$	0.199
0.25	$0.258x10^9$ $0.290x10^9$ $0.256x10^9$	$0.27x10^9$	0.27
0.50	$0.491x10^9$ $0.568x10^9$ $0.496x10^9$	$0.52x10^9$	0.52
0.80	$0.870x10^9$ $0.792x10^9$ $0.732x10^9$	$0.80x10^9$	0.80
1.00	$0.99x10^9$ $0.84x10^9$ $0.96x10^9$	$0.93x10^9$	0.93

*Values obtained from three replicates of each ATP concentration.

some heat-labile substance in plasma extract inhibited light production. Furthermore, slightly better stability and recovery of ATP were obtained with boiling Tris-HCl when compared to boiling $NaHCO_3$. Therefore, on the basis of these results, boiling Tris-HCl was chosen for the extraction of ATP from bacterial and fungal cultures.

ATP ANALYSIS IN A PURE BACTERIAL CULTURE

In a preliminary experiment, the efficiency of ATP extraction from a pure bacterial culture was examined using increasing volumes of cell suspension of the same density. A 36-hr culture of isolate #2788, grown in the balanced medium at 20°C, was used in this experiment. Aliquots of 1/100 dilution of the cell suspension were filtered and extracted for ATP as described. The cell density was estimated by standard plate count technique. The results of this experiment are summarized in Table 5.

It is evident from the results that samples of increasing volumes of the same density gave proportionately increasing values for ATP in the extracts (Fig. 2). In other words, the amount of ATP extracted was linearly related to the concentration of bacterial cells present in the suspension filtered. The range in the number of cells filtered was from $25.5x10^6$ to $8.5x10^7$, and the amount of ATP varied from $2.16x10^{-4}$ to $9.95x10^{-4}$ μg ATP/ml extracting solvent.

The calculated values of μg ATP/cell in the samples correlated very well with volume variations. These values

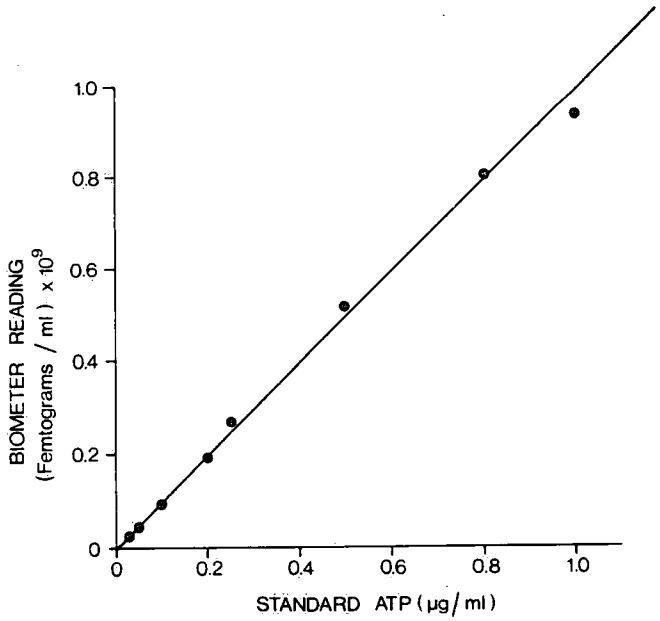

Figure 1. Standard curve for ATP.

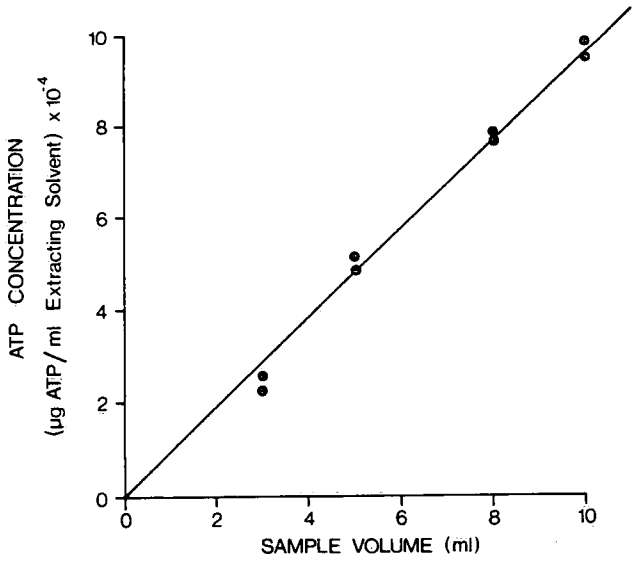

Figure 2. ATP concentration in sample volumes of a 1/100 diluted culture of a lake bacterium (#2788) grown in a synthetic liquid medium.

Table 4. Evaluation of the Stability and Recovery of a Standard ATP Solution* in Various Solvents.

Sample†	Solvent	Heat	Biometer Reading† 1	Biometer Reading† 2	Biometer Reading† Mean	Calculated ATP (μg/ml)
A_1	$NaHCO_3$	+	$9.54x10^8$	$9.56x10^8$	$9.55x10^8$	0.955
A_2	$NaHCO_3$	+	$9.56x10^8$	$9.54x10^8$		
A_3	$NaHCO_3$	–	$9.73x10^8$	$9.65x10^8$	$9.47x10^8$	0.947
A_4	$NaHCO_3$	–	$9.10x10^8$	$9.38x10^8$		
B_1	$NaHCO_3$ + Ethanol	+	$8.57x10^8$	$8.34x10^8$	$8.13x10^8$	0.813
B_2	$NaHCO_3$ + Ethanol	+	$7.49x10^8$	$8.12x10^8$		
B_3	$NaHCO_3$ + Ethanol	–	$9.08x10^8$	$8.61x10^8$	$8.65x10^8$	0.865
B_4	$NaHCO_3$ + Ethanol	–	$8.50x10^8$	$8.42x10^8$		
C_1	Tris-HCl	+	$0.98x10^9$	$0.96x10^9$	$9.80x10^8$	0.980
C_2	Tris-HCl	+	$0.96x10^9$	$1.02x10^9$		
C_3	Tris-HCl	–	$0.86x10^9$	$1.00x10^9$	$9.60x10^8$	0.960
C_4	Tris-HCl	–	$0.98x10^9$	$0.99x10^9$		
D_1	Tris-HCl + Ethanol	+	$0.91x10^9$	$0.90x10^9$	$9.00x10^8$	0.900
D_2	Tris-HCl + Ethanol	+	$0.89x10^9$	$0.92x10^9$		
D_3	Tris-HCl + Ethanol	–	$0.92x10^9$	$0.90x10^9$	$9.00x10^8$	0.900
D_4	Tris-HCl + Ethanol	–	$0.89x10^9$	$0.91x10^9$		

*1 μg/ml 0.01 *M* MOPS, pH 7.4 = $1.00x10^9$ femtograms/ml; injection volume 10 μl.
†Two replicates of each sample were analyzed for each treatment.
‡Femtogram/ml; each replicate was analyzed twice.

Table 5. Effect of Different Sample Volumes of the Same Density on ATP Recovery from a 36-hr Pure Culture of a Lake Bacterium.

Sample*	Sample Volume of 1/100 Dilution	Biometer Reading†	μg ATP/ml Extracting Solvents‡	Number of Cells Filtered§	μg ATP/Cell‖
A_1	3 ml	$2.56x10^5$	$2.56x10^{-4}$	$25.5x10^6$	$1.20x10^{-10}$
A_2	3 ml	$2.16x10^5$	$2.16x10^{-4}$	$25.5x10^6$	$1.18x10^{-10}$
B_1	5 ml	$4.84x10^5$	$4.84x10^{-4}$	$42.5x10^6$	$1.36x10^{-10}$
B_2	5 ml	$5.15x10^5$	$5.15x10^{-4}$	$42.5x10^6$	$1.45x10^{-10}$
C_1	8 ml	$7.81x10^5$	$7.81x10^{-4}$	$68.0x10^6$	$1.38x10^{-10}$
C_2	8 ml	$7.73x10^5$	$7.73x10^{-4}$	$68.0x10^6$	$1.38x10^{-10}$
D_1	10 ml	$9.95x10^5$	$9.95x10^{-4}$	$85.0x10^6$	$1.40x10^{-10}$
D_2	10 ml	$9.52x10^5$	$9.52x10^{-4}$	$85.0x10^6$	$1.35x10^{-10}$

*Two replicates of each sample were analyzed.
†Femtogram/ml; mean values obtained from two measurements of each replicate.
‡Calculated from: $\frac{\text{biometer reading}}{10^9}$ = μg ATP/ml extracting solvent.
§Estimated from viable plate count of $8.5x10^8$ cells/ml of original culture (i.e. $8.5x10^6$ cells/ml of 1/100 diluted culture).
‖Calculated from: $\frac{\mu\text{g ATP/ml extracting solvent} \times \text{volume of extracting solvent}}{\text{number of cells filtered}}$

For example, for sample A_1: $\frac{2.56x10^{-4} \times 12}{25.5x10^6} = 1.2x10^{-10}$ μg ATP/cell

ranged from 1.18 to 1.45 x 10^{-10} μg ATP/cell and compared favourably with the values reported by several other workers (Levin *et al.* 1968; Lee *et al.* 1971c; Owen *et al.* 1972).

The level of endogenous ATP across various phases of growth of a lake bacterium was examined in the next series of experiments. The isolate (#2788) was grown in the same balanced medium as previously described and aliquots of bacterial suspension were harvested after 4, 12, 17, 24, 30, 36 and 54 hr incubation for growth measurements and ATP extraction. The growth was measured by determining turbidity in Klett units. The relative concentration of ATP during various phases of growth was estimated from the total extractable ATP per Klett unit. The results of these experiments are given in Table 6 and are illustrated in Figure 3.

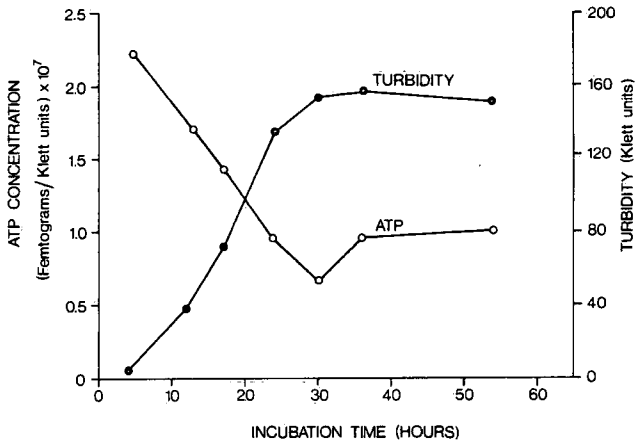

Figure 3. Growth curve and ATP concentration in a pure culture of a lake bacterium (#2788) in a synthetic liquid medium.

The data indicate that the concentration of cellular ATP varied considerably as the culture of this bacterium proceeded through the lag, exponential (log) and stationary phases of growth. The cellular ATP pool was highest during the late lag and early log phases of growth. Thereafter, cellular ATP levels gradually declined during the late exponential growth phase and began to stabilize as the cells entered the stationary phase. During the stationary phase the cells of this bacterium contained relatively constant amounts of ATP.

Table 6. ATP Concentration in a Lake Bacterium across Various Phases of Growth in a Balanced Synthetic Liquid Medium.

Sample*	Sample Volume (ml)	Dilution†	Incubation (hr)	Turbidity (Klett Units)‡	Femtograms ATP Extracted§	Femtograms ATP/Klett Unit	
						Values	Mean
A_1	1.0	1X	4	1	$2.36x10^7$	$2.36x10^7$	$2.20x10^7$
A_2	1.0	1X	4	1	$2.04x10^7$	$2.04x10^7$	
B_1	1.0	1X	12	38	$6.74x10^8$	$1.77x10^7$	$1.70x10^7$
B_2	1.0	1X	12	38	$6.11x10^8$	$1.61x10^7$	
C_1	5.0	1/100	17	71	$1.02x10^9$	$1.44x10^7$	$1.40x10^7$
C_2	5.0	1/100	17	71	$0.96x10^9$	$1.35x10^7$	
D_1	0.5	1X	24	137	$1.15x10^9$	$0.84x10^7$	$0.97x10^7$
D_2	0.5	1X	24	137	$1.51x10^9$	$1.10x10^7$	
E_1	5.0	1/100	30	152	$0.42x10^9$	$0.28x10^7$	$0.63x10^7$
E_2	5.0	1/100	30	152	$1.47x10^9$	$0.97x10^7$	
F_1	5.0	1/100	36	155	$1.32x10^9$	$0.85x10^7$	$0.97x10^7$
F_2	5.0	1/100	36	155	$1.67x10^9$	$1.08x10^7$	
G_1	5.0	1/100	54	150	$1.48x10^9$	$0.99x10^7$	$1.00x10^7$
G_2	5.0	1/100	54	150	$1.54x10^9$	$1.03x10^7$	

* Two replicates of each sample were analyzed.
† Bacterial suspensions were diluted only for ATP extraction.
‡ Turbidity measurements were made on undiluted bacterial suspension.
§ Total ATP extracted per 1 ml bacterial suspension (corrected for dilution) in 10 ml solvent.

As explained by Lee *et al.* (1971c), the high ATP values obtained during early stages of growth were probably due to increased cell biomass and to an increase in the ATP pool. Moreover, they were of the opinion that, "From a practical standpoint the (their) data emphasize that although in the early growth stages the ATP content of bacterial cells can reach levels greater than 10-fold higher than exist at less active growth stages, this high level is very short-lived and the ATP pool drops rapidly to equilibrate and be maintained well into the death phase within a relatively narrow range."

As pointed out earlier, there appears to be some controversy regarding the shifting ATP pool during various phases of bacterial growth. Our results are in agreement with those reported by several other investigators. For example, Forrest (1965) found that in a culture of *Streptococcus faecalis* the ATP content per cell was constant during the lag phase. He observed a continuous decrease in ATP levels during the exponential growth phase, which only leveled out as the cells entered the stationary phase. Hamilton and Holm-Hansen (1967) reported that the ATP content per cell in marine bacteria was highest during the log phase and rapidly declined to stabilize at a relatively constant level during the stationary phase. Our results also corroborate those of Lee *et al.* (1971b), who found that cells of the stationary phase of *Aerobacter aerogenes* contained relatively constant levels of ATP.

Our results are contradictory to those of D'Eustachio and Johnson (1968b) and D'Eustachio and Levin (1967, 1968), who examined the level of ATP in 13 different species of bacteria and across all phases of growth. Although these workers also found a slightly higher level of ATP during lag and early logarithmic phase, they concluded that the cellular concentration of ATP does not change appreciably and appears to be relatively constant during the various phases of growth. Further, they reported that ATP per viable cell variation between different species of bacteria examined was within one order of magnitude.

Several other workers have made some interesting observations concerning the ATP pools during different stages of growth of bacteria. Ausmus (1973) found that the ATP concentration was significantly higher during log phase than during lag or exhaustion phase for several species of bacteria. In addition, he found no appreciable differences in the ATP content during lag and exhaustion phase of any species. Kao *et al.* (1973), who studied ATP pools in pure and mixed cultures of *Escherichia coli* and *Pseudomonas aeruginosa,* found that the level of ATP increased with the increasing rate of growth through the lag and exponential phases. After reaching a maximum level, the ATP content declined rapidly when glucose was almost completely utilized by the organisms. The same authors noted an oscillatory variation of ATP concentration during the stationary phases of these bacteria. They also noted that interactions in mixed cultures of bacteria can cause a significant decrease in the ATP pool.

Chiu *et al.* (1973) observed changes and even a sudden drop in ATP levels in continuous cultures from one steady state value to another. They attributed the variation to microbial population shifts. Furthermore, they observed that in continuous cultures the dilution rate can also significantly affect the cellular ATP content. They concluded that, "The microbial population in continuous systems operated at high dilution rates had a high metabolic activity and a low ATP pool, while those species predominating at low dilution rates had a low metabolic activity and high ATP content."

Lazdunski and Belaich (1972), Bachi and Ettlinger (1973) and Montague and Dawes (1974) reported that exhaustion of the energy source is accompanied by a decrease of the ATP pool. On the other hand, several investigators (Strange *et al.* 1963; Forrest 1965; Cole *et al.* 1967; Knowles and Smith 1970; Holms *et al.* 1972) reported high ATP pools despite exhaustion of the energy source.

Gadkari and Stolp (1975) found that the ATP concentration was constant (9 nmoles/100 μg *N*) during the exponential growth phase of *Bdellovibrio bacteriovorus* indicating an equilibrium between the energy reactions. They observed that the ATP pool oscillates at regular intervals during endogenous respiration (starvation). Overproduction of ATP started only after the ATP concentration had declined to a minimum level of 6 nmoles/100 μg *N*. According to them, the alternating over- and under-production of ATP may serve as a special regulatory mechanism which allows the organism to make economic use of its cellular materials. They also noted that during starvation, decrease of viability is accompanied by a decrease of the ATP pool.

The influence of nutritional and environmental factors on the level of ATP was not examined in this investigation. However, it has been shown that the ATP pool in microorganisms varies with (or is influenced by) cultural conditions (Cole *et al.* 1967), pH changes (Brezonik *et al.* 1975), oxygen tension, energy control mechanism within the cell and substrate concentration (Strange *et al.* 1963; Kao *et al.* 1973), dilution rates and level of metabolic activity in continuous cultures (Chiu *et al.* 1973) and mixed culture population dynamics (Kao *et al.* 1973).

It is clear from the above discussion that the ATP responds rapidly to changes in a microorganism's physiological state, which in turn may be influenced by alterations in nutritional and environmental factors.

ATP ANALYSIS IN PURE CULTURES OF FUNGI

In a preliminary experiment, the growth patterns of *Aspergillus niger* (6-8), *Trichoderma viride* (14-7) and *Penicillium* sp. (4-8) were examined in three different synthetic liquid media. The cultures of these fungi were prepared and incubated as outlined earlier. For the measurement of growth, the cultures were harvested at 2-day intervals for 18 days when the experiment was terminated. The results of this experiment, summarized in Table 7, are graphically presented in Figures 4, 5 and 6.

In general, these fungi grew well on all three media. For *A. niger* and *T. viride*, best growth was observed on the balanced medium (SLM-1), followed by that on 'N' limiting (SLM-3) and 'C' limiting (SLM-2) media. Although the isolate of *Penicillium* sp. grew best on SLM-2, no striking difference was noticed in the growth of this isolate on all three media.

Like other filamentous fungi, these isolates followed a definite pattern of lag, log (linear) and decline (exhaustion) phases of growth. After a lag phase of approximately 24 hr, the growth of all three fungi increased rapidly in linear fashion until the 8th day of incubation in the case of *A. niger* and *Penicillium* sp. and until the 10-12th day in *T. viride*. Thereafter, as the cultures entered the exhaustion phase, an oscillatory variation of growth of these organisms was observed in all three media. The oscillation pattern of growth during the decline phase of these fungi may be due to any one or a combination of the following factors: (a) experimental error resulting from the fact that dry weights were determined from only one replicate of each treatment, (b) different rate of autolysis of these fungi, or (c) their differential ability to assimilate nutrients produced and accumulated in the medium as a result of their own metabolism.

On the basis of results of this experiment, it was decided to grow these fungi only on SLM-1 and to harvest cultures after 1, 2, 4, 6, 8, 9 and 14 days incubation for simultaneous measurements of their growth and ATP contents across different phases of growth cycle.

These measurements were made in the next series of experiments following procedures outlined earlier. After designated periods of incubation, three replicates of each organism were harvested for growth measurements and

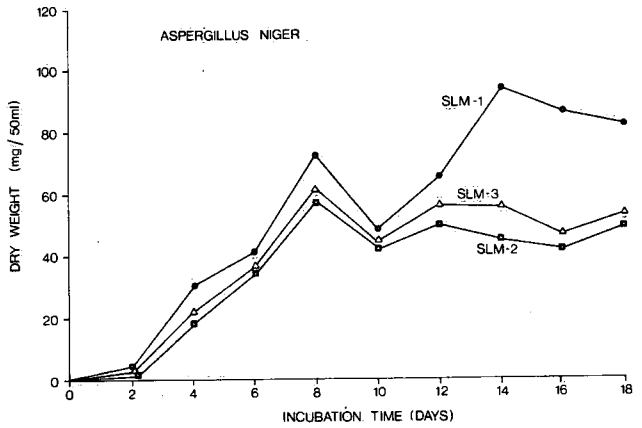

Figure 4. Growth curves of *Aspergillus niger* (6-8) on three different synthetic liquid media.

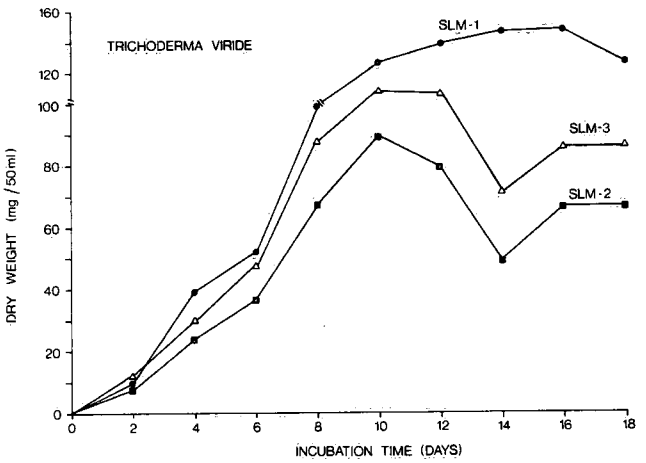

Figure 5. Growth curves of *Trichoderma viride* (14-7) on three different synthetic liquid media.

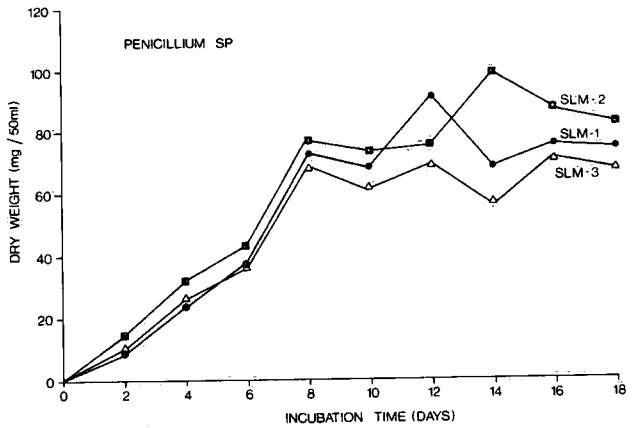

Figure 6. Growth curves of *Penicillium* sp. (4-8) on three different synthetic liquid media.

Table 7. Mycelial Dry Weight (mg/50 ml*) of Three Fungi Grown at 20°C in Three Different Synthetic Liquid Media over an 18-day Period.

Incubation (Days)	*Aspergillus niger* (6-8)			*Trichoderma viride* (14-7)			*Penicillium* sp. (4-8)		
	SLM-1†	SLM-2‡	SLM-3§	SLM-1	SLM-2	SLM-3	SLM-1	SLM-2	SLM-3
2	4.1	2.6	2.9	9.8	7.8	10.6	8.8	14.2	9.7
4	30.1	18.7	22.3	39.2	23.7	29.6	23.6	31.5	25.8
6	42.5	34.5	36.0	51.7	36.5	47.0	37.7	42.7	37.1
8	73.0	57.5	61.0	98.9	67.5	88.0	72.3	76.2	68.8
10	47.8	41.9	44.1	127.2	89.2	109.0	68.1	72.6	61.0
12	65.0	49.5	56.0	139.7	79.2	107.4	91.0	75.0	68.0
14	94.2	45.4	55.9	145.6	48.2	71.6	67.8	98.6	56.7
16	86.1	42.3	47.6	146.6	66.2	86.5	75.3	86.7	71.6
18	82.4	50.5	54.6	126.5	66.0	86.0	74.3	82.5	67.5

* Values obtained from one replicate of each culture.
† SLM-1: balanced medium.
‡ SLM-2: carbon limiting medium.
§ SLM-3: nitrogen limiting medium.

Table 8. Mycelial Dry Weight and ATP Concentration in a Pure Culture of *Aspergillus niger* (6-8) Grown in SLM-1 over a 14-day Period.

Incubation (Days)	Mycelial Dry Weight (mg/50 ml)			Biometer Reading (femtograms/ml)		μg ATP/ml Extracting Solvent†	μg ATP/mg Dry Weight‡	
	Replicate	Values	Mean	Replicate	Values*		Values	Mean
1	1	0.2	0.2	A	7.06×10^6	7.06×10^{-3}	0.4	0.4
	2	0.2		B	6.90×10^6	6.90×10^{-3}	0.4	
	3	0.2						
2	1	1.5	3.3	A	4.64×10^8	0.46	1.4	1.6
	2	5.6		B	6.08×10^8	0.61	1.8	
	3	2.7						
4	1	20.8	20.8	A	3.77×10^9	3.77	3.6	3.2
	2	24.2		B	2.86×10^9	2.86	2.8	
	3	17.5						
6	1	41.3	39.2	A	2.92×10^9	2.92	1.5	1.5
	2	39.2		B	2.78×10^9	2.78	1.4	
	3	37.2						
8	1	42.5	49.4	A	3.68×10^9	3.68	1.5	1.5
	2	66.0		B	3.49×10^9	3.49	1.4	
	3	39.5						
9	1	66.5	63.5	A	5.02×10^9	5.02	1.6	1.6
	2	61.0						
	3	63.0						
14	1	87.3	74.2	A	6.68×10^9	6.68	1.8	1.7
	2	63.7		B	5.71×10^9	5.71	1.5	
	3	71.6						

* Mean values of two measurements of each replicate.

† Calculated from: $\frac{\text{biometer reading}}{10^9}$ = μg ATP/ml extracting solvent.

‡Calculated from: $\frac{\mu\text{g ATP/ml} \times \text{volume extracting solvent}}{\text{mean dry weight}}$ = μg ATP/mg dry weight.

Table 9. Mycelial Dry Weight and ATP Concentration in a Pure Culture of *Trichoderma viride* (14-7) Grown in SLM-1 over a 14-day Period.

Incubation (Days)	Mycelial Dry Weight (mg/50 ml)			Biometer Reading (femtograms/ml)		μg ATP/ml Extracting Solvent†	μg ATP/mg Dry Weight‡	
	Replicate	Values	Mean	Replicate	Values*		Values	Mean
1	1	1.8	1.3	A	4.88×10^8	0.49	3.8	4.0
	2	1.2		B	5.32×10^8	0.53	4.1	
	3	0.9						
2	1	9.0	10.1	A	4.92×10^9	4.92	4.9	4.2
	2	14.0		B	3.52×10^9	3.52	3.5	
	3	7.3						
4	1	40.5	40.3	A	4.79×10^9	4.79	2.4	2.6
	2	32.6		B	5.57×10^9	5.57	2.8	
	3	48.0						
6	1	104.1	99.2	A	0.82×10^{10}	8.20	1.7	1.6
	2	94.8		B	7.38×10^9	7.38	1.5	
	3	98.8						
8	1	140.2	120.7	A	1.11×10^{10}	11.10	1.8	1.7
	2	103.8		B	0.98×10^{10}	9.80	1.6	
	3	118.3						
9	1	186.6	184.2	A	1.78×10^{10}	17.80	1.9	1.9
	2	183.5						
	3	182.5						
14	1	203.8	185.7	A	1.56×10^{10}	15.60	1.7	1.9
	2	187.6		B	1.95×10^{10}	19.50	2.1	
	3	165.6						

* Mean values of two measurements of each replicate.

† Calculated from: $\frac{\text{biometer reading}}{10^9}$ = μg ATP/ml extracting solvent.

‡ Calculated from: $\frac{\text{μg ATP/ml x volume extracting solvent}}{\text{mean dry weight}}$ = μg ATP/mg dry weight.

two were used for ATP analyses. For the extraction of ATP from 1- and 2-day-old cultures of these fungi, 10-ml aliquots of boiling Tris-HCl were used. The ATP was extracted from the rest of the cultures using 20 ml Tris-HCl to ensure or achieve maximum extraction.

The growth was measured by determining mycelial dry weight (mg/50 ml SLM-1). The relative ATP concentration during various stages of growth was estimated from the total extractable ATP per millilitre of the extracting solvent. From the values obtained, the ATP content per milligram dry weight was calculated and used for comparative analysis. The results of these experiments are described in Tables 8, 9 and 10 and are illustrated in Figures 7, 8 and 9.

As observed in the previous experiment, after a lag phase lasting about 24 hr, the growth of all three fungi increased in linear fashion and began to stabilize after 8-9 days incubation. The ATP level of these fungi varied during the three phases of growth and did not appear to be directly related to the amount of growth. The ATP pool of these fungi was highest during the lag and early log phase

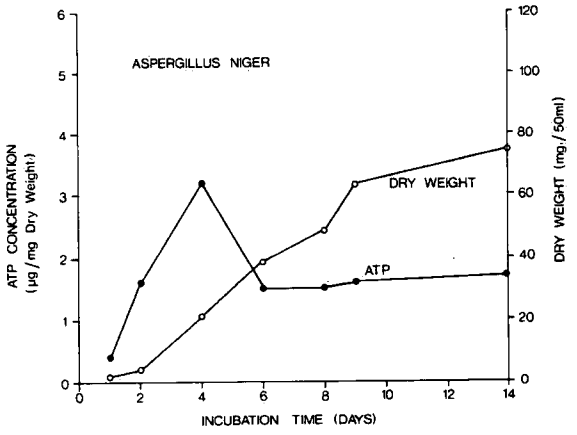

Figure 7. Growth curve and ATP concentration in a pure culture of *A. niger* (6-8) grown in SLM-1 over a 14-day period.

Table 10. Mycelial Dry Weight and ATP Concentration in a Pure Culture of *Penicillium* sp. (4-8) Grown in SLM-1 over a 14-day Period.

Incubation (Days)	Mycelial Dry Weight (mg/50 ml)			Biometer Reading (femtograms/ml)		µg ATP/ml Extracting Solvent†	µg ATP/mg Dry Weight‡	
	Replicate	Values	Mean	Replicate	Values*		Values	Mean
1	1	0.5	0.4	A	1.54×10^8	0.15	3.8	2.9
	2	0.4		B	8.30×10^7	0.08	2.0	
	3	0.3						
2	1	2.5	2.1	A	0.96×10^9	0.96	4.6	4.4
	2	2.1		B	0.87×10^9	0.87	4.1	
	3	1.8						
4	1	13.8	12.0	A	1.18×10^9	1.18	2.0	1.7
	2	12.6		B	0.86×10^9	0.86	1.4	
	3	9.5						
6	1	47.2	40.7	A	4.10×10^9	4.10	2.0	2.0
	2	35.9		B	3.92×10^9	3.92	1.9	
	3	39.0						
8	1	91.7	93.0	A	8.42×10^9	8.42	1.8	1.8
	2	92.2		B	8.38×10^9	8.38	1.8	
	3	95.2						
9	1	124.3	126.7	A	1.25×10^{10}	12.50	2.0	2.0
	2	130.8						
	3	125.1						
14	1	156.5	151.8	A	1.16×10^{10}	11.60	1.5	1.8
	2	150.8			1.49×10^{10}	14.95	2.0	
	3	148.1						

*Mean values of two measurements of each replicate.

†Calculated from: $\frac{\text{biometer reading}}{10^9}$ = µg ATP/ml extracting solvent.

‡Calculated from: $\frac{\text{µg ATP/ml x volume extracting solvent}}{\text{mean dry weight}}$ = µg ATP/mg dry weight.

of growth with the exception of *A. niger* where a slight variation was noted. However, as the growth continued to increase during the log phase, the ATP concentration continuously decreased and finally stabilized at a relatively constant level towards the end of the log phase. During the late log and stationary phases of growth, the ATP levels of all three fungi appeared to be relatively constant.

The maximal ATP levels registered in the present investigation for *Penicillium* sp., *T. viride* and *A. niger* were, respectively, 4.4, 4.2 and 3.2 µg ATP/mg dry

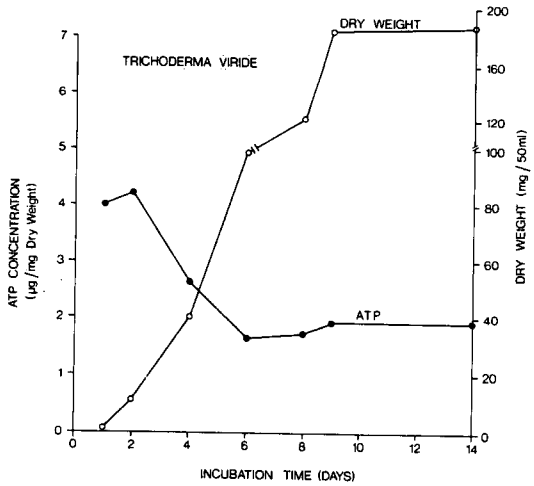

Figure 8. Growth curve and ATP concentration in a pure culture of *T. viride* (14-7) grown in SLM-1 over a 14-day period.

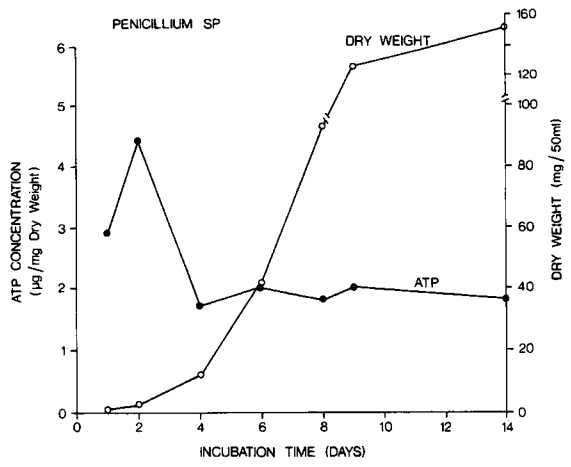

Figure 9. Growth curve and ATP concentration in a pure culture of *Penicillium* sp. (4-8) grown in SLM-1 over a 14-day period.

weight. Usually values of 1.5-2.0 μg ATP/mg dry weight were obtained.

The finding that ATP concentration is not constant during the maximum growth phase agreed with the results of Slayman (1973) and Johansson and Hägerby (1974). Our results contrast with those of Ausmus (1973), who found that the ATP concentration was significantly higher in the cultures of certain fungi during log phases than during lag or exhaustion phase. He concluded that the concentration of ATP was similar during the lag and exhaustion phases of growth of several fungi. In contrast, our results clearly indicate that the ATP levels were appreciably higher during lag and early log phases than during the exhaustion phase of growth except for *A. niger* where a slight variation was observed.

Johansson and Hägerby (1974) reported that in a culture of *Fommes annosus,* the cellular ATP pool increased with increasing growth, but declined later during the same exponential growth phase. In our studies, the isolate of *A. niger* followed this pattern of initial increase and later decreasing ATP concentration during the same log phase of growth (Fig. 7).

The high levels of ATP, as observed in our experiments, during the early stages of intensive synthesis of cellular material may be due to an abundant supply of the factors and the enzymes necessary for respiration, resulting in a very low ADP:ATP ratio. However, as the intensive metabolic activity continues, and with the increasing age of the culture, a relatively small number of cells are in a maximal energy-yielding state. Besides, as stated by Johansson and Hägerby (1974), "The energy consuming incorporation of reduced storage material (carbohydrates and fats) results in a changed ratio between the adenylates in the older cells, which is indicated by a decreased ATP concentration."

Conclusions

1. Problems with calibration and the readout reproducibility of the DuPont Biometer were encountered during ATP quantification. Consistent results can be obtained, however, if the final assay step of the ATP analysis is completed within 1 hr after allowing a 1-hr warm-up period for the biometer. Use of the biometer over longer periods results in considerable instrumental drift. It is important that the analyst develop and strictly follow a particular routine for carrying out ATP analyses. The analyst should also become thoroughly familiar with the biometer and its possible shortcomings.
2. Of the four solvent systems examined for stability of the ATP of known concentrations, boiling Tris-HCl buffer (0.02 *M*, pH 7.75) proved to be the best solvent with respect to the stability and recovery of ATP. It was also found to be an efficient system for ATP extraction from both bacterial and fungal cultures.
3. A linear relationship was observed between the amounts of ATP extracted from increasing volumes of bacterial suspension of the same density. The concentration of ATP/viable cell in the lake bacterium examined appeared to be in agreement with bacterial ATP contents reported by other investigators. The highest level of ATP/viable cell of this isolate occurred during the lag and early log phases of growth. The amount of cellular ATP then declined and remained relatively constant during the stationary phase of the growth cycle.
4. The ATP levels in three fungi examined varied considerably during their lag, log and stationary phases of growth with the highest ATP concentration being observed during the early log phase. The values for *Penicillium* sp., *Trichoderma viride* and *Aspergillus niger* were, respectively, 4.4, 4.2 and 3.2 μg ATP/mg dry mycelial weight. In all three, the concentration of cellular ATP was relatively constant during their stationary phases of growth.
5. Despite the inherent disadvantage of recording only peak light intensity, with careful operation the DuPont Biometer can be used successfully to determine ATP concentration. In our opinion, better instruments (e.g. JRB Photometer*), which can measure both the peak height and the exponential decay of the light, are now available and they are recommended for ATP analyses.
6. The ATP biomass estimation technique should be used with some reservations. Although the technique has been used extensively in diverse systems (see Appendix A—Bibliography), for meaningful results it is perhaps best to compare and correlate ATP data with those obtained using at least one other biomass estimating technique. There is no doubt that this technique is very sensitive and specific, but the overall procedure is still fairly lengthy, and at times, tedious. Extreme caution and care are therefore required during each step of the analysis.

* The JRB Photometer is now supplied by SAI Technology Company, San Diego, Calif., 92123.

CHAPTER 5

Background Information on the ATP Biomass Estimation Technique

Measurement of viable biomass and metabolic activity of various types of organisms is of considerable importance for many, if not all, ecological studies. Most of the conventional techniques, used routinely for biomass estimation, have proved to be expensive, slow, extremely laborious and often misleading due to inherent sources of error. Several workers (D'Eustachio and Johnson 1968b; Browne 1971; Strickland 1965, 1971; Holm-Hansen 1971, 1973b; Owen *et al.* 1972; Allen 1973; Ausmus 1973; Daly 1974; and Brezonik *et al.* 1975) have discussed the disadvantages and inadequacies of classical methods for the determination of biomass. Some of the problems associated with these techniques are cell clumping and aggregation, selective and restrictive growth conditions and the inability to distinguish viable and non-viable cells.

Of the many other methods that have been used to characterize biomass, adenosine triphosphate (ATP) seems to be the most suitable parameter. The rationale for using ATP as a criterion of biomass measurement is that it is characteristic of all living organisms (photosynthetic or heterotrophic) and the keystone of all cellular activities (Huennekens and Whitely 1960; Lehninger 1965; Atkinson 1971; Seitz and Neary 1974), and is degraded rapidly upon cell death (Holm-Hansen and Booth 1966). Adenosine triphosphate is present in relatively uniform concentration in all living cells, and does not exist in association with non-living, detrital material (Holm-Hansen and Booth 1966; Hamilton and Holm-Hansen 1967; Holm-Hansen 1970; Patterson *et al.* 1970). The uniqueness and specificity of ATP as an excellent measure of biomass has been the subject of several recent reviews (Strickland 1971; Holm-Hansen and Paerl 1972; Sorokin and Kadota 1972; Allen 1973; Ausmus 1973; Holm-Hansen 1973a; 1973b; Sharpe 1973; Cheer *et al.* 1974; Daly 1974; Stanley 1974).

Determination of biomass through measurement of ATP has been used in such diverse systems as marine water (Holm-Hansen and Booth 1966; Holm-Hansen 1969; Hobbie *et al.* 1972), fresh water (Rudd and Hamilton 1973; Burnison 1974), process water (Owen *et al.* 1972), spacecraft water (Levin *et al.* 1968), foods (Sharpe *et al.* 1970; Williams 1971), acidic basin peat (Greaves *et al.* 1973), sediments (Lee *et al.* 1971b, 1971c; Christan *et al.* 1974, 1975; Bancroft 1974; Bancroft *et al.* 1974; Karl and LaRock 1974, 1975), soils (Anderson and Davies 1973), and sewage sludge (Patterson *et al.* 1970; Brezonik and Patterson 1971; Weddle and Jenkins 1971; Chiu 1972). It has been used in virus detection (Chappelle 1971), cancer detection (Chappelle and Levin 1971), determination of seed viability and vigour (Ching 1973), as well as in laboratory-grown cultures of algae (St. John 1970; Holm-Hansen 1970; Browne 1971; Schmidt and Kamen 1971; Ausmus 1973; Cheer *et al.* 1974; Brezonik *et al.* 1975), bacteria (Strange *et al.* 1963; Forrest 1965; Cole *et al.* 1967; Hamilton and Holm-Hansen 1967; D'Eustachio and Johnson 1968a, 1968b; D'Eustachio and Levin 1967, 1968; Chappelle and Levin 1968; Harrison and Maitra 1968; Lee *et al.* 1971a; Ausmus 1973; Dhople and Hanks 1973; Kao *et al.* 1973) and fungi (Shropshire and Bergman 1968; MacGregor 1970; Somlo 1970; Bailey and Parks 1972; Ausmus 1973; Cocucci *et al.* 1973; Slayman 1973; Johansson and Hägerby 1974).

In addition, ATP analysis has been used and has a potential application in certain disciplines of other fields, such as clinical microbiology and chemistry, hematology, serology, urology, physiology, pharmacology, virology, limnology, biochemistry, immunology, in the fermentation industry, monitoring biocides, monitoring contamination in the food and cosmetic industries, and measurements of toxicity and nutrient bioassay studies (Allen 1973; Daly 1974; Brezonik *et al.* 1975; also see Appendix A—Bibliography).

Of the several methods available for quantitatively assaying ATP, the most sensitive and practical method utilizes the bioluminescent reaction occurring in the native firefly *Photinus pyralis* (McElroy 1947). The amount of light produced in the bioluminescent reaction is directly proportional to the amount of ATP present when all of the other reactants are in excess. Seliger and McElroy (1960) and Chappelle and Levin (1968) have shown that one photon (quantum) of light is emitted for each molecule of ATP hydrolyzed.

The detailed mechanism and the biochemistry of the firefly bioluminescent reaction have been described by several workers (Lyman and DeVincenzo 1967; Plant *et al.* 1968; Chappelle and Levin 1968; Strehler 1968; McElroy *et al.* 1969; Browne 1971; Gorden 1972; Allen 1973; Sharpe 1973; Daly 1974). The overall sequence of the firefly reaction can be summarized as follows:

$$\underset{\text{(Luciferin)}}{LH_2} + \underset{\text{(Luciferase)}}{E} + ATP^{Mg++}$$

$$\leftrightarrows \underset{\substack{\text{(Luciferyl} \\ \text{adenylate complex)}}}{E.L.H_2.AMP} + \underset{\text{(Pyrophosphate)}}{PP} \qquad (1)$$

$$E.LH_2.AMP + O_2 \rightarrow E + Product + CO_2 + AMP + hv\ (light) \qquad (2)$$

In the initial activation step (Equation 1) luciferin and ATP, catalyzed by luciferase, react to form a luciferase-luciferin-adenosine monophosphate complex (luciferyl adenylate complex) and inorganic pyrophosphate (PP). This complex reacts with molecular oxygen to produce light and a product molecule (oxyluciferin adenylate). According to McElroy *et al.* (1969), at a neutral or alkaline pH the quantum yield of light is near unity; however, at acidic pH (below 7) the quantum yield decreases and the yellow-green light shifts to a red emission (562 nm to 614 nm).

This reaction, although specific for ATP, is quite complicated, especially the mechanism of light reaction (Plant *et al.* 1968; McElroy *et al.* 1969; White *et al.* 1969, 1971). The luciferin from the native firefly has been shown to be D(-)-2-(6'-hydroxy-2-2'-benzothiazoyl)-Δ^2-thiazoline-4-carboxylic acid. Only the D(-) isomer of firefly luciferin is biologically active in the production of light; no light is produced if the L(+) isomer of luciferin is used because the oxidation of the intermediate $E.LH_2.AMP$ by molecular oxygen does not occur (McElroy *et al.* 1969).

It is well established that firefly lantern enzyme luciferase has a specific requirement for ATP (McElroy 1947; Strehler and McElroy 1957). Crude extracts of firefly contain transphosphorylase enzymes and may produce light in the presence of high energy phosphate molecules other than ATP. Holm-Hansen and Booth (1966) examined 13 different intracellular compounds to determine which of the high energy phosphate molecules might cause light emission when mixed with firefly extracts. They found that only adenosine diphosphate (ADP), cytidine-5'-triphosphate (CTP) and inosine-5'-triphosphate (ITP) influenced light emission. For example, the amount of light emitted upon the addition of ADP was less than 1% of that from an equivalent amount of ATP. The fact that some of the light emission may be due to nucleoside triphosphates other than ATP is probably not significant with regard to biomass estimation by bioluminescence because all ATP values in test samples are ultimately related to values obtained with a variety of microorganisms (Holm-Hansen and Booth 1966) and also because of the relative abundance of cellular ATP compared with other nucleoside triphosphates (Seitz and Neary 1974).

When a sample containing ATP is mixed with the luciferin-luciferase reaction mixture, there is an immediate emission of a brilliant flash of light in the range of 560-580 nm. The initial burst of light exponentially declines to a uniform level, however, addition of arsenate buffer to the reaction mixture decreases the initial light flash and produces an intermediate level of light which decays exponentially (arsenate buffer is now routinely added to many commercially available purified firefly extracts used for ATP assay). The measurement of either the peak height of the initial flash or the light which decays over a period of time gives a measure of ATP concentration in the original sample.

The light emission may be measured in several ways (Strehler 1968) and various workers have designed and used different instruments for this purpose: e.g., a combination of a photomultiplier tube, an amplifier and a recorder (Forrest 1965; Holm-Hansen and Booth 1966; Chappelle and Levin 1968; Strickland and Parsons 1968; Holm-Hansen 1969, 1970; St. John 1970); liquid scintillation spectrophotometers (Tal *et al.* 1964; Addanki *et al.* 1966; Stanley and Williams 1969 Schram 1970; Hammerstedt 1973; Rudd and Hamilton 1973; Johansson and Hägerby 1974; Cheer *et al.* 1974; Wiener *et al.* 1974; and Brunker 1975); and a modified GeMSAEC centrifugal photometric analyzer (Ausmus 1973).

Three commercial instruments are also available which measure the light produced during the firefly reaction: DuPont 760 Luminescence Biometer, American Instrument Company (AMINCO) Chem-Glow Photometer and the JRB* (also supplied by Lab-Line) ATP Photometer.

The detailed specifications and operating instructions of the DuPont Biometer have been provided by Anon.(1970), Allen (1973) and Daly (1974) and it has been used for ATP analyses by several investigators

* The JRB Photometer is now supplied by SAI Technology Company, San Diego, Calif., 92123.

(D'Eustachio and Johnson 1968a, 1968b; D'Eustachio and Levin 1968; Sharpe *et al.* 1970; Williams 1971; Owen *et al.* 1972; Chiu *et al.* 1975; Picciolo *et al.* 1975). Therefore, only a brief review of the DuPont Biometer's specificity and operation is given in Chapter 2, Materials and Methods.

The JRB Photometer is an ultrasensitive instrument especially designed to measure bioluminescent and chemiluminescent reactions. It is also designed and used for quantitative determination of ATP by measuring the amount of light emitted by its reaction with luciferin-luciferase preparations. This photometer utilizes time integration to measure the exponential decay of light produced during the firefly reaction. If desired, it can also measure the flash peak height of the firefly reaction. After a delay of 15 sec from the time of starting the assay switch and the mixing of the reactants, the JRB Photometer measures and integrates the area under the light curve for the next 60 sec. The instrument displays counts per minute (CPM) for an unknown sample, which then can be compared to a standard calibration curve to estimate the actual amount of ATP in the original sample. This approach allows for mixing of reactants outside of the light chamber and the use of crude enzyme. With the JRB Photometer variable sample volumes ranging from 10 μl to 10 ml can be analyzed. This analyzer is highly sensitive and can detect quantities of ATP down to 10^{-10} mg ATP (10^{-13} g ATP) with a range of 10^{-9} mg ATP/ml to 1 mg ATP/ml. Instructions for the operation of the JRB Photometer and its specifications are also available (Anon. 1974).

The AMINCO Chem-Glow Photometer is also designed to measure both bioluminescent and chemiluminescent reactions. It can be used manually or with automated systems. Two models are available for use with either 6 X 50 mm or 12 X 35 mm reaction tubes; either model may be adapted to accept the other size of tube. For high sensitivity the Chem-Glow Photometer requires the use of AMINCO's auxiliary components, including a repeating dispenser, an automated system adapter, a recorder, a standard light source and an integrator timer. The unit equipped with all accessories can determine peak height measurements simultaneously with integrated measurements. The instrument can measure quantities of ATP down to approximately 10^{-10} molar and samples as large as 0.5 ml can be assayed.

A detailed comparative account of the DuPont Biometer, JRB Photometer and AMINCO Chem-Glow Photometer has been provided by Wise (1974). The basic difference is that the DuPont Biometer measures only the peak intensity of the light produced during the firefly reaction, whereas both the JRB and AMINCO Photometers measure and integrate the long-term decaying luminescence and also have the option to determine the peak height. In addition, the DuPont Biometer requires the use of extremely purified firefly extract, while relatively crude preparations of firefly extract can be used with the JRB Photometer. As a result, the cost per analysis differs from about five cents (JRB Photometer) to about forty cents (DuPont Biometer). Furthermore, the operation and handling of the JRB Photometer is much simpler than the rather complicated and cumbersome procedure required for the use of the DuPont Biometer and the AMINCO Chem-Glow Photometer, especially when the latter is equipped with all of its accessories. No matter which manufacturer's analyzer is used, however, ATP determination for biomass estimation requires an identical five-step procedure of reagent preparation, instrument calibration, ATP extraction, measurement of ATP and data processing. Picciolo *et al.* (1975) tested these three commercial analyzers for use with liquid luminescence systems for an analytical assay of picomole quantities of ATP. They found that the three photometers were comparable in reproducibility, sensitivity and linearity.

CHAPTER 6

General Remarks on the ATP Biomass Estimation Technique

Biomass estimation via the determination of ATP has been used in various environments and in very diverse systems. There are, however, limitations to the technique. Some are similar to problems which arise in other estimators of biomass, including chlorophyll concentration, particulate organic carbon and nitrogen, protein, DNA, turbidity, packed cell volume, dry weight, and direct microscopic count. The greatest advantages of the ATP method over those listed above are its ability to distinguish between living and dead materials, and the high sensitivity and specificity of the assay procedure. However, ATP measurements should be compared with other "standard" estimators of biomass until sufficient data has been accumulated to show that the biomass estimate based on ATP determination is the method of choice.

Since ATP is present in all living cells, it is possible by size fractionation* to differentiate between zooplanktonic, phytoplanktonic and bacterial ATP (Burnison 1974, Rudd and Hamilton 1973), however, microscopic observations should also be made of the various size fractions to substantiate the degree of separation.

The methodology for ATP has been developed to the point where most of the problems arising during biomass estimation are associated with the varying concentrations of intracellular ATP. As pointed out earlier, several investigators have noted the change in ATP content per viable cell during the different growth phases of laboratory-grown cultures (Forrest 1965; Chapman *et al.* 1971; Lee *et al.* 1971b; Chiu *et al.* 1973; Kao *et al.* 1973). It must be kept in mind that ATP is a very dynamic cell constituent. The suggestion has been made that it is not only an indicator of biomass but also of metabolic activity (Brezonik *et al.* 1975). Chapman *et al.* (1971) pointed out that it is not the concentration of ATP but the ratio of its concentration to those of AMP and ADP that determine the rates of ATP-requiring reactions.

In view of these results a ratio of ATP to the total adenylate pool (ATP + ADP + AMP) or, even better, the adenylate energy charge (ATP + ½ADP/ATP + ADP + AMP) (Atkinson 1971; Atkinson and Walton 1967) should be used to measure the metabolic activity of a population. The biomass, however, seems to be best estimated by the concentration of ATP or the total adenylate pool. Both measurements of biomass are currently under investigation at the Canada Centre for Inland Waters, by B.K. Burnison of the Process Research Division.

---

* Using various screens and membrane filters.

References

Addanki, S., J.F. Sotos, and P.D. Rearick. 1966. Rapid determination of picomole quantities of ATP with a liquid scintillation counter. Analyt. Biochem. 14: 261-264.

Allen, P.D. 1973. Development of the Luminescence biometer for microbial detection. Dev. Ind. Microbiol. 14: 67-73.

Anderson, J.R., and P.I. Davies. 1973. Investigations on the extraction of adenosine triphosphate from soils. Bull. Ecol. Res. Comm. (Stockholm) 17: 271-273.

Anon. 1970. DuPont 760 Luminescence Biometer Instruction Manual. E.I. DuPont de Nemours & Co. (Inc.), Wilmington, Delaware 19898.

Anon. 1974. Lab-Line. ATP Photometer Instruction Manual No. 9140. Lab-Line Instruments Inc., Melrose Park, Illinois 60160. (Also JRB ATP Photometer Brochure for Model 2000. SAI Technology — Previously JRB Incorporated, San Diego, California 92123.)

Atkinson, D.E. 1971. Adenine nucleotide as universal stoichiometric metabolic coupling agents. *In* Advances in Enzyme Regulations, Vol. 9. Edited by G. Weber. Pergamon Press, N.Y., pp. 207-219.

Atkinson, D.E., and G.M. Walton. 1967. Adenosine triphosphate conservation in metabolic regulation: rat liver citrate cleavage enzyme. J. Biol. Chem. 242: 3239-3241.

Ausmus, B.S. 1973. The use of the ATP assay in terrestrial decomposition studies. Bull. Ecol. Res. Comm. (Stockholm) 17: 223-234.

Bächi, B., and L. Ettlinger. 1973. Influence of glucose on adenine nucleotide levels and energy change in *Acetobacter aceti*. Arch. Mikrobiol. 93: 155-164.

Bailey, R.B., and L.W. Parks. 1972. Response of the intracellular ATP pool of *Saccharomyces cerevisiae* to growth inhibition induced by excess L-methionine. (Abstr.) Am. Soc. Microbiol., P. 140.

Bancroft, K. 1974. Extraction and measurement of adenosine triphosphate from marine sediments with boiling bicarbonate solutions. M.Sc. Thesis, University of Georgia.

Bancroft, K., E.A. Paul, and W.J. Wiebe. 1974. Extraction of adenosine triphosphate from marine sediments with boiling sodium bicarbonate. (Abstr.) Am. Soc. Microbiol., p. 53.

Brezonik, P.L., and J.W. Patterson. 1971. Activated sludge ATP: Effects of environmental stress. J. Sanit. Eng. Div., Am. Soc. Civ. Eng. 97: 813-824.

Brezonik, P.L., F.X. Browne, and J.L. Fox. 1975. Application of ATP to plankton biomass and bioassay studies. Water Res. 9: 155-162.

Browne, F.X. 1971. ATP measurements in laboratory and field populations of lake plankton. Ph.D. Thesis, University of Florida, Gainesville, Fla.

Brunker, R. 1975. Autoinjector for the determination of picomolar quantities of ATP with a liquid scintillation counter. Anal. Biochem. 63: 418-422.

Burnison, B.K. 1974. Microbial ATP Studies. Proc. 19th Congress International Association of Limnology. p. 23.

Chapman, A.G., L. Fall, and D.E. Atkinson. 1971. Adenylate energy charge in *Escherichia coli* during growth and starvation. J. Bacteriol. 108: 1072-1086.

Chappelle, E.W. 1971. Virus detection. U.S. Patent 3575812, p. 5.

Chappelle, E.W., and G.V. Levin. 1968. The use of the firefly bioluminescent reaction for rapid detection and counting of bacteria. Biochem. Med. 2: 41-52.

Chappelle, E.W. and G.V. Levin. 1971. Cancer detection. U.S. Patent 3575811, p. 4.

Cheer, S., J.H. Gentile, and C.S. Hegre. 1974. Improved methods for ATP analysis. Anal. Biochem. 60: 102-114.

Ching, T.M. 1973. Adenosine triphosphate content and seed vigor. Plant Physiol. 51: 400-402.

Chiu, S.Y. 1972. Modeling of kinetics and significance of ATP measurements in an activated sludge system. Ph.D. Thesis, Kansas State University, pp. 158.

Chiu, S.Y., I.C. Kao, L.E. Erickson, and L.T. Fan. 1973. ATP pools in activated sludge. J. Water Pollut. Control Fed. 45: 1746-1758.

Christan, R.R., K. Bancroft, and W.J. Wiebe. 1974. Distribution of microbial adenosine triphosphate in saltmarsh sediments. (Abstr.) Am. Soc. Microbiol. p. 54.

Christan, R.R., K. Bancroft, and W.J. Wiebe. 1975. Distribution of microbial adenosine triphosphate in saltmarsh sediments at Sapelo Island, Georgia. Soil Sci. 119: 89-97.

Cocucci, M., M.C. Cocucci, and E. Marre. 1973. Growth inhibition without ATP decrease in *Rhodotorula gracilis* cultured under reduced oxygen pressure. Plant Sci. Lett. 1: 425-431.

Cole, H.A., J.M. Wimpenny, and D.E. Hughes. 1967. The ATP pool in *Escherichia coli*. I. Measurement of the pool using a modified luciferase assay. Biochim. Biophys. Acta, 143: 445-453.

Daly, K.F. 1974. The luminescence biometer in the assessment of water quality and wastewater analysis. American Laboratory, 6: 38-44.

D'Eustachio, A.J., and D.R. Johnson. 1968a. Adenosine triphosphate content of bacteria. Fed. Proc. 27: 761.

D'Eustachio, A.J., and D.R. Johnson. 1968b. An instrumental approach to rapid microbiology. DuPont Internal Study. p. 10.

D'Eustachio, A.J., and G.V. Levin. 1967. Levels of adenosine triphosphate during bacterial growth. (Abstr.) Bacteriol. Proc., Am. Soc. Microbiol. pp. 121-122.

D'Eustachio, A.J. and G.V. Levin. 1968. Rapid assay of bacterial populations. (Abstr.) Bacteriol. Proc., Am. Soc. Microbiol. p. 13.

Dhople, A.M., and J.H. Hanks. 1973. Quantitative extraction of adenosine triphosphate from cultivable and host-grown microbes: calculation of adenosine triphosphate pools. Appl. Microbiol. 26: 399-403.

Forrest, W.W. 1965. Adenosine triphosphate pool during the growth cycle in *Streptococcus faecalis*. J. Bacteriol. 90: 1013-1016.

Gadkari, D., and H. Stolp. 1975. Energy metabolism of *Bdellovibrio bacteriovorus* I. Energy Production, ATP pool, Energy charge. Arch. Microbiol. 102: 179-185.

Gorden, R.W. (Editor). 1972. ATP and its ecological significance. *In* Field and Laboratory Microbial Ecology. Wm. C. Brown Co., Dubuque, Iowa. pp. 13-15.

Greaves, M.P., R.E. Wheatley, H. Shepherd, and A.H. Knight. 1973. Relationship between microbial populations and adenosine triphosphate in a basin plot. Soil Biol. Biochem. 5: 685-687.

Hamilton, R.D., and O. Holm-Hansen. 1967. Adenosine triphosphate content of marine bacteria. Limnol. Oceanogr. 12: 319-324.

Hammerstedt, R.H. 1973. An automated method for ATP analysis utilizing the luciferin-luciferase reaction. Anal. Biochem. 52: 449-455.

Harrison, D.E.F., and P. Maitra. 1968. The role of ATP in the control of energy metabolism in growing bacteria. J. Gen. Microbiol. 53: vii.

Hobbie, J.E., O. Holm-Hansen, T.T. Packard, L.R. Pomeroy, R.W. Sheldon, J.P. Thomas, and W.J. Wiebe. 1972. Distribution and activity of microorganisms in ocean water. Limnol. Oceanogr. 17: 544-555.

Holm-Hansen, O. 1969. Determination of microbial biomass in ocean profiles. Limnol. Oceanogr. 14: 740-747.

Holm-Hansen, O. 1970. ATP levels in algal cells as influenced by environmental conditions. Plant Cell Physiol. 11: 689-700.

Holm-Hansen, O. 1971. Determination of microbial biomass in deep ocean profiles. *In* Fertility of the Sea. Edited by J.D. Costlow, Jr. Gordon and Breach Ltd., London. pp. 197-207.

Holm-Hansen, O. 1973a. The use of ATP determinations in ecological studies. Bull. Ecol. Res. Comm. (Stockholm) 17: 215-222.

Holm-Hansen, O. 1973b. Determination of total microbial biomass by measurement of adenosine triphosphate. *In* Estuarine Microbial Ecology. Edited by H.L. Stevenson and R.R. Colwell. University of South Carolina Press, Columbia, S.C. pp. 73-89.

Holm-Hansen, O., and C.R. Booth. 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. Oceanogr. 11: 510-519.

Holm-Hansen, O., and H.W. Paerl. 1972. The applicability of ATP determination for estimation of microbial biomass and metabolic activity. *In* Detritus and its Ecological Role in Aquatic Ecosystems. Mem. 1st Ital. Idrobiol. 29: 149-168.

Holms, W.H., I.D. Hamilton, and A.G. Robertson. 1972. The rate of turnover of the adenosine triphosphate pool of *Escherichia coli* growing aerobically in simple defined media. Arch. Microbiol. 83: 95-109.

Holmsen, H., I. Holmsen, and A. Bernhardsen. 1966. Microdetermination of adenosine diphosphate and adenosine triphosphate in plasma with the firefly luciferase system. Anal. Biochem. 17: 456-473.

Huennekens, F.M., and H.R. Whitely. 1960. Phosphoric acid anhydrides and other energy-rich compounds. *In* Comparative Biochemistry. Vol. 1. Edited by M. Florkin and H.S. Mason. Academic Press Inc., New York, N.Y. pp. 107-180.

Johansson, M., and E. Hägerby. 1974. Influence of growth conditions, metabolic inhibitors, and phenolic compounds on the ATP pool in *Fommes annosus*. Physiol. Plant. 32: 23-32.

Kao, I.C., S.Y. Chiu, L.T. Fan, and L.E. Erickson. 1973. ATP pools in pure and mixed cultures. J. Water Poll. Control Fed. 45: 926-931.

Karl, D.M., and P.A. LaRock. 1974. Extraction and measurements of adenosine triphosphate in sediments and sands. (Abstr.) Am. Soc. Microbiol. p. 55.

Karl, D.M., and P.A. LaRock. 1975. Adenosine triphosphate measurements in soil and marine sediments. J. Fish. Res. Board Can. 32: 599-607.

Knowles, C.J., and L. Smith. 1970. Measurements of ATP levels of intact *Azotobacter vinelandii* under different conditions. Biochim. Biophys. Acta, 197: 152-160.

Lazdunski, A., and J.P. Belaich. 1972. Uncoupling in bacterial growth: ATP pool variation in *Zymomonas mobilis* cells in relation to different uncoupling conditions of growth. J. Gen. Microbiol. 70: 187-197.

Lee, C.C., R.F. Harris, J.K. Syers, and D.E. Armstrong. 1971a. Adenosine triphosphate content of *Selenastrum capricornutum*. Appl. Microbiol. 21: 957-958.

Lee, C.C., R.F. Harris, J.D.H. Williams, D.E. Armstrong and J.K. Syers. 1971b. Adenosine triphosphate in lake sediments: I. Determination. Soil Sci. Soc. Am. Proc. 35: 82-86.

Lee, C.C., R.F. Harris, J.D.H. Williams, J.K. Syers, and D.E. Armstrong. 1971c. ATP in lake sediments: II. Origin and significance. Soil Sci. Soc. Am. Proc. 35: 86-91.

Lehninger, A.L. 1965. Bioenergetics. Benjamin, Inc., New York, N.Y. pp. 258.

Levin, G.V., E. Usdin, and A.R. Slonim. 1968. Rapid detection of microorganisms in aerospace water systems. Aerosp. Med. 39: 14-16.

Lyman, G.E., and T.P. DeVincenzo. 1967. Determination of picogram amounts of ATP using the luciferin-luciferase enzyme system. Anal. Biochem. 21: 435-443.

MacGregor, A.N. 1970. Amount of chemical extracted ATP from intact fungi and an actinomycetes. (Abstr.) Bacteriol. Proc., Am. Soc. Microbiol. p. 14.

McElroy, W.D. 1947. The energy source for bioluminescence in an isolated system. Proc. Nat. Acad. Sci. U.S. 33: 342-345.

McElroy, W.D., H.H. Seliger, and E.H. White. 1969. Mechanism of bioluminescence, chemi-luminescence and enzyme function in the oxidation of firefly luciferin. Photochem. Photobiol. 10: 153-170.

Montague, M.D., and E.A. Dawes. 1974. The survival of *Peptococcus prevotii* in relation to the adenylate energy charge. J. Gen. Microbiol. 80: 291-299.

Owen, E.M., P.C. Scarry, and M.L. Vasers. 1972. Use of the biometer for evaluation of process. Water Dev. Ind. Microbiol. 13: 257-263.

Patterson, J.W., P.L. Brezonik, and H.D. Putnam. 1970. Measurement and significance of adenosine triphosphate in activated sludge. Environ. Sci. Technol. 4: 569-575.

Picciolo, G.L., D.A. Nibley, and E.W. Chappelle. 1975. Characteristics of three (3) commercial photometers for use with liquid luminescence systems. (Abstr.) ATP Methodology Conference, San Diego, California, pp. 5-6.

Plant, P.J., E.H. White, and W.D. McElroy. 1968. The decarboxylation of luciferin in firefly bioluminescence. Biochem. Biophys. Res. Commun. 31: 98-103.

Rudd, J.W.M., and R.D. Hamilton, 1973. Measurement of adenosine triphosphate (ATP) in two Precambrian Shield lakes of Northwestern Ontario. J. Fish. Res. Board Can. 30: 1537-1546.

St. John, J.B. 1970. Determination of ATP in *Chlorella* with the luciferin-luciferase enzyme system. Anal. Biochem. 37: 409-416.

Schmidt, G.L., and M.D. Kamen, 1971. Control of chlorophyll synthesis in *Chromatium vinosum*. Arch. Mikrobiol. 76: 51-64.

Schram, E. 1970. Use of scintillation counters for bioluminescence assay of adenosine triphosphate (ATP). *In* The Current Status of Liquid Scintillation Counting. Edited by E.D. Bransome, Jr., Grune and Stratton, N.Y. pp. 129-133.

Seitz, W.R. and M.P. Neary. 1974. Chemiluminescence and bioluminescence in chemical analysis. Anal. Chem. 46: 188A-202A.

Seliger, H.H., and W.D. McElroy. 1960. Spectral emission and quantum yield of firefly bioluminescence. Arch. Biochem. Biophys. 88: 136-141.

Sharpe, A.N. 1973. Automation and instrumentation developments for the bacteriology laboratory. *In* Sampling — Microbiological Monitoring of Environments. Edited by R.G. Board and D.W. Lovelock. Soc. Appl. Bacteriol. Tech. Ser. 7: 197-232.

Sharpe, A.N., M.N. Woodrow, and A.K. Jackson. 1970. Adenosine triphosphate (ATP) levels in foods contaminated by bacteria. J. Appl. Bacteriol. 33: 758-767.

Shropshire, W. and K. Bergman. 1968. Light induced concentration changes of ATP from *Phycomyces sporangiophores:* A re-examination. Plant Physiol. 43: 1317-1318.

Shropshire, W., and R.H. Gettens. 1966. Light induced concentration changes of adenosine triphosphate in *Phycomyces sporangiophores*. Plant Physiol. 41: 203-207.

Slayman, C.L. 1973. Adenine nucleotide levels in *Neurospora,* as influenced by conditions of growth and by metabolic inhibitors. J. Bacteriol. 114: 752-766.

Somlo, M. 1970. Respiration-linked ATP formation by an oxidative phosphorylation mutant of yeast. Arch. Biochem. Biophys. 136: 122-133.

Sorokin, Y.I., and H. Kadota (Editors). 1972. Total microbial biomass estimation by the ATP method. *In* Techniques for the Assessment of Microbial Production and Decomposition in Fresh Waters. IBP Handbook No. 23. Blackwell Scientific Publications, Oxford, Eng. pp. 71-76.

Stanley, P.E. 1974. Analytical bioluminescence assays using the liquid scintillation spectrometer: a review. *In* Liquid Scintillation Counting, Vol. 3. Edited by M.A. Crook and P. Johnson. Heyden and Son Ltd., London (New York). pp. 253-272.

Stanley, P.E., and S.G. Williams. 1969. Use of the liquid scintillation spectrometer for determining ATP by luciferase enzyme. Anal. Biochem. 29: 381-392.

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.

Strehler, B.L. 1968. Bioluminescence assay: principles and practice. *In* Methods of Biochemical Analysis, Vol. 16. Edited by D. Glick. Interscience Publishers, John Wiley and Sons, Inc. N.Y. pp. 99-181.

Strehler, B.L., and W.D. McElroy. 1957. Assay of adenosine triphosphate. *In* Methods in Enzymology. Vol. 3. Edited by S.P. Colowick and N.O. Kaplan. Academic Press, Inc., N.Y. pp. 871-873.

Strickland, J.D.H. 1965. Production of organic matter in the primary stages of the marine food chain. *In* Chemical Oceanography. Vol. 1. Edited by J.P. Riley and G. Skirrow. Academic Press Inc., N.Y.

Strickland, J.D.H. 1971. Microbial activity in aquatic environments. *In* Microbes and Biological Productivity. 21st Symposium of the Society of General Microbiology, Cambridge University Press, Cambridge, Eng. pp. 231-253.

Strickland, J.D.H., and T.R. Parsons. 1968. Determination of adenosine triphosphate (ATP). *In* A Practical Handbook of Seawater Analysis. Fish Res. Board Can., Bull. 167, Queen's Printer, Ottawa. pp. 245-249.

Tal, E., S. Dickstein, and F.G. Sulman. 1964. ATP determination with the Tricarb scintillation counter. Experientia, 20: 652.

Weddle, C.L., and D. Jenkins. 1971. The viability and activity of activated sludge. Water Res. 5: 621.

White, E.H., E. Rapaport, T.A. Hopkins, and H.H. Seliger. 1969. Chemi- and bioluminescence of firefly luciferin. J. Am. Chem. Soc. 91: 2178-2180.

White, E.H., E. Rapaport, H.H. Seliger, and T.A. Hopkins. 1971. The chemi- and bioluminescence of firefly luciferin: An efficient chemical production of electronically excited states. Bioorg. Chem. 1: 92-122.

Wiener, S., R. Wiener, M. Urivetzky, and E. Meilman. 1974. Coprecipitation of ATP with potassium perchlorate: the effect on the firefly enzyme assay of ATP in tissue and blood. Anal. Biochem. 59: 489-500.

Williams, M.L.B. 1971. The limitations of the DuPont Luminescence Biometer in the microbiological analysis of food. Can. Inst. Food Technol. J. 4: 187-189.

Wise, R.H. 1974. Off the shelf analyzers for measuring adenosine triphosphate (ATP) in activated sludge. EPA-670/2 74-034, National Environmental Research Centre, U.S. Environmental Protection Agency, Cincinnati, Ohio, 45268.

The references included in this bibliography are not cited in the text of this report.

The references are arranged in alphabetical order and the names of serials are abbreviated in the form given in the *1974 Bibliographic Guide for Editors and Authors,* published by the American Chemical Society, Washington, D.C.

Appendix A
Bibliography

Bibliography

Airth, R.L., G.E. Foerster, and R. Hinde. 1970 Bioluminescence. *In* Photobiology of Microorganisms. Edited by P. Halldal. John Wiley & Sons, Inc. N.Y., pp. 417-446.

Albaum, H., T. Cayle, and A. Shapiro. 1951. Blood levels of adenine nucleotides. J. Clin. Invest. 30: 525-530.

Aledort, L.M., R.I. Weed, and S.B. Troup. 1966. Ionic effects of firefly bioluminescence assay of red blood cell ATP. Anal. Biochem. 17: 268-277.

Anon. 1970. Bacterial cell counts. Process. Biochem. 5:35.

Atkinson, D.E. 1968a. Citrate and the citrate cycle in the regulation of energy metabolism. *In* The Metabolic Roles of Citrate. Edited by T.W. Goodwin. Academic Press, N.Y., pp. 23-40.

Atkinson, D.E. 1968b. The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. Biochemistry, 7:4030-4034.

Atkinson, D.E. 1968c. Regulation of energy metabolism. Symp. Fundam. Cancer Res. 22: 397-413.

Atkinson, D.E. 1969. Regulation of enzyme function. Annu. Rev. Microbiol. 23: 47-68.

Atkinson, D.E. 1971. Adenine nucleotides as stoichiometric coupling agents in metabolism and as a regulatory modifier. The adenylate energy charge. *In* Metabolic Pathways, Vol. 5. Edited by D.M. Greenberg. Academic Press, N.Y., pp. 1-23.

Atkinson, D.E., and N.J. Ball, Jr. 1975. Adenylate energy charge in *Saccharomyces cerevisiae* during starvation. J. Bacteriol. 121: 975-982.

Atkinson, D.E., and L. Fall. 1967. Adenosine triphosphate in biosynthetic regulation. *Escherichia coli* phosphoribosylopyrophosphate synthase. J. Biol. Chem. 292: 3241-3242.

Ausmus, B.S. 1971. Measurement of microbial activity using the luciferin-luciferase for adenosine triphosphate. U.S. IBP Eastern Deciduous Forest Biome, Memo Report No. 71:72, Oak Ridge Natl. Lab.

Bagnara, A.S., and L.R. Finch. 1972. Quantitative extraction and estimation of intracellular nucleoside triphosphates of *Escherichia coli*. Anal. Biochem. 45: 24-34.

Balch, N. 1972. ATP content of *Calanus finmarchicus*. Limnol. Oceanogr. 17: 906-909.

Balharry, G.J.E., and D.J.D. Nicholas. 1970. ATP-sulphurylase in spinach leaves. Biochim. Biophys. Acta, 220: 513-524.

Balharry, G.J.E. and D.J.D. Nicholas. 1971. New assay for ATP-sulphurylase using the luciferin-luciferase method. Anal. Biochem. 40: 1-17.

Bancroft, K., E.A. Paul, and W.J. Wiebe. 1976. The extraction and measurement of ATP from marine sediments. Limnol. Oceanogr. 21:473-480.

Bancroft, K., J.E. Yetka, and W.J. Wiebe. 1975. Oxygen uptake, adenosine triphosphate content, and bacterial growth state in estuarine waters. (Abstr.) Am. Soc. Microbiol. p. 186.

Barrett, J. 1973. Nucleoside triphosphate metabolism in the muscle tissue of *Ascaris lumbricoides* (Nematoda). Int. J. Parisitol. 3: 393-400.

Bauchop, T., and S.R. Elsden. 1960. The growth of microorganisms in relation to their energy supply. J. Gen. Microbiol. 23: 457-469.

Bedell, G.W. II, and Govindjee. 1973. Photophosphorylation in intact algae: Effects of inhibitors, intensity of lights, electron acceptor and donors. Plant Cell Physiol. 14: 1081-1097.

Berland, B.R., D.J. Bonin, R.A. Daumas, P.L. Laborde et S.Y. Maestrini. 1970. Variations du comportement physiologique de l'algue *Monallantus salina* (xanthophycée) en culture. Mar. Biol. 7: 82-92.

Berland, B.R., D.J. Bonin, P.L. Laborde, and S.Y. Maestrini. 1972. Variations de quelques facteurs estimatifs de la biomass, et en particulier de l'ATP, chez plusieurs algues marines planctoniques. Mar. Biol. 13: 338-345.

Bernardi, A. 1974. A fast method of analysis of the 5' terminal nucleotides of deoxyriboligonucleotides. Anal. Biochem. 59: 501-507.

Beutler, E., and M.C. Baluda. 1964. Simplified determination of blood adenosine triphosphate using the firefly system. Blood, 23: 688-696.

Beutler, E., and O. Duron. 1965a. Red cell ATP determinations. Blood, 25: 625.

Beutler, E., and O. Duron. 1965b. Effect of pH on preservation of red cell ATP. Transfusion, 5: 17-24.

Beutler, E., and K. Mathai. 1967. A comparison of normal RCB ATP levels as measured by the firefly system and the hexokinase system. Blood, 30: 311-320.

Billen, D., B.L. Strehler, G.E. Stapleton, and E. Brigham. 1953. Post irradiation release of ATP from *Escherichia coli*. Arch. Biochem. Biophys. 43: 1-10.

Biomass Determination — A New Technique for Activated Sludge Control, Water Pollution Control Research Series 17050E0Y 01/72. Environmental Protection Agency PB 211-127. Supt. of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

Biometer Flashes, E.I. DuPont Nemours & Co. Inc., Wilmington, Delaware 19898. (i) Number 760BF1. 1970. Water ecology/waste water treatment. (ii) Number 760BF2. 1971. Monitoring cooling tower and water and biocide control. (iii) Number 760BF3. 1972a. Bacteria in cutting oils and hydraulic fluids. (iv) Number 760BF4. 1972b. Rapid determination of BOD problem. (v) Number 760BF5. 1972c. Bacterial screening of urine.

Biometer Flash Number BF6 (P.D. Allen). Fermentation monitoring. E.I. DuPont Nemours & Co. Inc., Wilmington, Delaware 19898.

Bitler, B., and W.D. McElroy. 1957. The preparation and properties of crystalline firefly luciferin. Arch. Biochem. Biophys. 72: 358-368.

Bock, R.M., N. Ling, S.A. Morell, and S.H. Lipton. 1956. Ultraviolet absorption spectra of ATP and related 5'-ribonucleosides. Arch. Biochem. Biophys. 62: 253-264.

Bomsel, J.L., and A. Pradet. 1967. Étude des adénosine 5' mono-, di- et tri-phosphates dans les tissus végétaux. II. Évolution in vivo de l'ATP, l'ADP et l'AMP dans les feuilles de blé en fonction de différentes conditions du milieu (AMP, ADP, and ATP in plant tissues. II. In vivo levels of the phosphate in wheat leaves as a function of different growth conditions). Physiol. Veg. 5: 223-236.

Bomsel, J.L., and A. Pradet. 1968. Study of adenosine 5'-mono-, di-, and triphosphate in plant tissues. IV. Regulation of the level of nucleotides, in vivo, by adenylate kinase: theoretical and experimental study. Biochim. Biophys. Acta, 162: 230-242.

Bott, T.L. 1973. Bacteria and the assessment of water quality. Biological Methods for the Assessment of Water Quality, ASTM STP, 528, American Society for Testing and Materials, pp. 61-75.

Brewer, G.J., and J.W. Eaton. 1971. Erythrocyte metabolism: Interaction with oxygen transport. Science, 171: 1205-1211.

Brewer, G.J., and C.A. Knutsen. 1966. A technique for the processing of blood samples for subsequent analysis of ATP, and an investigation of the method of standardization of the firefly luciferase ATP assay. Clin. Chim. Acta, 14: 836-839.

Brintzinger, H. 1963. The structures of adenosine triphosphate metal ion complexes in aqueous solution. Biochim. Biophys. Acta, 77: 343-345.

Brokaw, C.J. 1967. Adenosine triphosphate usage by flagella. Science, 156: 76-78.

Brooks, D.E. 1970. Content of ATP and ADP in bull spermatozoa using the firefly luciferase system. J. Reprod. Fertil. 23: 525-528.

Brooks, D.E., and C. Lutwak-Maunn. 1971. Content of ATP and ADP in rabbit blastocysts. Nature, 229: 202-203.

Brundage, R.S., R.L. Karpel, K. Austin, and J. Weisel. 1972. Relaxation of spectra of copper II — ATP complexes at low pH. Biochim. Biophys. Acta, 267: 258-267.

Buck, J.B. 1948. The anatomy and physiology of the light organ in fireflies. Ann. N.Y. Acad. Sci. 49: 397.

Buisson, D.H., and H. Sigel. 1972. Protection of adenosine 5'-triphosphate toward hydrolysis by the formation of a mixed ligand metal ion complex. Angew. Chem. Internat. Ed. 11: 1025.

Burn, G.P. 1962. ATP content and glucose uptake of human erythrocytes and the influence of insulin. Biochim. Biophys. Acta, 59: 347-354.

Butler, E., and C.K. Mattai. 1967. A comparison of normal red cell ATP levels as measured by the firefly system and the hexokinase system. Blood, 30: 311-319.

Carlucci, A.F., O. Holm-Hansen, and P.M. Williams. 1971. Distribution, biochemical activity, and chemical composition of plankton in Antarctic waters. Antarct. J. U.S. 6:

Chan, P.S., C.T. Black and B.J. Williams. 1973. Separation of cyclic 3', 5'-AMP from ATP, ADP, and 5'-AMP by precipitation with inorganic compounds. Anal. Biochem. 55: 16-25.

Chapman, C., and W. Bartley. 1969. Adenosine phosphate and the control of glycolysis and gluconeogenesis in yeasts. Biochem. J. 111: 609-613.

Chapman, J.D., R.G. Webb, and J. Borsa. 1971. ATP pool levels in synchronously growing Chinese hamster cells. J. Cell. Biol. 49: 229-233.

Chappelle, E.W. 1969. Use of hexokinase for the reduction of inherent light levels in luciferase preparations. U.S. Patent 3437560, pp. 3.

Chappelle, E.W., and D.G. Hoffman. 1970. Apparatus for detecting and measuring light emitted as a result of the ATP-luciferin-luciferase biochemical reaction. U.S. Patent 3520660, pp. 6.

Chappelle, E.W., and G.L. Picciolo. 1971a. Assay of flavine mononucleotide (FMN) and flavine adenine dinucleotide (FAD) using the bacterial luciferase reaction. *In* Methods in Enzymology. Edited by S. Colowick and N.O. Copeland. 18, Part B, Academic Press, N.Y., pp. 381-385.

Chappelle, E.W., and G.L. Picciolo. 1971b. Bacterial adenosine phosphate as a measure of urinary tract infection. NASA Tech. Brief. 71-10051, GSFC, Greenbelt, Maryland.

Chappelle, E.W., G.L. Picciolo, and R.H. Altland. 1967. A sensitive assay for flavin mononucleotide using the bacterial bioluminescence reaction. Biochem. Med. 1: 252-260.

Chase, A.M. 1960. The measurement of luciferin and luciferase. *In* Methods of Biochemical Analysis. Vol. 8. Edited by D. Glick. Interscience Publishers, John Wiley & Sons, Inc., N.Y., pp. 61-118.

Chase, A.M. 1962. The measurement of luciferin and luciferase. Methods Biochem. Anal. 8: 61-117.

Cheng, S. 1961. A sensitive assay method for ADP and the determination of ATP, ADP, and CrP in single nerve trunks. J. Neurochem. 7: 271-277.

Ching, T.M., and K.K. Ching. 1972. Content of adenosine phosphate and adenylate energy charge in germinating ponderosa pine seeds. Plant Physiol. 50: 536-540.

Ching, T.M., and R. Danielson. 1972. Seedling vigor and adenosine triphosphate level of lettuce seeds. Proc. Assoc. Off. Seed Anal. 62: 116-124.

Ching, T.M., and W.E. Kronstad. 1972. Varietal differences in growth potential adenylate energy level and energy charge of wheat. Crop Sci. 12: 785-789.

Classman, T.A., C. Cooper, L.W. Harrison, and T.J. Swift. 1971. A proton magnetic resonance study of metal ion-adenic ring interactions in metal ion-complexes with ATP. Biochemistry, 10: 843-851.

Clegg, T.J., and J.L. Koenevig. 1974. The effect of four chlorinated hydrocarbon pesticide and one organophosphate pesticide on ATP levels in three species of photosynthesizing fresh water algae. Bot. Gaz. 135: 368-372.

Cockburn, W. 1974. Simultaneous measurement of oxygen evolution and endogenous ATP levels in isolated intact chloroplast suspensions. J. Exp. Bot. 25: 111-120.

Cohn, M., and T.R. Hughes. 1972. Nuclear magnetic resonance spectra of ADP and ATP. II. Effect of complexing with divalent metal ions. J. Biol. Chem. 237: 176-180.

Coombs, J., P.J. Halicki, O. Holm-Hansen, and B.E. Volcani. 1967a. Studies on the biochemistry and fine structure of silica shell formation in diatoms. I. Changes in concentration of nucleoside triphosphates during synchronized division of *Cylindrotheca fusiformis* Reimann and Lewin. Exp. Cell. Res. 47: 302-314.

Coombs, J., P.J. Halicki, O. Holm-Hansen, and B.E. Volcani. 1967b. Studies on the biochemistry and fine structure of silica shell formation in diatoms. II. Changes in concentration of nucleoside triphosphate in silicon-starvation synchrony of *Navicula pelliculose* (Breb.) Hilse. Exp. Cell. Res. 47: 315-328.

Cooney, D.A., H.N. Jayram, E.R. Homan, and C.F. Motley. 1974. A facile radiometric technique for measuring ATP. Anal. Biochem. 62: 157-165.

Cormier, M.J., and J.R. Totter. 1964. Bioluminescence. Annu. Rev. Biochem. 33: 431-458.

Cormier, M.J., J. Lee, and D.M. Hercules. (Editors) 1973. Chemiluminescence and Bioluminescence, Plenum Press, N.Y.

Damoglou, A.P., and E.A. Dawes. 1967. Studies on the lipid content and phosphate requirement of glucose- and acetate-grown *Escherichia coli*. Proc. Biochem. Soc. 467th meeting, 5-6 Jan. (Biochem. J. 102: 32P)

Daumas, R.A. 1973. Évaluation de la teneur en adénosine triphosphate des organismes marins: possibilités actuelles et limites de la méthode. Tethys, 5: 71-80.

Daumas, R., and M. Fiala. 1969. Évaluation de la matière organique vivante dans les eaux marines par la mesure de l'adénosine triphosphate. Mar. Biol. 3: 243-246.

Dawes, E.A., and P.J. Large. 1970. Effect of starvation on the viability and cellular constituents of *Zymomonas anaerobia* and *Zymomonas mobilis*. J. Gen. Microbiol. 60: 31-42.

De Leo, P., and G.G. Sermanni. 1968. Interference of myokinase in the determination of ATP by luciferin assay. Biochim. Biophys. Acta, 170: 208-210.

DeLuca, M. 1969. Hydrophobic nature of the active site of firefly luciferase. Biochemistry, 8: 160-166.

DeLuca, M., and M.E. Dempsey. 1972. Mechanism of bioluminescence and chemiluminescence enicidated by use of oxygen. Chemiluminescence and Bioluminescence, 18: 345-360.

DeLuca, M., and W.D. McElroy. 1965. The hydrolase properties of firefly luciferase. Biochem. Biophys. Res. Commun. 18: 836-842.

DeLuca, M., L. Brand, T.A. Cebula, H.H. Seliger, and A.F. Makula. 1971. Nanosecond time-resolved proton transfer studies with dehydroluciferin and its complex with luciferase. J. Biol. Chem. 246: 6702-6704.

DeLuca, M., G.W. Wirtz, and W.D. McElroy. 1964. Role of sulfhydryl groups in firefly luciferase. Biochemistry, 3: 935-939.

Denburg, J., and M. DeLuca. 1968. Pseudo-allosteric behavior of firefly luciferase. Biochem. Biophys. Res. Commun. 31: 453-460.

Denburg, J.L., and W.D. McElroy. 1970. Catalytic subunit of firefly luciferase. Biochemistry, 9: 4619-4624.

Denburg, J.L., R.T. Lee, and W.D. McElroy. 1969. Substrate binding properties of firefly luciferase. Arch. Biochim. Biophys. 134: 381-394.

DeWeer, P., and A.C. Lowe. 1973. Myokinase equilibrium; An enzymatic method for the determination of stability constant of Mg^{++} complexes with ATP, ADP, and AMP in media of high ionic strength. J. Biol. Chem. 298: 2829-2835.

Dhople, A.M., and J.H. Hanks. 1973. Extraction of bacterial ATP from host-grown organisms. (Abstr.) Am. Soc. Microbiol. p. 15.

Dhople, A.M. and J.H. Hanks. 1974. Energetics (ATP) and limited extracellular growth of *Mycobacterium lepraemurium* (Abstr.) Am. Soc. Microbiol. p. 43.

Douglas, W.W., and A.M. Poisner. 1966a. Evidence that the secreting adrenal chromaffin cell releases catechol amines directly from ATP-rich granules. J. Physiol. 183: 236-248.

Douglas, W.W., and A.M. Poisner. 1966b. Relation between ATP splitting and secretion in the adrenal of chromaffin cell: extrusion of ATP (unhydrolyzed) during release of catechol amines. J. Physiol. 183: 249-256.

Doxtader, K.G. 1969. Estimation of microbial biomass in soil on the basis of ATP measurements. (Abstr.) Bacteriol. Proc., Am. Soc. Microbiol. P. 14.

Doxtader, K.G., J.W. Nyhan, and E.M. Bautista. 1972. Microbial respiration and ATP levels in grassland soil. (Abstr.) Am. Soc. Microbiol. p. 13.

Dufault, L.B., and J.A. Cameron. 1973. Changes in liver and platelet poor plasma ATP in pregnant mice following challenge with *Salmonella typhimurium* endotoxin. (Abstr.) Am. Soc. Microbiol. p. 89.

Dufresne, L., and H.J. Gitelman. 1970. A semiautomated procedure for determination of ATP. Anal. Biochem. 37: 402-408.

Ebadi, M.S., B. Weiss, and E. Costa. 1971. Microassay of adenosine-3', 5'-monophosphate (cyclic AMP) in brain and other tissues by the luciferin-luciferase system. J. Neurochem. 18: 183-192.

Eppley, R.W., A.F. Carlucci, O. Holm-Hansen, D. Kiefer, J.J. McCarthy, and P.M. Williams. 1970. Evidence for eutrophication in the sea near Southern California coastal sewage outfalls. Calif. Mar. Res. Commun. 16: 74-83.

Eppley, R.W., A.F. Carlucci, O. Holm-Hansen, D. Kiefer, J.J. McCarthy, E. Venrick, and P.M. Williams. 1971. Phytoplankton growth and composition in shipboard cultures supplied with nitrate, ammonium, or urea as the nitrogen source. Limnol. Oceanogr. 16: 741-751.

Erkenbrecher, C.W., and L.H. Stevenson. 1974. The effect of tides on the microbial biomass in salt-marsh creeks. (Abstr.) Am. Soc. Microbiol. p. 54.

Ernst, W. 1970. ATP als indikator für die biomasse mariner sedimente. Oecologia (Berlin), 5: 56-60.

Estabrook, R.W., and P.K. Maitra. 1962. A fluorimetric method for the quantitative microanalysis of adenine and pyridine nucleotides. Anal. Biochem. 3: 369-382.

Euler, U.S., F. Lishajko, and L. Stjarne. 1963. Catecholamines and ATP in isolated adrenergic nerve granules. Acta Physiol. Scand. 59: 495-496.

Fanica-Gaignier, M., J. Clement-Metral, and M.D. Kamen. Adenine nucleotide levels and photopigment synthesis in a growing photosynthetic bacterium. Biochim. Biophys. Acta, 226: 135-143.

Ferguson, R.L., and M.B. Murdoch. 1972. Microbial biomass in the Newport River Estuary as measured by the luciferin-luciferase-ATP reaction: Instrumentation, design, calibration and preliminary data. *In* Center for Estuarine and Menhaden Research Annual Report to the Atomic Energy Commission. pp. 271-277.

Ferguson, R.L., and M.B. Murdoch. 1973. Microbiology of the Newport River Estuary. N.O.A.A. Nat. Mar. Fish. Ser. Annual Report to Atomic Energy Commission.

Forrest, W.W., and D.J. Walker. 1965a. Synthesis of reserve materials for endogenous metabolism in *Streptococcus faecalis*. J. Bacteriol. 89: 1448-1452.

Forrest, W.W., and D.J. Walker. 1965b. Control of glycolysis in washed suspensions of *Streptococcus faecalis*. Nature, 207: 46-48.

Fox, J.L., and M.S. Moyer. 1973. Some effects of a power plant on marine microbiota. Chesapeake Sci. 14: 1-10.

Franzen, J.S., and S.B. Binkley. 1961. Comparison of the acid soluble nucleotides in *E. coli* at different growth rates. J. Biol. Chem. 236: 515-519.

Frei, J. 1962. Métabolisme énergétique du leucocyte. I. Détermination enzymatique par fluorimétrie des di- et triphosphates d'adénosine des leucocytes péritonéaux du cobaye. Enzymol. Biol. Clin. 2: 175-187.

Frie, F. , and A. Haspelova-Horvatovicova. 1967. Ein beitrag zur schnellen quantitativen bestimmung von ATP, ADP und AMP in grünen pflanzenorganen. Phytochemistry, 6: 633-640.

Gajdos, A., and M. Gajdos-Torok. 1969. The quantitative regulation of the biosynthesis of porphyrins by intracellular ATP concentration. Biochem. Med. 2: 372-388.

Gemerden, H. van. 1968. On the ATP generation by *Chromatium* in darkness. Arch. Mikrobiol. 64: 170-176.

Gibson, J., and S. Morita. 1967. Changes in adenine nucleotides of intact *Chromatium* produced by illumination. J. Bacteriol. 93: 1544-1550.

Green, A.A., and W.D. McElroy. 1956. Crystalline firefly luciferase. Biochim. Biophys. Acta, 20: 170-176.

Grylls, F.S.M. 1961. The chemical composition of yeasts. *In* Biochemists Handbook. Edited by C. Long. Van Nostrand, N.Y., pp. 1050-1053.

Grys, S., and A. Malinowska. 1972. The effect of tetracycline on the level of nicotinamide and adenine nucleotides and of flavins in the tissues of piglets. Pol. Arch. Weter. 15: 783-788.

Guinn, G., and M.P. Eidenbock. 1972. Extraction, purification, and estimation of ATP from leaves, floral buds, and immature fruits of cotton. Anal. Biochem. 50: 89-97.

Guard, J.W., and P.G. Scholefield. 1971. The energy metabolism of *Novikoff ascites* hepatoma cells: I. The effects of glucose on the intracellular level of ATP, ADP, and AMP. Can. J. Biochem. 49: 686-694.

Hamilton, R.D., O. Holm-Hansen, and J.D.H. Strickland. 1968. Notes on the occurrence of living microscopic organisms in deep water. Deep Sea Res. 15: 651-656.

Harrison, D.E., and P.K. Maitra. 1969. Control of respiration and metabolism in growing *Klebsiella aerogenes*. Biochem. J. 112: 647-656.

Hastings, J.W. 1968. Bioluminescence. Annu. Rev. Biochem. 37: 597-630.

Hempling, H.G., C.C. Stewart, and G. Gasic. 1969. The effect of exogenous ATP on the electrolyte content of TA_3 ascites tumor cells. J. Cell. Physiol. 73: 133-140.

Hernandez, E., and M.J. Johnson. 1967. Energy supply and cell yield in aerobically grown microorganisms. J. Bacteriol. 94: 996-1001.

Ho, K.P., and V.M. Suny. 1975. Yield and ATP pool of *Candida lipolytica* cells grown on glucose and acetate. (Abstr.) Am. Soc. Microbiol. p. 197.

Hobson, P.N., and R. Summers. 1972. ATP pool and growth yield in *Selenomanas ruminantium*. J. Gen. Microbiol. 70: 351-360.

Holm-Hansen, O. 1968. Ecology, physiology, and biochemistry of Blue-green Algae. Annu. Rev. Microbiol. 22: 47-70.

Holm-Hansen, O. 1970a. Determination of microbial biomass in deep ocean water. *In* Organic Matter in Natural Waters. Edited by D.W. Hood. Institute of Marine Science, Univ. of Alaska Press, College, Alaska, pp. 287-300.

Holm-Hansen, O. 1970b. Microbial distribution in ocean water relative to nutrients and food sources. *In* Biological Sound Scattering in the Ocean. Edited by G.B. Farquhar. Marine Center for Ocean Science, Washington, D.C., pp. 149-157.

Holm-Hansen, O., T.T. Packard, and L.R. Pomeroy. 1970. Efficiency of the reverse flow filter technique for concentration of particulate matter. Limnol. Oceanogr. 15: 832-835.

Holm-Hansen, O., J.D.H. Strickland, and P.M. Williams. 1966. A detailed analysis of biologically important substances in a profile off Southern California. Limnol. Oceanogr. 11: 548-561.

Holmsen, H., E. Storm, and H.J. Day. 1972. Determination of ATP and ADP in blood platelets; a modification of the firefly luciferase assay for plasma. Anal. Biochem. 46: 489-501.

Hopkins, T.A., H.H. Seliger, E.H. White, and M.W. Cass. 1967. The chemiluminescence of firefly luciferin. A model for the bioluminescent reaction and identification of the product excited state. J. Am. Chem. Soc. 89: 7148-7150.

Horska, S., and D. Sofrova. 1971. Relative variations of ATP, ADP, and AMP levels in the blood of mother and fetus exhibiting signs of intrauterine hypoxia during childbirth. Biol. Neonate, 19: 194-199.

Horska, S.D., D. Sofrova, and Z.K. Stembera. 1969. Variation of the ATP content of foetal blood after hypoxia in childbirth. Biol. Neonate, 14: 279-285.

Hoskins, D.D., D.T. Stephens, and E.R. Casillas. 1971. Enzymic control of fructolysis in primate spermatozoa. Biochim. Biophys. Acta, 237: 227-238.

Howell, S.L., and R.B.L. Ewart. 1973. Synthesis and secretion of growth hormone in the rat anterior pituitary. J. Cell. Sci. 12: 23-35.

Hulett, H.R. 1970. Non-enzymatic hydrolysis of adenosine phosphates. Nature, 225: 1248-1249.

Imshenetskii, A.A., M.D. Evdokimova, and G.C. Sotnikov. 1971. Methods of decomposition of microbial cells for determining their ATP content. Mikrobiologiya, 40: 730-734.

Imshenetskii, A.A., S.V. Lysenko, G.G. Sotnikov, and S.S. Abyzov. 1973. Influence of ultra low temperatures on the ATP of microorganisms. Mikrobiologiya, 42: 579-581.

Iwamura, T., T. Kanazawa, and K. Kanazawa. 1963. Nucleotide metabolism in *Chlorella*. II. Quantitative changes in component nucleotides of the acid soluble fraction of algae cells during their life cycle. *In* Japanese Soc. Plant Physiol. Studies on microalgae and photosynthetic bacteria. Univ. Tokyo Press, Tokyo., pp. 587-596.

Izzo, R.S. Firefly assay method for adenosine triphosphate. Amino Reprint 454. Am. Inst. Co., Silver Spring, Maryland 2091.

Jaffe, M.J. 1973. The role of ATP in mechanically stimulated rapid closure of the Venus's Flytrap. Plant Physiol. 51: 17-18.

Jallet, P.M.M., P. Laget, H. Guérin, et J. Pieri. 1972. Biochimie — Détermination de la constante d'équilibre du complexe MgATP à l'aide du système luciférine-luciférase. C.R. Acad. Sci. Paris, p. 274.

Janglova, Z., J. Suchy, and Z. Vanek. 1969. Intracellular ATP concentration in *Streptomyces aureofaciens*. Folia Microbiol. 14: 208-210.

Jannasch, H.W., and G.E. Jones. 1959. Bacterial populations in sea water as determined by different methods of enumeration. Limnol. Oceanogr. 4: 128-139.

Johnson, F.H., H.C. Edward, and Y. Haneda. 1961. The luciferin luciferase reactions. *In* A Symposium on Light and Life. Edited by W.D. McElroy and B. Glass. John Hopkins Press, Baltimore, Md. pp. 206-218.

Johnson, R., J.H. Gentile, and S. Cheer. 1974. Automatic sample injector — Its application in the analysis of triphosphate. Anal. Biochem. 60: 115-121.

Johnson, R.A., J.G. Hardman, A.E. Broadus, and E.W. Sutherland. 1970. Analysis of adenosine 3', 5'-monophosphate with luciferase luminescence. Anal. Biochem. 35: 91-97.

Jones, P.C.T. 1969. The effect of temperature on ATP levels in cells of an ascitic hepatoma. J. Cell. Physiol. 73: 37-42.

Jones, P.C.T. 1970a. The effect of light, temperature, and anaesthetics on ATP levels in the leaves of *Chenopodium rubrum* and *Phaseolus vulgaris*. J. Exp. Bot. 21: 58-63.

Jones, P.C.T. 1970b. The interaction of light and temperature in determining ATP levels in the myxamoeba of the cellular slime mould *Dictyostelium discoideum* Acr. 12. Cytobios, 2: 89-94.

Jones, P.C.T. 1970c. Studies on the nature of circadian rhythms: I. Nucleoside phosphate levels in the tissues of wakeful and sleeping golden hamsters. Cytobios, 2: 175-179.

Kalbhen, D.A., and H.J. Koch. 1967. Methodische untersuchungen zur quantitativen mikrobestimmung von ATP in biologischen material mit dem firefly-enzyme system. Z. Klin. Chem. Klin. Biochem. 5: 299-311.

Karl, D.M., P.A. LaRock, W. Sturges, and J.W. Morse. 1975. Adenosine triphosphate in the North Atlantic and its relationship to the oxygen minimum layer. Submitted for publication to Deep Sea Res.

Karl, D.M., J.W. Morse, R. Scott, and L. Butler. 1974. ATP and DOC measurements in the Mid-Atlantic. Trans. Am. Geophys. Union, 55: 294.

Kelly, D.P., and P.J. Syrett. 1966. Energy coupling during sulfur compound oxidation by *Thiobacillus*. J. Gen. Microbiol. 43: 109-118.

Kennard, O., N.W. Isaacs, J.C. Coppola, A.J. Kirby, S. Warren, W.D.S. Motherwell, D.G. Watson, D.L. Wampler, D.H. Chenery, A.C. Larson, K.A. Kerr, and L.R. Di Sanseverion. 1970. Three dimensional structure of ATP. Nature, 225: 333-336.

Keys, A.J. 1968. The intracellular distribution of free nucleotides in the tobacco leaf. Biochem. J. 108: 1-8.

Kilbert, L.H. Jr., M.S. Schiff, and P.P. Foa. 1972. The measurement of ATP in pancreatic islets by means of the luciferin-luciferase reaction. Horm. Metab. Res. 4: 242-244.

Kilburn, D.G. 1969. The energetics of mammalian cell growth. J. Cell. Sci. 4: 645-654.

Klofat, W., G. Picciolo, E.W. Chappelle, and E. Freese. 1969. Protection of ATP in normal cells and sporulation mutants of *Bacillus subtilis*. J. Biol. Chem. 244: 3270-3276.

Kullmann, L., G.E. Staal, and H.W. Wouters. 1972. Increased ATP concentration in erythrocytes in neurofibromatosis. Clin. Sci. 42: 775-779.

Kylin, A., and J.E. Tillberg. 1967. The relation between total photophosphorylation, level of ATP and oxygen evolution in *Scenedesmus* as studied with DCMU and antimycin A. Z. Pflanzenphysiol. 58: 165-174.

Laborde, P.L. 1972. L'adénosine triphosphate des micro-organismes marins planctoniques. Rapports avec la biomasse et la productivité primaire. Thèse de Doctorat, Université d'Aix-Marseille. p. 108.

Laborde, P.L., and H.J. Minas. 1974. Influence des conditions hivernales sur les productions phyto-et zooplanctoniques en Méditerranée Nord-Occidentale. IV. Distribution verticale des taux d'adénosine triphosphate et de production primaire. Mar. Biol. 25: 203-212.

LaRock, P.A., R.E. Hodson, D.J. Shultz, D.M. Karl, and M.D. Sand. 1975. Microbial activity in the Cariaco Trench. Submitted for publication to Deep Sea Res.

Lee, R., and W.D. McElroy. 1969. Role and reactivity of sulfhydryl groups in firefly luciferase. Biochemistry, 8: 130-136.

Lee, R.T., and W.D. McElroy. 1971a. Effects of 5'-adenylic acid on firefly luciferase. Arch. Biochem. Biophys. 145: 78-84.

Lee, R.T., and W.D. McElroy. 1971b. Isolation and partial characterization of a peptide derived from the luciferin binding site of firefly luciferase. Arch. Biochem. Biophys. 146: 551-556.

Lee, R.T., J.L. Denburg, and W.D. McElroy. 1970. Substrate-binding properties of firefly luciferase. II. ATP-binding site. Arch. Biochem. Biophys. 141: 38-52.

Leenders, H.J., A. Kemp, A.F. Koninky, and J. Rosing. 1974. Changes in cellular ATP, ADP, AMP levels following treatments affecting cellular respiration and the activity of certain nuclear genes in *Drosophila* salivary glands. Exp. Cell. Res. 86: 25-30.

Leese, H.J., and J.R. Bronk. 1972. Automated fluorometric analysis of micromolar quantities of ATP, glucose, and lactic acid. Anal. Biochem. 45: 211-221.

Lehninger, A.L. 1973. Bioenergetics, 2nd ed. W.A. Benjamin, Inc., Menlo Park, Calif. 245 pp.

Leonard, F., and A.F. Hegyeli. 1973. A mechanism for topical cutaneous calcinosis. Calcif. Tissue Research, 13: 331-334.

Leonard, N.J., and R.A. Laursen. 1965. Synthesis and properties of analogs of adenosine diphosphate, adenosine triphosphate, and nicotinamide adenine dinucleotide derived from 3-β-D-ribofuranosyladenine (3-isoadenosine). Biochemistry, 4: 365-376.

Levin, G.V., and A.H. Heim. 1965. Gulliver and Diogenes — Exobiological antitheses. *In* Life and Space, Research Vol. III. Edited by M. Florkin. Academic Press, N.Y., pp. 105-119.

Levin, G.V., C.S. Chen, and G. Davis. 1967. Development of the firefly bioluminescence assay for the rapid, quantitative detection of microbial contamination in water. Aerosp. Med. Res. Lab., TR-67-71.

Levin, G.V., J.R. Clendenning, E.W. Chappelle, A.H. Heim, and E. Rocek. 1964. A rapid method for detection of microorganisms by ATP assay; its possible application in virus and cancer studies. Bioscience, 14: 37-38.

Lin, S., and H.P. Cohen. 1968. Measurement of adenosine triphosphate content of crayfish stretch receptor cell preparation. Anal. Biochem. 24: 531-540.

Loos, J.A., R.C.H. vanDoorn, and D. Roos. 1973. A sensitive mechanized determination of ATP and ADP. Anal. Biochem. 53: 309-312.

Loppert, H.G. and E. Broda. 1973. ATP-Gehalt and ATP-Umsatz von *Chlorella* in Abhängigkeit von Belichtung und Belfütung. Z. Allg. Mikrobiol. 13: 499-506.

Lutkenhaus, J., J. Ryan, and M. Konrad. 1973. Kinetics of phosphate incorporation into adenosine triphosphate and guanosine triphosphate in bacteria. J. Bacteriol. 116: 1113-1123.

MacLeod, N.H., E.W. Chappelle, and A.M. Crawford. 1969. ATP assay of terrestrial soils: A test of an exobiological experiment. Nature, 223: 267-268.

Mague, T.H., N.M. Weare, and O. Holm-Hansen. 1974. Nitrogen fixation in the North Pacific Ocean. Mar. Biol. 24: 109-119.

Manandhar, M.S.P., and K.V. Dyke. 1974a. Adenosine tetraphosphate analysis: Polyethyleneimine (PEI) thin layer purification and firefly extract liquid scintillation quantitation. Anal. Biochem. 58: 368-375.

Manandhar, M.S.P., and K.V. Dyke. 1974b. Guanosine triphosphate analysis. PEI — thin layer purification and luciferin-luciferase liquid scintillation quantitation. Anal. Biochem. 60: 122-129.

Manandhar, M.S.P., and K.V. Dyke. 1974c. Delayed luminescence analysis (DLA) of purine and pyrimidine ribose and deoxyribose nucleotide triphosphate in picomole quantities. Microchem. J. 19: 42-51.

Masterson, T.S., A.D. Mason, and W.L. Brown. 1964. Instrument for the quantitation of ATP utilizing firefly bioluminescence. Am. J. Med. Electron. 3: 283-285.

Matsumura, P., and R.E. Marquis. 1975. Adenosine triphosphate supply and barotolerance of streptococci. (Abstr.) Am. Soc. Microbiol. p. 191.

McCapra, F. 1973. Chemistry of bioluminescence. Endeavour, 32: 139-145.

McClare, C.W.F. 1972. In defence of the high energy phosphate bond. J. Theor. Biol. 35: 233-246.

McElroy, W.D. 1956. Cypridina and firefly luciferase. *In* Methods in Enzymology, Vol. II. Edited by S.P. Colowick and N.O. Kaplan. Academic Press, Inc., N.Y., pp. 851-861.

McElroy, W.D. 1963a. Crystalline firefly luciferase. *In* Methods in Enzymology, Vol. VI. Edited by S.P. Colowick and N.O. Kaplan. Academic Press, Inc., N.Y., pp. 445-448.

McElroy, W.D. 1963b. Preparation and properties of firefly luciferyl and oxyluciferyl adenylic acid. *In* Methods in Enzymology, Vol. VI. Edited by S.P. Colowick and N.O. Kaplan. Academic Press, Inc., N.Y., pp. 775-782.

McElroy, W.D., and M. DeLuca. 1972. Chemical and enzymatic mechanisms of firefly luminescence. *In* Chemiluminescence and Bioluminescence. Edited by M.J. Cormier, D.M. Hercules, and J. Lee. Plenum Press, N.Y., pp. 285-312.

McElroy, W.D., and H.B. Glass. (Editors) 1961. A Symposium on Light and Life, John Hopkins Univ. Press., Baltimore, Md.

McElroy, W.D., and C.S. Rainwater. 1948. Spectral energy distribution of the light emitted by firefly extracts. J. Cell. Comp. Physiol. 32: 421-425.

McElroy, W.D., and H.H. Seliger. 1966. Firefly bioluminescence. *In* Bioluminescence in Progress. Edited by F.H. Johnson and Y. Haneda. Princeton University Press, Princeton, N.J., pp. 427-458.

McElroy, W.D., and B.L. Strehler. 1949. Factors influencing the response of the bioluminescent reaction of ATP. Arch. Biochem. Biophys. 22: 420-433.

Milekhina, E.I., E.E. Rakhleeva, G.C. Sotnikov, and I.O. Ivanov. 1970. Use of the luciferin-luciferase method for ATP determination in the cells of *Azotobacter vinelandii*. Mikrobiologiia (Microbiology), 39: 722-726.

Miovic, M.L., and J. Gibson. 1973. Nucleotide pools and adenylate energy charge in balanced and unbalanced growth of *Chromatium*. J. Bacteriol. 114: 86-95.

Mitchell, G.W., and J.W. Hastings. 1971. A stable, inexpensive, solid-state photomultiplier photometer. Anal. Biochem. 39: 243-250.

Morton, R.A., T.A. Hopkins, and H.H. Seliger. 1968. The spectroscopic properties of firefly luciferin and related compounds. An approach to product emission. Biochemistry, 8: 1598-1607.

Munch-Petersen, A., and J. Neuhard. 1964. Studies on the acid-soluble nucleotide pool in thymine-requiring mutants of *Escherichia coli* during thymine starvation. I. Accumulation of deoxyadenosine triphosphate in *Escherichia coli*. Biochim. Biophys. Acta, 80: 542-551.

Nakao, M., T. Nakao, M. Tatibana, and H. Yoshikawa. 1960. Phosphorus metabolism in human erythrocyte. III. Regeneration of adenosine triphosphate in long-stored erythrocyte by incubation with inosine and adenine. J. Biochem. 47: 661-671.

Nakao, T., T. Wada, T. Kamiyama, M. Nakao, and K. Nagano. 1962. A direct relationship between ATP levels and in vivo viability of erythrocytes. Nature, 194: 877-878.

Navon, S., and A. Agrest. 1963. ATP content in the central nervous system of rats exposed to chronic hypercapnea. Am. J. Physiol. 205: 957-958.

Nazar, R.N., H.G. Lawford, and J.T. Wong. 1970. An improved procedure for extraction and analysis of cellular nucleotides. Anal. Biochem. 35: 305-313.

Neilson, A.H., O. Holm-Hansen, and R.A. Lewin. 1972. An obligately autotrophic mutant of *Chlamydomonas dysosmos:* a biochemical eluicidation. J. Gen. Microbiol. 71: 141-148.

Nielsen, R., and H. Rasmussen, 1968. Fractionation of extracts of firefly tails by gel filtration. Acta Chem. Scand. 22: 1757-1762.

Nigam, V.N. 1969. ATP level and control of glycolysis in Novikoff ascites-hepatoma cells. Enzymologia, 36: 257-268.

Nilsson, L. 1970. The effect of anesthetics on the tissue lactate, pyruvate, phosphocreatine, ATP, and AMP concentrations and on intracellular pH in the rat brain. Acta Physiol. Scand. 80: 142-144.

Nobel, P.S., D.T. Chang, C. Wong, S.S. Smith, and D.E. Barcus. 1968. Initial ATP formation, NADP reduction, CO_2 fixation, and chloroplast flattening upon illuminating pea leaves. Dept. of Botanical Sciences & Molecular Biology Inst., Univ. of Calif.

Numgester, W.J., L.J. Paradise, and J.A. Adair. Loss of cellular ATP as a measure of cytolytic activity of antiserum. Proc. Soc. Exp. Biol. Med. 132: 582-586.

Obendorf, R.L., and A. Marcus. 1974. Rapid increase in adenosine 5'-triphosphate during early wheat embryo germination. Plant Physiol. 53: 779-781.

Obendorf, R.L., S. Spiegel, and A. Marcus. 1974. ATP, messenger RNA and ribosomal RNA synthesis during early wheat embryo germination. Plant Physiol. Suppl. 38.

O'Brien, R.W., and J.G. Morris. 1971. Oxygen and the growth and metabolism of *Clostridium acetobutylicum*. J. Gen. Microbiol. 68: 307-318.

Oelze, J., and M.C. Kamen. 1971. Adenosine triphosphate cellular levels in *Rhodospirillum rubrum* during transition from aerobic to anaerobic metabolism. Biochim. Biophys. Acta, 234: 137-143.

Oleniacz, W.S., M.A. Pisano, M.H. Rosenfeld, and R.L. Elgart. 1968. Chemiluminescent method for detecting microorganisms in water. Environ. Sci. Tech. 2: 1030-1033.

Olson, O.E. and S.A. Kuby. 1964. Studies on ATP transphosphorylases. J. Biol. Chem. 239: 460-467.

Palmiano, E.P., and B.O. Juliano. 1972. Biochemical changes in the rice grain during germination. Plant Physiol. 49: 751-756.

Parve, E.S. 1953. Determination of ATP in yeast and blood. Biochim. Biophys. Acta, 10: 121-127.

Patterson, J.W., P.L. Brezonik, and H.D. Putnam. 1969. Sludge activity parameter and their application to toxicity measurements in activated sludge. Proc. 24th Purdue Industrial Waste Conference, Purdue University, Lafayette, Indiana.

Pedersen, T.A., M. Kirk, and J.A. Bassham. 1966. Light-dark transients in levels of intermediate compounds during photosynthesis in air-adapted *Chlorella*. Physiol. Plant. 19: 219-231.

Phillips, R.C., P. George, and R.J. Ruman. 1969. Thermodynamic data for the hydrolysis of ATP as a function of pH, Mg^2 ion concentration and ionic strength. J. Biol. Chem. 244: 3330-3342.

Picciolo, G.L., B.N. Kelbough, E.W. Chappelle, and A.J. Fleig. 1971a. An automated luciferase assay of bacteria in urine. NASA, GSFC X-641-71-163.

Picciolo, G.L., B.N. Kelbough, E.W. Chappelle, and A.J. Fleig. 1971b. Automatic bio-sample bacteria detection system. NASA Tech. Brief 71-10055. GSFC, Greenbelt, Maryland.

Polakis, E.S., and W. Bartley. 1966. Changes in the intracellular concentrations of adenosine phosphates and nicotinamide nucleotides during the aerobic growth cycle of yeast on different carbon sources. Biochem. J. 99: 521-533.

Ponnamperuma, C., S. Sagan, and R. Mariner. 1963. Synthesis of ATP under possible primative earth conditions. Nature, 199: 220-226.

Porumb, S. 1972. Influenta cimpurilor electromagnetice asupra continutului in ATP si ADP in hematile de iepure. Stud. Cercet. Biol. Ser. Zool. 24: 165-168.

Pradet, A., and J.L. Bomsel. 1968. In vivo control of adenylic nucleotides by adenylate kinase. C. R. Acad. Sci., Paris, Ser. D, 266: 2416-2418.

Pradet, A., A. Narayanan, and J. Vermeersch. 1968. Plant tissue AMP, ADP, and ATP. III. Energy metabolism during the first stages of germination of lettuce seeds. Bull. Soc. Fr. Physiol. Veg. 14: 107-114.

Pradet, A. 1967a. Études des adénosines-5'-mono, di- et tri-phosphates dans les tissus végétaux. I. Dosage enzymatique (AMP, ADP, and ATP in plant tissues. I. Enzymatic determination). Physiol. Veg. 5: 209-221.

Pradet, A. 1967b. Étude des adénosines-5'-mono, di- et tri-phosphates dans les tissus végétaux. V. Effet in vivo, sur le niveau de la charge énergétique, d'un déséquilibre induit entre fourniture et utilisation de l'énergie dans les semences de laitue. (Adénosine 5'-mono-, di-, and triphosphates in plant tissues. V. Induction of an imbalance between utilization and regeneration of energy and its influence on the energy levels, studied in vivo in lettuce seeds). Physiol. Veg. 7: 261-275.

Proceedings ATP Methodology Seminar, March 3-5, 1975. SAI Technology Company, San Diego, Calif. 92121. 616 pp.

Prydz, H., and L.O. Froholm. 1964. The use of a recording Beckman DB spectrophotometer for the assay of ATP with a luciferin-luciferase reagent. Acta Chem. Scand. 18: 554-556.

Quammen, M.L., P.A. LaRock, and J.A. Calder. 1973. Environmental effects of pulp mill wastes. *In* Estuarine Microbiol. Ecology. Edited by H. Stevenson and R.R. Colwell. Univ. of South Carolina Press, Columbia, South Carolina, pp. 329-343.

Racker, E. 1972. Bioenergetics and the problem of tumor growth. Am. Sci. 60: 50-63.

Ramaiah, A., and J.D. Nicholas. 1964. The synthesis of ATP and the incorporation of ^{32}P by cell free preparations from *Nitrosomonas europaea*. Biochim. Biophys. Acta, 86: 459-465.

Ramirez, J. and L. Smith. 1968. Synthesis of adenosine triphosphate in intact cells of *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides* on oxygenation or illumination. Biochim Biophys. Acta, 153: 466-475.

Rasmusen, H., and R. Nielsen. 1968. An improved analysis of ATP by the luciferase method. Acta Chem. Scand. 22: 1745-1762.

Raymond, P., A. Narayanan and A. Pradet. 1973. Evidence for the presence of cyclic AMP in plant tissues. Biochem. Biophys. Res. Commun. 53: 1115-1121.

Reasons, R.H., and R.S. Hikida. 1973. Biochemistry of adenosine triphosphate activity of avian fast and slow muscle. Exp. Neurol. 38: 27-32.

Rhodes, W.C., and W.D. McElroy. 1958. The synthesis and function of luciferyl-adenylate and oxyluciferyl-adenylate. J. Biol. Chem. 233: 1528-1536.

Robertson, A.M., and R.S. Wolfe. 1970. Adenosine triphosphate pools in *Methanobacterium*. J. Bacteriol. 102: 43-51.

Rooney, D.W., and J.J. Eiler. 1969. Effects of division-synchronizing hypoxia and hyperthermic shocks upon *Tetrahymena* respiration and intra cellular ATP pools. J. Cell. Biol. 41: 145-153.

Rottino, A., G.T. Hoffman, and H. Albaum. 1952. Adenine nucleotide content of red blood cells of normal individuals and of patients suffering from Hodgkin's disease and other diseases. Blood, 7: 836-841.

Ryther, J.H., and W.M. Dunstan. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science, 171: 1008-1013.

St. John, J.B. 1971. Comparative effects of diuron and chloropropham on ATP levels in *Chlorella*. Weed Sci. 19: 274-276.

Sandmeier, M., and J. Ivart. 1972. Modification du taux des nucléotides adényliques (ATP, ADP, AMP), par un éclairement de lumière rouge clair (660 nm). Photochem. Photobiol. 16: 51-59.

Santarius, K.A., and U. Heber. 1965. Changes in the intracellular levels of ATP, ADP, AMP and Pi and regulatory function of the adenylate system in leaf cells during photosynthesis. Biochim. Biophys. Acta, 102: 39-54.

Schlegel, J.U., G.E. Pipkin, and G.N. Shultz. 1969. The etiology of bladder tumors. Brit. J. Urol. 41: 718-723.

Schmidt, G.L., and M.D. Kamen. 1970. Variable cellular composition of *Chromatium* in growing cultures. Arch. Mikrobiol. 73: 1-18.

Schon, G. 1969. Der einflub der kulturbedingungen auf den ATP, ADP, und AMP spiegel bei *Rhodospirillum rubrum*. Arch. Mikrobiol. 66: 348-364.

Schram, E., and H. Roosens. 1972. Semi-automated microtransferator and cell for the bioluminescence assay of ATP and reduced NAD with scintillation counters. *In* Liquid Scintillation Counting, Vol. 2. Edited by M.A. Crook, P. Johnson, and B. Scales. Heyden and Son Ltd., London (New York), pp. 115-120.

Schultz, J., and J.W. Daly. 1973. Cyclic adenosine 3'-5'-monophosphate in guinea pig cerebral cortical slices. J. Biol. Chem. 248: 843-852.

Seliger, H.H. 1972. Applications of bioluminescence and chemiluminescence. *In* Chemiluminescence and Bioluminescence. Edited by M.J. Cormier, D.M. Hercules, and J. Lee. Plenum Press, N.Y., pp. 461-478.

Seliger, H.H., and W.D. McElroy. 1959. Quantum yield in the oxidation of firefly luciferin. Biochem. Biophys. Res. Commun. 1: 21-24.

Seliger, H.H., and W.D. McElroy. 1964. The colors of firefly bioluminescence. Enzyme configuration and species specificity. Proc. Nat. Acad. Sci. 52: 75-81.

Seliger, H.H., and W.D. McElroy. 1965. Light: Physical and Biological Action. Academic Press, N.Y., pp. 417.

Seliger, H.H., J.B. Buek, W.G. Fastie, and W.D. McElroy. 1964. Spectral distribution of firefly light. J. Gen. Physiol. 48: 94.

Seliger, H.H., W.D. McElroy, E.H. White, and G.F. Field. 1961. Stereospecificity and firefly bioluminescence, a comparison of natural and synthetic luciferins. Proc. Nat. Acad. Sci. 47: 1129-1134.

Senez, J.C. 1962. Some considerations of the energetics of bacterial growth. Bacteriol. Rev. 26: 95-107.

Shen, L., L. Fall, G.M. Walton, and D.E. Atkinson. 1968. Interaction between energy charge and metabolite modulation in the regulation of enzymes of amphibolic sequences. Phosphofructokinase and pyruvate dehydrogenase. Biochemistry, 7: 4041-4045.

Shoaf, W.T., and B.W. Lium. 1976. The measurement of adenosine triphosphate in pure algal cultures and natural aquatic samples. J. Res. U.S. Geol. Survey, 4: 241-245.

Simple Chamber facilities chemiluminescent detection of bacteria. Technical support package for Tech. Brief 70-10525, Technology Utilization Office, NASA Langley Research Center, Hampton, Virginia 23365.

Smith, R.C., and O. Maaloe. 1964. Effect of growth rate on the acid soluble nucleotide composition in *Salmonella typhimurium*. Biochim. Biophys. Acta, 86: 229-234.

Sofrova, D., and S. Leblova. 1970. Determination of ATP in plant tissue. Photosynthetica, 4: 162-184.

Sojka, G.A., and H. Gest. 1968. Integration of energy conservation and biosynthesis in the photosynthetic bacterium *Rhodopseudomonas capsulata*. Proc. Natl. Acad. Sci. 61: 1486-1493.

Sojka, G.A., and H. Gest. 1970. Effects of exogenous ATP on growing cells of *Rhodopseudomonas capsulata*. (Abstr.). Bacteriol. Proc., Am. Soc. Microbiol. pp. 64-65.

Sojka, G.A., G.A. Din, and H. Gest. 1967. Integration of energy conversion and biosynthetic processes in bacterial photosynthesis. Nature, 216, 1021-1022.

Spiro, T.G., W.A. Kjellstrom, M. Zeydel, and R.A. Butow. 1968. On the kinetics of metal catalyzed ATP dephosphorylation. Biochemistry, 7: 859-865.

Stadelman, P., and M. Munawar. 1974. Biomass parameters and primary production at a nearshore and midlake station of Lake Ontario during IFYGL. Proc. 17th Conf. Great Lakes Res. 109-119.

Stanley, P.E. 1971. Determination of subpicomole levels of NADH and FMN using bacterial luciferase and the liquid scintillation spectrometer. Anal. Biochem. 39: 441-453.

Stenlid, G. 1970. Flavonoids as inhibitors of the formation of adenosine triphosphate in plant mitochondria. Phytochemistry, 9: 251-256.

Stenlid, G. 1971. The effect of some inhibiting sugars upon the content of

ATP in wheat roots. Physiol. Plant. 25: 397-401.
Stewart, J.M., and G. Guinn. 1969. Chilling injury and changes in adenosine triphosphate of cotton seedlings. Plant Physiol. 44: 605-608.
Strehler, B.L. 1953. Firefly luminescence in the study of energy transfer mechanisms. II. ATP and photosynthesis. Arch. Biochem. Biophys. 43: 67-79.
Strehler, B.L., and J.R. Totter. 1952. Firefly luminescence in the study of energy transfer mechanisms. I. Substrate and enzyme determination. Arch. Biochem. Biophys. 40: 28-41.
Strehler, B.L., and J.R. Totter. 1954. Determination of ATP and related compounds. *In* Methods of Biochemical Analysis, Vol. 1. Edited by D. Glick. Interscience Publishers, John Wiley & Sons, Inc., N.Y., pp. 341-356.
Strickland, J.D.H., O. Holm-Hansen, R.W. Eppley, and R.J. Linn. 1969. The use of a deep tank in plankton ecology. I. Studies of the growth and composition of phytoplankton crops at low nutrient levels. Limnol. Oceanogr. 14: 23-34.
Sunderland, D.W., and M.J. Merrett. 1967. Respiration rate, adenosine diphosphate and triphosphate concentrations of leaves showing necrotic local lesions following infection by tobacco mosaic virus. Physiol. Plant. 20: 368-372.
Suzuki, N., and T. Goto. 1972a. Studies of firefly bioluminescence. II. Tetrahedron, 28: 4075-4082.
Suzuki, N., and T. Goto. 1972b. Synthesis of 4-thiazolone derivatives related to firefly oxyluciferin. Agr. Biol. Chem. 12: 2213-2221.
Suzuki, N., M. Sato, and T. Goto. 1972. Studies on firefly bioluminescence. I. Synthesis and spectral properties of firefly oxyluciferin, a possible emitting species in firefly bioluminescence. Tetrahedron, 28: 4065-4075.
Syrett, P.J. 1958. Respiration rate and internal adenosine triphosphate concentration in *Chlorella*. Arch. Biochem. Biophys. 75: 117-124.
Tetas, M., and J.M. Lowenstein. 1963. The effect of bivalent metal ions on the hydrolysis of ADP and ATP. Biochemistry, 2: 350-357.
Travis, J., and W.D. McElroy. 1966. Isolation and sequence of an essential sulfhydryl peptide at the active site of firefly luciferase. Biochemistry, 5: 2170-2176.
Turnock, G.S., S.U. Erickson, B.A.C. Ackrell, and B. Birch. 1972. A mutant of *Escherichia coli* with a defect in energy metabolism. J. Gen. Microbiol. 70: 507-515.
Van Dyke, K., R. Stitzel, R. McClellan, and C. Szustkiewicz. 1969a. An automated procedure for the sensitive and specific determination of ATP. Clin. Chem. 15: 3-14.
Van Dyke, K., R. Stitzel, R. McClellan, and C. Szustkiewicz. 1969b. Automated analysis of ATP; its application to on line continuous-flow incubation and measurement of ATP coupled enzyme system. Advan. Automat. Anal. Technicon Int. Congr. 1: 47-53.
Vechet, B., H. Brana and H. Kupkova. 1971. The ATP pool and the adenyl cyclase activity in *Escherichia coli* strains with different UV sensitivity. Folia Microbiol. 16: 31-34.
Vellend, H., S.A. Tuttle, M. Burza, L. Weinstein, G.L. Picciolo, and E.W. Chappelle. 1974. A rapid method for the determination of microbial sensitivity using the firefly luciferase assay for adenosine triphosphate. (Abstr.) Am. Soc. Microbiol. p. 84.
Wahl, R., and L.J. Kozloff. 1962. The nucleoside triphosphate content of various bacteriophages. J. Biol. Chem. 237: 1953-1960.
Watanabe, S., L. Evenson, and I. Gulz. 1963. Effect of MG^{++} and CA^{++} ions on the fluorescence of the protonated ring of adenine, adenosine, AMP, ADP, and ATP. J. Biol. Chem. 238: 324-327.
Weber, C.I. 1970. New plankton biomass parameters. Newsletter #4. Analytical Quality Control Laboratory, Federal Water Pollution Control Administration, Environ. Prot. Agency, Cincinnati, Ohio.
Weber, C.I. 1973a. Recent developments in the measurement of the response of plankton and periphyton to changes in their environment. Environ. Prot. Agency Bioassay Techniques and Environmental Chemistry, pp. 119-138.
Weber, C.I. 1973b. Biological monitoring of the Aquatic Environmental Protection Agency, *In* Biological Methods for the Assessment of Water Quality, ASTM STP, American Society for Testing and Materials, pp. 46-60.
Weiss, B., R. Lehne, and S. Strada. 1972. Rapid micro assay of adenosine 3', 5' monophosphate phosphodiesterase activity. Anal. Biochem. 45: 222-235.
Welsch, F., and L. Smith. 1969. Kinetics of synthesis and utilization of ATP by intact cells of *Rhodospirillum rubrum*. Biochemistry, 8: 3403-3411.
Wettermark, G., E. Borglund, and S.E. Brolin. 1968. A regenerating system for studies of phosphoryl transfer from ATP. Anal. Biochem. 22: 211-218.
Wettermark, G., H. Stymne, S.E. Brolin, and B. Petersson. 1975. Substrate analyses in single cells. I. Determination of ATP. Anal. Biochem. 63: 293-307.
White, E.H., F. McCapra, and G.F. Field. 1963. The structure and synthesis of firefly luciferin. Am. Chem. Soc. J. 85: 337-343.
White, E.H., F. McCapra, G.F. Field, and W.D. McElroy. 1961. The structure and synthesis of firefly luciferin. Am. Chem. Soc. J. 83: 2402-2403.
Whittam, R. 1958. Potassium movements and ATP in human red cells. J. Physiol. 140: 479-497.
Williams, S.G. 1970. Role of phytic acid in the wheat grain. Plant Physiol. 45: 376-381.
Witteveen, S.A.G.J., B.E. Sobel, and M. DeLuca. 1974. Kinetic properties of the isoenzyme of human creatine phosphokinase. Proc. Natl. Acad. Sci. 71: 1384-1387.
Wolf, P.L., T. Kearne, J. Neuhoff, and J. Lauridson. 1974. Identification of CPK Isoenzyme MB in Myocardial Infarction. Lab. Med. 5.
Wood, L., and E. Beutler. 1974. The effect of ascorbate and dihydroxy acetone on the 2,3-diphosphoglucerate and ATP levels of stored human red cells. Transfusion, 14: 272-277.
Yamada, S. 1971. The effect of ATP contents of human erythrocytes on immune hemolysis. J. Nara. Med. Assoc. 22: 28-35.
Zwetnow, N.N. 1970. The influence of an increased intracranial pressure on the lactate, pyruvate, bicarbonate, phosphocreatine, ATP, ADP, and AMP concentrations of the cerebral cortex of dogs. Acta Physiol. Scand. 79: 158-166.