MICROBIOLOGICAL REVIEWS, Dec. 1980, p. 739-796
0146-0749/80/04-0739/58 $02.00/0

Vol. 44, No. 4

214

Cellular Nucleotide Measurements and Applications in Microbial Ecology [9 .

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INTRODUCTION

Ecology is physiology under the worst possible conditions

T. Brock, 1966

Experimental microbial ecology represents one of the least developed areas of microbiological research. The organization and dynamics of microbial communities are such that they should be studied as integrated units if an understanding of the mechanisms and regulation of vital biogeochemical cycles is to be achieved. The primary objective of ecosystems research, or synecology, is to study and ultimately understand the exchange of energy and matter between and among the various living and nonliving compartments. A detailed quantitative assessment of the total amount of living carbon (i.e., biomass) and of the magnitude of community metabolic activity and growth is fundamental to our understanding of natural processes. One motivation for measuring biomass and activity is to estimate the total amount of energy stored in the biota and to monitor the rate of material flux through the living system.

Ecology is defined as the integrated study of biological relationships among organisms sharing a common habitat and of the interactions occurring between the biotic and abiotic components of that environment. Although natural ecosystems are of primary concern to the experimental microbial ecologist, environments designed, constructed, and primarily controlled by human activities (e.g., industrial and agricultural complexes and urbanized areas) are also of considerable interest in applied microbial ecology. Therefore, the field of microbial ecology extends beyond the study of soil and aquatic environments and includes investigations of such areas as microbial disease and infection, contamination of processed foods, wastewater and sewage treatments, purification of potable water supplies, and even the search for extraterrestrial life, to name a few.

In the context of this review, the term *microbial* shall encompass all organisms less than ~200 μm in greatest dimension. Accordingly, bacteria, actinomycetes, unicellular algae, yeasts and fungi, protozoa, and many metazoan taxa are all regarded as components of the natural microbial assemblage. Their small size (i.e., high surface-to-volume ratio) is the sole unifying characteristic of this heterogeneous group; the result is an intimate contact and potentially large interaction with their abiotic environment. It is important to remember that microorganisms live in microenvironments defined on scales of micrometer and millimeter dimensions and that the physical and chemical characteristics of these microhabitats are generally quite different from those of the ambient macroenvironments.

Classical microbiological methods generally have not been suitable for enumerating the sparse populations or for assessing the relatively slow growth rates that occur in most natural environments. Even more recently developed laboratory methods for estimating biomass (e.g., fresh or dry weight determinations, optical density, total protein or cell carbon, and direct microscopy) are generally unsatisfactory when applied to natural populations due to the heterogeneity and variable size spectrum of the individual microbial assemblages, the presence of dead cells, and the varying proportion of nonliving organic materials (i.e., detritus) which are closely associated with the living cells. The development of reliable techniques which can be used unequivocally to measure biomass and to estimate the specific growth rates of autochthonous microbial assemblages is still considered to be the most fundamental research objective of experimental microbial ecology. Extensive laboratory studies have been conducted in an attempt to characterize the physiology and biochemistry of bacteria isolated from soil, freshwater, and marine ecosystems; however, it is unknown whether or not these metabolic potentials are ever realized in situ among the naturally occurring microbial populations. The experimental microbial ecologist must ultimately have a "commitment to nature" (38) and sooner or later must complement laboratory studies with field observations and measurements.

No single approach to the study of microbial ecology is universally accepted. One option is to investigate a single group of organisms or a single metabolic process through an analytical breakdown of the natural environment. The second, and perhaps more difficult, route is to study the complex ecosystem in toto. This review emphasizes the latter approach and specifically is organized around a presentation and discussion

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of the use (and misuse) of nucleotide measurements in experimental microbial ecology. Previous reviews concerning the pathways of nucleotide metabolism (129), the role of adenine nucleotides in the regulation of cellular metabolism (8, 10, 47, 48, 125, 194), and selected topics in cellular bioenergetics (118) have been most helpful in formulating the concept of "nucleotide fingerprinting" of complex microbial communities.

In spite of the large number of papers published under the general theme of cellular nucleotide measurements and applications in microbial ecology, no comprehensive review of the subject has been written. The present effort is intended to: (i) offer a brief review of the fundamental physiological principles which provide the motivation for considering nucleotide measurements in experimental microbial ecology, (ii) outline the principles and current limitations of the analytical procedures used to extract and quantify nucleotides from microbial assemblages in nature, (iii) appraise the available laboratory and field data concerning predictable effects of the environment on steady-state levels of cellular nucleotides, and (iv) present specific applications and interpretations of the measurement of nucleotide fingerprints in naturally occurring populations of microorganisms. These goals are sought by organizing and discussing the ecological data within an appropriate framework of microbial physiology and nucleotide metabolism. It is hoped that this review will stimulate and facilitate the application of nucleotide fingerprinting of microbial populations in nature as a means of assessing biomass, growth, and activity and concurrently provide caution for the interpretation of existing data.

NUCLEOTIDES, NUCLEOSIDES, AND RELATED INTRACELLULAR COMPOUNDS

Cellular Bioenergetics

All living cells contain an identical suite of molecules, referred to collectively as nucleosides and nucleotides, which are essential for viability and growth. Although in the strictest sense nucleosides and nucleotides are derived from nucleic acids, the terms are now used in a much broader sense to include structurally related compounds as well (129). More than 100 different nucleosides and nucleotides have now been isolated and identified (167); however, the 5′-ribonucleotides of the bases adenine, guanine, uracil, and cytosine compose the bulk (90 to 95%) of the free nucleotide derivatives of most cells. Less abundant and more exotic derivatives, such as the deoxyribonucleotides, higher-level polyphosphates, 3′,5′ cyclic monophosphates, adenine-sulfuryl nucleotides, conjugated nucleotides, naturally occurring nucleoside and nucleotide antibiotics, vitamins, and coenzymes also have specific functions in cellular metabolism. In addition, the 16S and 23S ribosomal ribonucleic acid (RNA) and transfer RNA components of many procaryotes are known to contain structurally modified nucleosides as well as "natural" bases. The chemistry and biochemistry of these modified nucleotides have been concisely reviewed by Hall (120).

It is now well established that nucleotides have at least four functions, including: (i) storage and transport of cellular metabolic energy, (ii) synthesis of deoxyribonucleic acid (DNA) and RNA, (iii) activation and transfer of precursors for cellular biosynthesis, and (iv) control and regulation of cellular metabolism. The central role of the adenine nucleotides, and especially adenosine 5′-triphosphate (ATP), as intermediate carriers of chemical energy linking catabolism and biosynthesis has been known since the publication of Lipmann's classic paper in 1941 (221). The production of ATP, however, should be viewed not as a mechanism for the storage of chemical potential energy, but rather as a system for rapid and specific mobilization of cellular energy. Turnover times for the intracellular ATP pool of 0.1 to 1.0 s are not uncommon for growing bacteria (47, 126, 151, 195, 241). Therefore, the amount of chemical energy represented by the intracellular ATP pool is only a very small percentage of the potential energy flux through the cell.

Bauchop and Elsden (25) have proposed that the extent of growth of a microorganism is proportional to the amount of ATP available to it from the degradation of an energy source. This allows for the calculation of a yield coefficient, Y_{ATP}, defined as the number of grams (dry weight) of cells produced per mole of ATP generated from catabolism (103). Several discussions of Y_{ATP} have since appeared (103, 239), including a challenge to the universality of this concept (301, 302). Furthermore, it is not known whether yield studies conducted in the laboratory have predictive value for estimating microbial growth yields in nature. Tempest (314) has raised an interesting question regarding the importance of growth yield as a selective force in the evolution of microorganisms. He has concluded that the ability to scavenge low levels of nutrients probably represents a more important selection factor than the ability to metabolize them optimally. Currently, it is unknown whether ATP production and growth are uncou-

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pled in naturally occurring populations of microorganisms or whether natural microbial assemblages are functioning at or near their maximum theoretical Y_{ATP} limits.

Regulation of Cellular Metabolism

Since the maximum potential rate of energy production in microorganisms is much greater than the requirement for ATP, living systems have evolved mechanisms to ensure integration, correlation, and control of cellular processes. Bacterial metabolism is regulated by mechanisms which function at various biochemical levels and time scales. These range from coarse genetic control of enzyme synthesis and activity (i.e., induction, repression, derepression, etc.) to fine metabolic tuning effected through kinetic and thermodynamic aspects of the system (163). Several examples of the latter involve cellular nucleotides.

Adenylate energy charge. In vitro studies have revealed that the activities of certain enzymes are affected by the concentration of ATP, others are affected by adenosine 5'-diphosphate (ADP) or adenosine 5'-monophosphate (AMP), and still others are affected by the ATP/AMP or ATP/ADP ratios. Present usage expresses the cumulative effects of the adenosine phosphates on the rate of cellular metabolism in terms of the "adenylate energy charge" parameter (12). The adenylate energy charge (EC_A) is equal to one-half of the number of anhydride-bound phosphate groups per adenine moiety, or

$$EC_A = \frac{[ATP] + 1/2[ADP]}{[ATP] + [ADP] + [AMP]}$$

and is equivalent to a linear measure of the total amount of chemical potential energy momentarily stored in the adenine nucleotide pool. In vitro rate responses of several "key" enzymes in cellular metabolism to variations in EC_A have provided the background data for this control hypothesis. A discussion of the unique metabolic significance of the adenylate system in stoichiometric coupling of cellular energy and a review of the extensive laboratory data supporting these theoretical considerations have been presented in detail previously (7–11, 47, 48, 125, 194).

The EC_A is unitless, which limits the usefulness of this parameter in supplying information about intracellular nucleoide concentrations or the rate of ATP turnover; these can vary significantly at a fixed EC_A (194). Furthermore, Lowry et al. (226) have argued that the EC_A is an insensitive metabolic indicator, since small changes in EC_A actually disguise much larger changes in the absolute ATP/ADP or ATP/AMP ratios to which the enzymes are actually responding. The validity of the EC_A concept as a mechanism for the control of cellular metabolism has also been attacked on more theoretical grounds (108, 265). This criticism pertains to the state of equilibrium of the adenylate kinase reaction and to the intracellular availability of HPO_4^{2-} and Mg^{2+}. Furthermore, conceptual problems arise in the interpretation of the EC_A control hypothesis as a result of known examples of barrier and kinetic compartmentation of adenine nucleotide pools. Failure of the EC_A theory in encompassing certain thermodynamic considerations of biological energy flow is the basis for additional criticisms. However, Atkinson (10) has recently emphasized that the component parts of a cell are far from equilibrium and that the kinetic, and not thermodynamic, controls provide the stabilizing influence on cellular metabolism. Despite these objections, an overwhelming proportion of the published data indicate that the relative molar concentrations of the adenine nucleotides in actively metabolizing cells are maintained within the stringent limits predicted by the EC_A hypothesis (for compilations, see references 47, 48, 125, and 194).

Phosphorylation state. Independent of the EC_A hypothesis, the phosphorylation state ($[ATP]/[ADP][HPO_4^{2-}]$) has been proposed as a fundamental regulatory parameter controlling the oxidation-reduction state of the cytoplasmic nicotinamide adenine dinucleotide (NAD)-reduced NAD (NADH) couple and the rates of respiration and oxidative phosphorylation in mitochondria (308, 335). More recently, Reed (269) found a positive linear correlation between EC_A and phosphorylation state and postulated that the phosphorylation state is regulated by the EC_A.

Oxidation-reduction state. Even though the majority of energy-producing and energy-requiring reactions of a cell are coupled directly to the formation or hydrolysis of ATP, many important metabolic processes proceed as an indirect result of the adenylate system. The pyridine nucleotides (NAD, NAD-phosphate [NADP]), and specifically the numerical values of the oxidized-reduced pyridine nucleotide couples (i.e., [oxidized NAD]/[NADH] and [oxidized NADP]/[NADPH]), affect (and are affected by) cellular dehydrogenase activity, the oxidative phosphorylation state, and the energy charge of the adenylate system. The intracellular oxidation-reduction state of the pyridine nucleotides may also be an important factor in the network of metabolic control. Andersen and von Meyenburg (4) have defined the catabolic reduction charge ([NADH]/[NADH + oxidized

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NAD]) and the anabolic reduction charge ([NADPH]/[NADPH + oxidized NADP]) as important regulatory parameters in cellular metabolism.

Indirect energy coupling. In addition to direct utilization of ATP in cellular metabolism, the free energy of hydrolysis of the γ-phosphate group of ATP can be reversibly transferred to the diphosphates of additional intracellular nucleotides as required for specific biosynthetic reactions. This "phosphate shuttle" is catalyzed by the enzyme nucleosidediphosphate kinase. Since the $\Delta G_0'$ values for the hydrolyses of all nucleoside triphosphates (NTPs) are comparable, the nucleosidediphosphate kinase reaction has a K_{eq} of 1.0 and is therefore freely reversible. With a few exceptions, the activity of nucleosidediphosphate kinase is absolutely essential for the continued supply of the nonadenine NTPs. This indirect coupling of chemical energy (i.e., use of guanosine 5′-triphosphate (GTP), cytidine 5′-triphosphate, uridine 5′-triphosphate, etc., rather than ATP directly) is required exclusively for the synthesis of new cellular materials. Thompson and Atkinson (317) have speculated that the use of nonadenine NTPs specifically for growth allows the cells to preferentially suspend most endergonic biosynthetic reactions during periods of nutrient and energy depletion, thereby conserving their scarce metabolic resources for the maintenance of homeostasis. They also determined that the activity of nucleosidediphosphate kinase is directly coupled to the cellular EC_A in such a way that the flow of phosphate into the nonadenine NTP pools is sharply curtailed when EC_A ratios fall below a critical minimum level (317). Since the nucleosidediphosphate kinase reaction represents a significant potential drain on the available cellular ATP, it may be one of the most important enzymatic reactions in the regulation of cellular bioenergetics.

Cyclic adenosine 3′,5′-monophosphate, guanosine 5′-triphosphate, and guanosine tetraphosphate. Three additional cellular nucleotides which may exert a significant influence on the control of metabolic processes are cyclic adenosine 3′,5′-monophosphate (cAMP), GTP, and guanosine tetraphosphate. Since the classic work of Makman and Sutherland in 1965 (232), cAMP has been implicated as an active component of many important microbial regulatory mechanisms. One of the most important functions of cAMP is its effect (in concert with the appropriate inducer molecule) on the synthesis of inducible enzymes. The ability of exogenous cAMP to overcome the effects of catabolite repression is also noteworthy. The appearance of cAMP in cells has been regarded by Tomkins (322) as an important "metabolic symbol" for carbon starvation and as a biochemical mechanism for modulating the growth and development of both procaryotic and eucaryotic cells.

The guanine nucleotides may also play a unique and important role in the regulation of RNA and protein syntheses. GTP has been shown to be an essential factor for the initiation and the aminoacyl-transfer RNA binding processes and for the polypeptide elongation sequence of protein biosynthesis. Karl (169, 174) has recently proposed that the intracellular GTP/ATP ratio may serve as a specific nucleotide fingerprint which can be used to estimate the rate of protein synthesis within naturally occurring microbial populations. A second important guanine ribonucleotide, guanosine tetraphosphate, first isolated by Cashel and Gallant in 1969 (44), has since been implicated in the control of ribosomal RNA synthesis (99, 208) as well as protein synthesis (200).

Undoubtedly, the fine-tuning mechanisms for the control and integration of cellular energy production and utilization are complex and extend well beyond our formulations of charge ratios, nucleotide fingerprints, and metabolic symbols. Nevertheless, it should be realized that endeavors to think in functional terms have provided a conceptual and experimental framework around which to conduct meaningful physiological studies on the role of nucleotides in living cells.

Analytical Procedures

An overwhelming and justified emphasis on methodology is a familiar feature in the literature pertaining to environmental nucleotide applications. Numerous investigators have developed methods for extraction of cellular nucleotides; however, no single procedure has emerged as the most efficient. Undoubtedly, it would be useful to have a single or even a few standard techniques which could be used for analyses of a wide spectrum of sample materials and microbial habitats. Unfortunately, extreme variability in the physicochemical and microbiological characteristics of individual habitats precludes the existence and mitigates the usefulness of any "universally" applicable technique. Therefore, the procedure that is ultimately selected for routine nucleotide extraction and analysis will depend on the nature of the sample material. Innumerable improvements in nucleotide extraction and assay procedures have surfaced in recent years, and the continuing state of flux of "preferred methods" tends to limit the usefulness of any personal recommendations. The best advice that can be given is that several different techniques should be evaluated and compared,

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using natural representative sample materials, before selecting the preferred extraction method. Most deleterious effects of sampling, extraction, and analysis of NTPs generally result in decreased concentrations of NTPs, especially ATP. A high yield of NTPs and high EC_A values should therefore be taken as primary criteria for evaluating the relative extraction efficiencies of various methods. In comparative studies of this nature, it is imperative to be aware of, and correct for, specific interferences which may be unique to a given chemical extractant, extraction procedure, or particular environmental substratum.

In view of the potential variability in extraction procedures and efficiencies, a brief discussion of the following points seems warranted: (i) criteria for efficient nucleotide extraction, (ii) common sources of analytical interference, and (iii) limitations of our "state of the art" techniques. In this context, readers should direct their attention towards various symposia and workshops (33, 34, 52, 281) which have attempted to resolve a few of the outstanding inadequacies of existing techniques and to formulate concise, universally acceptable methodologies.

The procedures for measuring environmental nucleotide concentrations can be conveniently divided into three equally important analytical aspects: (i) sampling, (ii) extraction, and (iii) analysis.

Sampling. No technique for sampling microorganisms in the field is more rapid than reported rates of ATP turnover. Consequently, quantitative measurements of intracellular adenine nucleotides are accurate only if concentrations remain constant throughout the sampling period. Biochemists and microbial physiologists have designed sophisticated sampling devices to minimize the time elapsed between sampling from the culture and the extraction. Spring-loaded syringes precharged with the appropriate reagents can now efficiently and reproducibly remove cells from growth chambers and extract all the intracellular nucleotides in less than 1 s. Unfortunately, microorganisms from natural ecosystems must first be collected, transported, often sorted, and frequently concentrated before extraction. To cite an example, marine microbiologists samplingthe deep-sea environment (depths of ≥2,000 m) usually wait a minimum of 30 min merely for the collected material to reach the ocean surface, in addition to the time required for shipboard manipulations and for nucleotide extraction. In situ growth conditions should be maintained as constant as possible during this sampling period. If this cannot be achieved, caution must be exercised in subsequent interpretation of the data.

Romano and Laborde (275) have compared the ATP and EC_A values of water samples extracted in situ with the results obtained by following the more conventional sampling and shipboard extraction procedures. Their results from a single station in the Mediterranean Sea indicated that there were no deleterious sampling effects to depths of 30 to 40 m. However, more extensive and systematic studies are necessary to further assess the quantitative effects of sampling, transportation, storage, and additional experimental manipulations of sample materials on the resultant intracellular nucleotide levels. Transitions in light, temperature, pH, pressure, moisture, and dissolved gases may all affect the physicochemical properties of the sample and thereby directly or indirectly influence the intracellular nucleotide concentrations (and their ratios).

Most aqueous environmental samples require concentration before nucleotide extraction, although certain eutrophic ecosystems (and most laboratory cell cultures) may be processed by direct injection of the sample into the extraction medium (165). The most convenient and practical method for concentrating cell material is via vacuum filtration; however, reverse filtration techniques (149), dialysis plates (160), hollow-fiber concentrator systems (160), and centrifugation (61, 87) have also been used. The choice of membrane filter and pressure differential will be dictated by the size and fragility of the cells, and all efforts should be taken to minimize filter clogging and cell lysis during the concentration process.

Several investigators have reported a deleterious effect of vacuum filtration on the final ATP concentrations of sample extracts (165, 180, 271, 293, 309). Karl and Holm-Hansen (179, 180) have recently reexamined this "filtration effect" in terms of ATP, total adenine nucleotides (A_T = ATP + ADP + AMP), and EC_A. Their data indicated that both the total intracellular ATP concentration and the EC_A decreased, when normalized to a standard volume, as the amount of water filtered increased. This response was attributed to metabolic stress imposed by vacuum filtration. Since the A_T concentration was found to be conservative (i.e., A_T per unit of volume was independent of volume filtered), these data discount cell lysis as an important factor. The observed filtration effect was not a linear function of the volume filtered, but instead was found to be hyperbolic, with the asymptote presumably corresponding to some maximum level of metabolic stress (EC_A = 0.5–0.6). It has previ-

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ously been demonstrated that centrifugation also has a detrimental effect on the ATP levels of *Escherichia coli* (61), suggesting a common physiological mechanism. In addition, Karl and Holm-Hansen (179, 180) have reported that the magnitude of this filtration effect is influenced by the species composition and cell density of the microorganisms, as well as by the in situ community EC_A. Similarly, Sutcliffe et al. (309) have discovered that only certain coastal marine waters are affected by this phenomenon and that open-ocean samples (i.e., presumably slower-growing microbial populations) are relatively free from this source of interference. In freshwater ecosystems, Rudd and Hamilton (276) were unable to demonstrate any effect of vacuum filtration on ATP levels during an investigation of two Precambrian shield lakes, and Riemann (271) was only able to elicit this response in the most eutrophic of four lakes in central Denmark. At present, the variable response of natural populations to the effects of vacuum filtration, especially with regard to measurements and interpretations of EC_A data, is one of the most serious, and as yet unresolved, problems in the application of nucleotide determinations in aquatic ecosystems. A thorough understanding of the precise physiological mechanism(s) occurring at the cellular level is needed to eliminate the analytical interference caused by vacuum filtration.

Sampling artifacts influencing the determination of environmental EC_A values are not restricted to filtration effects. Skjoldal and Båmstedt (294) have demonstrated a significant "capture stress" associated with net collections of planktonic metazoans. The EC_A values of various zooplankton species were shown to decrease to ~0.2 immediately upon collection, but recovered to 0.8 after 24 h in an aquarium. Therefore, in many environmental applications the use of total adenylates may be preferable to ATP measurements in order to circumvent the higher level of variability which may be introduced by transient fluctuations in cellular ATP levels.

Extraction. Numerous methods are available for the extraction of nucleotides from microbial cells. Media for initial extraction may be divided into the following categories: (i) boiling aqueous buffers, (ii) inorganic acids, (iii) organic acids, (iv) organic solvents, and (v) inorganic bases. Under all circumstances, however, the basic criteria for a successful extraction procedure are identical. The most important requisites include rapid cell death and lysis, complete nucleotide release, complete and irreversible inactivation of enzyme activity, and long-term stability of the extracted nucleotides (i.e., no chemical or enzymatic hydrolysis). In addition, there may be problems arising from adsorption (especially onto soil and sediment particles), ionic interferences (both organic and inorganic), color quenching, turbidity, and poor extraction efficiencies of microorganisms associated with nonliving organic and inorganic materials.

Table 1 lists the most commonly used nucleotide extraction procedures, and Table 2 presents a summary of the numerous comparative studies which have been conducted to optimize ATP extraction from various sample materials. Although several investigators have recommended identical extraction procedures for similar sample materials (e.g., cold H_2SO_4 for soils and sediments), it is apparent that no single extraction method can be regarded as universally acceptable (Table 2). Nevertheless, a few general comments can be made concerning the extraction of nucleotides from natural microbial assemblages. (i) Boiling buffers have consistently yielded variable and inconsistent results when applied to soils, sediments, and metazoans. One explanation which has been offered for these observations is termed the "heat gradient hypothesis," which is a consideration of thermal extraction efficiencies based on the kinetics of heat flow in two-phase systems (177, 182, 183). (ii) Strongly alkaline conditions should be avoided during the extraction process due to the chemical lability of many polynucleotides, nucleoside diphosphate esters of sugars, aminoacyl adenylates, and related compounds. This is especially important in the determination of EC_A values, where the production of ADP (from the hydrolysis of ADP sugars) or AMP (from the breakdown of NAD or nucleic acids) may yield falsely low EC_A values. (iii) Internal standards should always be used to correct for losses (or apparent losses) of nucleotides resulting from hydrolysis, adsorption, coprecipitation, organic and inorganic ionic interferences, turbidity, color quench, pH spectral shifts, and other factors. Internal standards have been added as aqueous ATP-salt solutions, bacterial cells, and radiolabeled (^{14}C, ^{3}H, ^{32}P) ATP. It should be emphasized that the function of the internal standard is not to assess the efficiency of a given extraction medium, but rather to aid in evaluating the "apparent" and "real" losses resulting from various sources of analytical interference. Therefore, the most accurate method of introducing the internal standard is through the use of aqueous ATP solutions. Transient fluctuations in cellular ATP levels with environmental perturbations render the use of live microbial cells inadequate as a primary internal standard. Radioisotopic tracer tech-

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TABLE 1. *Spectrum of methods available for extraction of cellular nucleotides*[a]

Menstruum	Conditions of extraction	Reference
Aqueous buffers		
TRIS	pH 7.75, 20 mM, 100°C	146
TRIS-EDTA	pH 7.75, 20 mM-2 mM, 100°C	228, 254
TRIS-arsenate-EDTA-butanol	pH 7.4, 100 mM-10 mM-10 mM-6%, 100°C	50, 228
Glycine-EDTA	pH 10, 10 mM-5 mM, 100°C	321
Sodium bicarbonate	pH 8.5, 100 mM, 100°C	24
Phosphate (Sorensen)	pH 7.7, 65 mM, 100°C	40
Phosphate-citrate (McIlvaine)	pH 7.7, 40 mM-20 mM, 100°C	40
HEPES	pH 7.5, 25 mM, 100°C	65
Water	pH 5 to 8, 55 M, 100°C	19
Inorganic acids		
Nitric	0.1 N, 20°C	258
Perchloric	0.4 M, 0°C	19
Perchloric-EDTA	2.3 M-67 mM, 0°C	228
Phosphoric-EDTA	1 M-48 mM, 4°C	176
Sulfuric-charcoal adsorption	0.6 N, 4°C	135
Sulfuric-EDTA	0.6 N-48 mM, 4°C	182
Sulfuric-ion exchange	0.6 N-Na^+ resin, 4°C	210
Sulfuric-ion exchange-ethanolamine	0.6 N-Na^+ resin-5 N, 25°C	88
Triton X-100-phosphoric-sulfuric	1.2% (vol/vol)-0.75 M-0.075 M	Christensen and Devol[b]
Sulfuric-oxalic	1 to 2 N-62 mM, 4°C	65
Organic acids		
Acetic	1 M, freeze-thaw	244
Formic	pH 3, 2 M, 0°C	19
Formic-EDTA	0.46 M-2 mM, 0°C	192
Trichloroacetic	5 to 10% (wt/vol), 0°C	19
Trichloroacetic-EDTA	0.5 M-17 mM, 0°C	228
Trichloroacetic-phosphate-paraquat	0.5 M-0.25 M-0.1 M, 4°C	161
Organic solvents		
Acetone	90% (vol/vol)	58
Acetone-trichloroacetic acid-ether	90%-10%-100%	58
N-Bromosuccinimide-EDTA	10 mM-10 mM, 25°C	230
Formamide	10%, 25°C	196
Dichloromethane	90%, 25°C	196
Methanol	100%, 25°C	196
Butanol-EDTA	25% (vol/vol)-15 mM, 20°C	62
Butanol-octanol	50 to 90% (vol/vol)-100%	83, 284
Chloroform	23% (vol/vol), 98°C	78
Chloroform-EDTA	100%-100 mM, 20°C	228
Chloroform phosphate	pH 11.7, 23% (vol/vol)-10 mM, 25°C	321
Dimethyl sulfoxide	90% in 50 mM TRIS	210
Dimethyl sulfoxide-sulfuric acid	90% in 0.1 N H_2SO_4	210
Ethanol-EDTA	pH 7, 96% (vol/vol)-4 mM, 78°C	300, 228
Inorganic bases		
Potassium hydroxide-EDTA	10 mM-2 mM, 100°C	228

[a] Abbreviations: TRIS, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

[b] R. Christensen and A. Devol, submitted for publication.

niques do not allow for a reliable assessment of all possible sources of interference, since the instrumentations and methodologies used for detecting the labeled ATP and the total ATP extracted from the cells are not the same. The theory and application of internal ATP standards have been thoroughly reviewed by Strehler (304) and will not be further discussed in this review.

Measurement. Presently, there are a variety of methods available for the quantitative determination of cellular nucleotides. ATP has been the most frequently measured nucleotide because of its central role in cellular energetics and

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TABLE 2. *Summary of comparative studies to optimize ATP extraction from microbial cultures and environmental samples*

Sample material	Methods[a]		Comments	Reference
	Tested	Recommended		
Activated sludge	TRIS Gly-EDTA $HClO_4$ Acetone DMSO $CHCl_3$-Gly $CHCl_3$-PO_4	Gly-EDTA	Selected because it resulted in complete extraction and no hydrolytic loss of ATP	321
Activated sludge	BuOH $HClO_4$ TCA TRIS	TRIS	Selected on the basis of simplicity, reproducibility, and ATP yield	219
Algal cultures	Acetone-TCA-E Acetone TRIS	Acetone-TCA-E	Acetone gave higher ATP yields than did TRIS	58
Algal cultures	H_2O TRIS Gly-EDTA EtOH TCA $HClO_4$	TCA or $HClO_4$	$HClO_4$ and TCA yielded the highest ATP/ADP and EC_A ratios; however, the variability between methods was species dependent	205
Algal cultures and natural water samples	DMSO TRIS BuOH-OcOH	DMSO	All three techniques were equivalent with regard to ATP yield, but DMSO was selected due to convenience	286
Aufwuchs communities	TRIS Acetone Acetone-TCA-E	Acetone-TCA-E	Gave most consistent extraction efficiencies; ATP yields were not significantly different between TRIS and acetone-TCA-E	58
Bacterial cultures	TRIS-EDTA KOH TRIS-arsenate-EDTA-BuOH EtOH BuOH $CHCl_3$ HCOOH H_2SO_4 TCA $HClO_4$	TCA	Evaluation based upon ATP and total adenylate yield and EC_A; however, TCA was not universally acceptable for all species	228
Beach sand	TRIS $NaHCO_3$ H_2SO_4-EDTA	H_2SO_4-EDTA	Evaluation based upon a comparison of ATP, total adenylates, and EC_A	177
Copepods (2 species)	TRIS H_2SO_4-EDTA	H_2SO_4-EDTA	Evaluation based on a comparison of ATP, total adenylates, and EC_A	177
Escherichia coli cultures	$HClO_4$ TCA HCOOH CH_3COOH H_2O	$HClO_4$	"Weaker" organic acids require longer periods for extraction and result in lowered ATP yields	19
Escherichia coli cultures	CH_3COOH $HClO_4$ TCA	CH_3COOH	Highest yield of NTPs	244

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TABLE 2. *Continued*

Sample material	Methods[a]		Comments	Reference
	Tested	Recommended		
Lake sediments	HEPES $NaHCO_3$ H_2SO_4-IE H_2SO_4-EDTA H_2SO_4-Ox	H_2SO_4-Ox	Oxalate precipitate removed interfering Ca^{2+} and humic substances and yielded a lower coefficient of variation than did other methods; H_2SO_4-IE, however, resulted in higher ATP yields	65
Lake sediments	TRIS *N*-bromo DMSO DMSO-acid $HClO_4$ H_2SO_4-IE	H_2SO_4-IE	Selected on the basis of recovery of added ATP; ATP recovery not related to $CaCO_3$, Fe_2O_3, organic C, clay, or P-sorption potential of the sediment	210
Lake sediments	H_2SO_4-IE H_2SO_4-CA $CHCl_3$-PO_4	$CHCl_3$-PO_4	Provided improved ATP recovery and long-term stability of extracted ATP and resulted in minimal interference with enzymatic reaction over other methods tested	321
Marine and freshwater sediments	McIlvaine H_2SO_4-EDTA H_3PO_4-EDTA	H_3PO_4-EDTA	Selected on the basis of ability to repress residual alkaline phosphatase activity which is commonly encountered in environmental nucleotide extracts and on a comparative evaluation of ATP and A_T yields, EC_A, and GTP/ATP ratios	176
Marine sediments	TRIS $NaHCO_3$ BuOH-OcOH $HClO_4$ H_2SO_4	$NaHCO_3$	H_2SO_4 gave the highest ATP yield, but a high coefficient of variation	24
Marine sediments	TRIS $NaHCO_3$ McIlvaine Sorensen	McIlvaine	Gave high recoveries of ATP from sediments and seawater; extraction at mildly alkaline pH also avoids problems with high cation concentrations in extracts	40
Marine sediments	TRIS H_2SO_4-IE H_2SO_4-CA	H_2SO_4-CA	Efficiency of ATP recovery was superior with CA purification after H_2SO_4 extraction because ionic interference with the luciferase reaction or ATP precipitation upon neutralization of acid extracts was avoided	135
Marine sediments	H_2SO_4 Triton X-100–H_3PO_4–H_2SO_4	Triton X-100–H_3PO_4–H_2SO_4	Triton X-100–H_3PO_4–H_2SO_4 resulted in higher uncorrected yield of ATP but after internal standard corrections, there were no statistical differences in ATP yield	Christensen and Devol[b]
Metazoa	TRIS Injection Sonication Homogenization H_2SO_4-EDTA Injection Sonication Homogenization	H_2SO_4-EDTA, homogenization	Evaluation based on comparison of ATP, total adenylates, and EC_A	177

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TABLE 2. *Continued*

Sample material	Methods[a]		Comments	Reference
	Tested	Recommended		
Rumen fluids	TRIS $CHCl_3$ $NaHCO_3$-$CHCl_3$ H_2SO_4	H_2SO_4	H_2SO_4 was the most efficient and was better suited for extracting particulate samples	104
Seawater and bacterial-algal cultures	HNO_3 TRIS		HNO_3 best for bacteria, TRIS best for algae; the two techniques are comparable for most seawaters	Deming[c]
Soil	TCA H_2SO_4-IE-E DMSO $HClO_4$ TRIS	H_2SO_4-IE-E	TRIS ATP was less than 3% of the H_2SO_4-IE-E ATP yield	88
Soil	HCOOH *N*-bromo TRIS BuOH-OcOH	BuOH-OcOH	Selected on basis of highest ATP yield	62
Soil	$HClO_4$ TRIS DMSO-H_2SO_4 DMSO *N*-bromo H_2SO_4 BuOH	H_2SO_4	BuOH and H_2SO_4 both yielded the highest extraction efficiency, but H_2SO_4 is routinely used because it is more rapid and convenient for processing large numbers of samples	13
Soil	H_2SO_4 TCA TCA-PO_4 TCA-PO_4-paraquat	TCA-PO_4-paraquat	Paraquat binds to clay and competes with ATP adsorption sites	161
Soil	H_2SO_4 $NaHCO_3$ $NaHCO_3$-$CHCl_3$	$NaHCO_3$-$CHCl_3$	$CHCl_3$ prevented adsorption of ATP to sediment particles	255
Spiroplasma citri cultures	TRIS $HClO_4$ TCA	TCA	TRIS yielded greater ATP values but lower EC_A than the acidic procedures	278
Urinary tract pathogens	$CHCl_3$ MeOH EtOH DMSO Formamide BuOH CH_2Cl_2 Acetone $HClO_4$ HNO_3	Acetone or HNO_3	Acetone and HNO_3 were equivalent with regard to ATP yield	196

[a] See Table 1. Abbreviations: TRIS, tris(hydroxymethyl)aminomethane hydrochloride; Gly, glycine; EDTA, ethylenediaminetetraacetic acid; BuOH, butanol; DMSO, dimethyl sulfoxide; TCA, trichloroacetic acid; OcOH, octanol; E, ether; EtOH, ethanol; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; IE, ion exchange; OX, oxalic acid; CA, charcoal adsorption; *N*-bromo, *N*-bromosuccinimide; MeOH, methanol.

[b] R. Christensen and A. Devol, submitted for publication.

[c] J. Deming, unpublished data.

relatively high intracellular concentrations (compared with the less abundant cellular nucleotides). ATP can be separated and detected by various chromatographic, radioisotopic, and enzymatic techniques; however, the firefly bioluminescence method is the most rapid, sensitive, and reproducible assay. As a result of these attributes, the firefly bioluminescence assay for

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ATP has become the most widely accepted method for ecological studies.

(i) Firefly bioluminescence. The light emission reaction catalyzed by firefly luciferase (EC 2.7.—),

$$\text{ATP} + \text{reduced luciferin} + O_2 \xrightarrow{\text{firefly luciferase}} \text{AMP} + \text{inorganic pyrophosphate} + CO_2 + \text{product} + \text{light}$$

has been studied extensively since the pioneering work by McElroy in 1947 (238). Excellent reviews describing the purification of firefly luciferase and its physical and chemical properties and reaction mechanism have been published recently (71, 74).

Although the essence of the firefly bioluminescence assay of ATP has remained unchanged since its first description by Strehler and Totter (305), several minor modifications have been described for specific applications. Of particular interest are the preparation, handling, and use of the luciferin-luciferase reagents. In general, the enzyme preparations can be divided into two categories: crude and "purified." The term *purified* as used in this context does not necessarily denote a crystalline enzyme preparation. Purified reagents can be prepared by ammonium sulfate precipitation and adsorption onto calcium phosphate gels (116), Sephadex G-100 gel chromatography (268), diethylaminoethyl-Sephadex chromatography (29), or isoelectric focusing (227), or they may be purchased commercially from several sources. Purified reagents are generally more sensitive and specific, but are much more expensive to use. Increased sensitivity in the ATP bioluminescence assay is of considerable importance in many environmental applications (165, 178); however, the choice of reagents is generally dictated by the specific application. Commercially available crude luciferase reagents have been used most extensively in ecological studies (>90% of the published data). Additional analytical considerations with respect to ATP measurements, including (i) the relative merits of peak height versus integrated light measurements (169, 174), (ii) luciferase enzyme reaction specificity (71, 72) and kinetics (73), (iii) computer-assisted data reduction methods (31, 91, 188, 273), (iv) automated methods of ATP analysis (122, 162, 259), and (v) general limitations and trouble-shooting of the method (148, 181), have been discussed elsewhere as referenced and will not be considered in this review.

In addition to direct measurements of ATP, the firefly luciferin-luciferase bioluminescence assay can also be used to quantify additional cellular nucleotides which can be enzymatically coupled to the production or hydrolysis of ATP. Many other intracellular metabolites and enzyme activities which are unique to, or specifically associated with, nucleotide metabolism may also be assayed with firefly luciferase. ADP and AMP are frequently measured after conversion to equimolar concentrations of ATP (48, 180, 264). Recently a method has been described for the quantitative determination of guanosine nucleotides (guanosine 5′-monophosphate, guanosine 5′-diphosphate, and GTP) by the firefly reaction (170, 172). This technique is based upon selective enzymatic hydrolysis of ATP and uridine 5′-triphosphate in cell extracts, followed by quantitative determination of GTP based on ATP-generated light emission resulting from a nucleosidediphosphate kinase-firefly luciferase coupled reaction i.e.,

$$\text{GTP} \xrightarrow{\text{nucleosidediphosphate kinase}} \text{ATP} \xrightarrow{\text{firefly luciferase}} \text{light}$$

Guanosine 5′-diphosphate and guanosine 5′-monophosphate may also be coupled to GTP production, and hence to firefly luciferase light emission, by using enzyme reactions analogous to those described previously for the coupled phosphorylation of ADP and AMP (180). This GTP assay procedure may also be used for measuring uridine 5′-triphosphate concentrations in mixed nucleotide extracts or for cytidine 5′-triphosphate, inosine 5′-triphosphate, and thymidine 5′-triphosphate analyses in chromatographically purified fractions, although it was not specifically developed for these applications. An example of this latter application has been described by Manandhar and Van Dyke (232). Cyclic mononucleotides (cAMP and cyclic guanosine 3′,5′-monophosphate) and the activities of cyclic nucleotide phosphodiesterases have been measured by using appropriate firefly luciferin-luciferase coupled assay systems (98). Finally, recent work in our laboratory has indicated that the firefly bioluminescence reaction may also be used to assay the concentration of the sulfur nucleotide adenosine 5′-phosphosulfate in sulfate adenyltransferase-firefly luciferase coupled reaction (D. B. Craven and D. M. Karl, unpublished data).

(ii) Bacterial bioluminescence. Bacterial bioluminescence also provides a unique system for the quantitative determination of specific metabolites and enzyme activities that are cou-

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pled to these metabolites. Bacterial luciferase requires reduced flavin mononucleotide (reduced FMN) in addition to several other reactants. Under suitable reaction conditions, the rate of light emission is proportional to the concentration of reduced FMN (299). Most commercial and laboratory preparations of bacterial luciferase also contain NADH:FMN oxidoreductase (dehydrogenase) activity, in addition to luciferase, thereby extending the versatility of the assay to include quantitative determinations of NADH, NADPH, and FMN (299). The specific procedures for sample and enzyme preparations, detection of light emission, and general analytical considerations have been described by DeLuca (74). Chappelle and Picciolo (51) have also devised a technique for the determination of flavin adenine dinucleotide which requires hydrolysis to FMN before the bacterial bioluminescence reaction.

(iii) *Renilla* bioluminescence. The discovery that 3',5'-diphosphoadenosine is required for light emission in the bioluminescence system of the anthozoan *Renilla reniformis* has led to the development of a sensitive and specific quantitative assay for 3',5'-diphosphoadenosine and adenosine 3'-phosphate-5'-phosphosulfate. The description and application of these methods have been published (5).

(iv) Immobilized enzymes. In recent years, the technology required for the immobilization of soluble enzymes and intact viable bacterial cells onto solid supports has advanced tremendously. The immobilization of an enzyme generally results in an increased level of stability and yields the advantage of recycling while retaining its inherent substrate specificity, sensitivity, reaction kinetics, and general usefulness as an analytical tool (158). Methods for the immobilization of firefly and bacterial luciferases onto arylamine-glass beads (which can then be cemented onto glass rods) have been devised and applied to detect low concentrations of ATP, NADH, and NADPH (119, 157, 211). The new immobilized systems offer a rapid and inexpensive biochemical assay technique and will undoubtedly be selected for future ecological applications.

NUCLEOTIDE LEVELS IN MICROBIAL CELLS: EFFECTS OF GROWTH RATE, CULTURE CONDITIONS, NUTRIENT STRESS, AND ENVIRONMENTAL PERTURBATIONS

Adenine Nucleotides and Energy Charge Ratios

Within the past two decades numerous investigators have attempted to define pool sizes, turnover rates, and concentration ratios of cellular nucleotides (and related compounds) in microorganisms. The overwhelming majority of the published data have emphasized ATP concentrations (in terms of nanomoles of ATP per milligram [dry weight] of protein or cell N) and adenine nucleotide concentration ratios (i.e., ATP/ADP, ATP/AMP, or EC_A) in bacteria and, to a lesser extent, in eucaryotes. A discussion of the occurrence and probable significance of cAMP, cyclic guanosine 3',5'-monophosphate, guanosine tetraphosphate, and other less abundant nucleotides in microbial cells is beyond the scope of this review; however, excellent current summaries of the distribution of guanosine tetraphsphate (292), cyclic guanosine 3',5'-monophosphate (113), and cAMP (270) and their involvement in metabolic regulation in microbial cells have appeared recently.

Although intracellular concentrations of NTPs are often referred to as "pools," it is essential to bear in mind that the rapid and continuous turnover of these molecules (at least several times per second for ATP in bacteria) precludes the existence of a true energy reserve. Therefore, static measurements of ATP pools and adenine nucleotide ratios are by themselves insufficient to fully characterize the rate of cellular metabolism. Additional kinetic parameters of catabolism or growth must be used to corroborate ATP pool measurements before an assessment of total cellular energy flux or an evaluation of the efficiency of metabolic energy coupling can be made.

Procaryotes. Table 3 presents some representative ATP levels, EC_A values, and carbon-to-ATP (wt/wt) ratios in growing bacteria. Previous reviews, by Chapman and Atkinson (47), Knowles (194), Chapman et al. (48), and Weber (327), have compiled similar data for ATP and EC_A in microorganisms and higher plant and animal tissues. The C/ATP ratios will be discussed subsequently in this review.

The similarity in the values reported for intracellular ATP pools and adenine nucleotide ratios for various bacterial species growing under different culture conditions is noteworthy (Table 3) in light of numerous potential difficulties with ATP analysis and interlaboratory differences in experimental protocol. In general, the ATP pool in exponentially growing cells appears to be regulated around a mean of 2.0 to 6.0 nmol of ATP per mg (dry weight) of cells, regardless of the mode of nutrition. Although the ATP pool appears to be constant during logarithmic growth, the absolute value attained is dependent somewhat on the specific composition of the culture

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TABLE 3. *Representative ATP levels, EC_A values, and C/ATP ratios in exponentially growing procaryotic microorganisms*

Species	Medium, growth conditions	ATP (nmol/mg of dry wt)[a]	EC_A	C/ATP[a]	Reference
Acetobacter aceti	Ethanol-mineral salts, aerobic, batch	7.6	0.87	130[b]	18
Anacystis nidulans	Inorganic salts, constant light, 30°C, aerobic, batch	2.9	0.97	340[b]	205
Arthrobacter crystallopoietes	Glucose-mineral salts, aerobic, batch (sphere morphology)	3.8–6.7	0.95	149–259[b]	215
	Glucose-mineral salts-casein, aerobic, batch (rod morphology)	2.2–5.6		179–448[b]	216
Azotobacter vinelandii	Mannitol-mineral salts (N-free)				
	Aerobic, batch	4.8–6.2		159–205[b]	125
	Anaerobic, batch	2.0		493[b]	
Bacillus brevis	Aerobic, batch	7.7	0.87	128[b]	199
Bacillus cereus	Nutrient broth, aerobic, batch	3.3	0.89	299[b]	228
Bacillus sp.	Nutrient broth, aerobic, batch	2.9–9.4 ($\bar{x}$ = 5.7)		105–340[b] ($\bar{x}$ = 173)	219
Beneckea harveyii	Glycerol-mineral salts, aerobic, batch	13–16	0.86	62–76[b]	186
Beneckea natriegens	Succinate-mineral salts, aerobic, batch	7.0	0.90	141	246
	Glucose-mineral salts, aerobic, batch	6.0	0.90	165	
Chromatium strain D	High light (2.4 mW/cm^2)	4.2–7.8	0.76	127–235	241
	Medium light (0.4 mW/cm^2)	4.9–5.0	0.82	197–201	
	Low light (0.2 mW/cm^2)	3.3–4.0	0.83	247–299	
Citrobacter freundii	Chemostat cultures				
	Galactose-limited	5.1–7.2	0.91	137–193[b]	81
	N-limited (galactose)	5.5–7.0	0.96	141–179[b]	
	Glucose-limited	5.0–8.5	0.87	116–197[b]	
	N-limited (glucose)	5.2–6.7	0.90	147–190[b]	
Clostridium acetobutylicum	Defined, anaerobic, batch	3.7		267[b]	248
Clostridium kluyveri	Ethanol-acetate-bicarbonate, anaerobic	5.48	0.79	180[b]	70
	Crotonate-bicarbonate, anaerobic	7.01	0.76	141[b]	
Escherichia coli	Glucose-mineral salts, aerobic, batch	3.5–7.0	0.85	145–281[b]	48
Escherichia coli	Glucose-mineral salts, aerobic, batch	4.0	0.76	246[b]	79

medium and on coupling conditions of growth (61, 101, 102, 207; Table 3).

Studies on the effect of growth rate on the intracellular ATP pools have produced conflicting results. Several investigators have claimed that the ATP concentration in bacteria (expressed as nanomoles of ATP per milligram of dry weight) is independent of the cellular growth rate (101, 107, 133, 195, 296), whereas others have indicated a positive correlation between

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TABLE 3. *Continued*

Species	Medium, growth conditions	ATP (nmol/mg of dry wt)[a]	EC_A	C/ATP[a]	Reference
Escherichia coli	Acetate-mineral salts, aerobic, batch	4.1	0.77	235[b]	226
	Glucose-mineral salts, aerobic, batch	6.1	0.85	162[b]	
Escherichia coli	Glucose-mineral salts, aerobic, batch	3.26 (±9.5%)		303[b]	235
	Succinate-mineral salts, aerobic, batch	3.82 (±9%)		258[b]	
Escherichia coli	Nutrient broth, aerobic, batch	2.6–3.8 ($\bar{x}$ = 3.2)		260–379[b] ($\bar{x}$ = 308)	219
Klebsiella aerogenes (*K. pneumoniae*)	Glucose-limited, dissolved O_2 tension (mmHg[c]) of:				
	420	8.3		119[b]	126
	220	6.6		149[b]	
	57	5.9		167[b]	
	5.3	6.1		162[b]	
	3.0	6.5		152[b]	
	<0.2	3.7		267[b]	
	0	3.7		267[b]	
Klebsiella pneumoniae	Nutrient broth, aerobic, batch	2.6	0.80	379[b]	228
Photobacterium fischeri	Glycerol-complete, aerobic, batch				
	Bright strain (MJ-1)	13.8	0.87	72[b]	186
	Inducer (−) mutant (B-61)	13.7		72[b]	
	B-61 plus inducer	12.9		76[b]	
Pseudomonas aeruginosa	Nutrient broth, aerobic, batch	3.6	0.97	274[b]	228
Rhodospirillum rubrum	Light-saturated, batch	1.8–2.5		394–547[b]	331
Selenomonas ruminantium	C-limited, continuous culture	2.3		448[b]	133
Staphylococcus aureus	Nutrient broth, aerobic, batch	6.9	0.92	143[b]	228
Zymomonas mobilis	Anaerobic, batch, 30°C				
	Complex	3.04 (±10%)		324[b]	207
	Defined	3.06 (±14%)		322[b]	
	Minimal	7.0 (± 5%)		141[b]	
	Defined-limiting panothenate	6.2 (±13%)		159[b]	
	Minimal-limiting panothenate	8.9 (± 5%)		111[b]	
Five species of bacteria	Not given	1.97 (±10%)		450–500	13
Seven species of marine bacteria	Continuous culture	3.0–10.8[b] ($\bar{x}$ = 6.9)		91–333 ($\bar{x}$ = 143)	121
Three species of marine bacteria	Continuous culture	1.9–3.9		250–510[b]	146

ATP pools and rate of growth (20, 245). Recently, Dolezal and Kaprálek (81) reported that the ATP pool in *Citrobacter freundii* was independent of growth rate up to a critical value of ≥0.6 of the maximum growth rate (μ_{max}), but the ATP concentration increased by ~50% near μ_{max}.

Although the precise molar ATP concentra-

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TABLE 3. *Continued*

Species	Medium, growth conditions	ATP (nmol/mg of dry wt)[a]	EC_A	C/ATP[a]	Reference
Six species of actinomycetes	Not given	4.54[b]		217 (±13%)	13
Nine species of rumen bacteria	Batch, exponential	2.2–34.7 ($\bar{x}$ = 9.7)		28–448[b] ($\bar{x}$ = 102)	104

[a] Percentages in parentheses represent coefficients of variation.
[b] Assumed: organic carbon = 0.50 × dry weight (229).
[c] 1 mmHg = 133.3 Pa.

tion in microbial cells is difficult to determine due to technical problems associated with estimating intracellular volumes, most reported values are within the range of 1 to 5 mM (18, 22, 79, 295). It appears that the intracellular concentrations of adenine nucleotides are probably at or near saturating levels for the activities of most respiratory and metabolic enzymes.

Under steady-state conditions of growth, there is a tightly regulated balance between ATP utilization and regeneration. Consequently, the rate of ATP turnover should be positively correlated with growth rate (i.e., ATP utilization), assuming a constant Y_{ATP}. As the growth rate decreases, however, the proportion of ATP required for maintenance increases, and the expected relationship between ATP turnover rate and growth rate deviates from experimental values. By comparison, the turnover rate of the total adenine nucleotide pool is positively correlated with growth, at both fast and slow rates (47), and this pool appears to "turn over" (i.e., is used up in biosynthesis of macromolecules and is regenerated) approximately 30 to 50 times per generation (data reviewed in reference 47).

The EC_A in growing procaryotic cells appears to be regulated within the well-defined limits of the EC_A theory. Figure 1 serves to emphasize that many of the data collected prior to 1970 are probably unreliable due to improper considerations of turnover times and environmental perturbations. The EC_A values of microbial cells have not increased since 1948, but rather the accuracy and precision of the methods used to measure cellular adenine nucleotides undoubtedly have improved. Most chemostat studies have revealed that there is no correlation between EC_A and growth rate (81, 311; D. M. Karl, unpublished data), although certain exceptions have been reported (131; K. P. Ho and V. Munk, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, O33, p. 197). Nevertheless, both in vitro and in vivo studies have indicated that ATP-requiring reactions are curtailed at low EC_A values (i.e., $EC_A \leq 0.8$). Therefore, a positive correlation

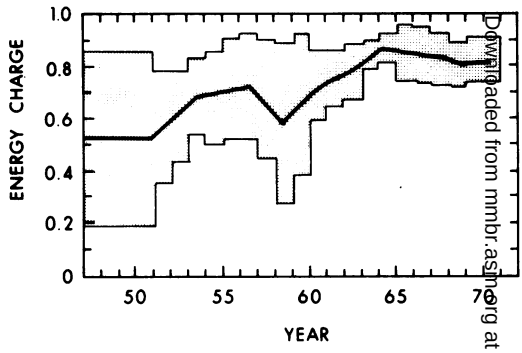


FIG. 1. *EC_A (energy charge) values tabulated by Chapman et al. (48), plotted against the years in which the analyses were reported. The curve drawn is a 3-year moving average of EC_A values. The shaded portion covers one apparent standard deviation on each side of the line. These data emphasize the fact that only recently have proper considerations been given to the analytical procedures required for the extraction and analysis of cellular nucleotides. From Atkinson (10).*

must exist between the growth potential, or functional capacity, of a cell and its EC_A.

Based on a computer simulation model constructed from phosphofructokinase activity data, Goldbeter (114) has proposed that the cellular EC_A is maintained at a constant high value (>0.9) for most of the time, but periodically (about every 200 s) exhibits a brief drop to a value of 0.5. This oscillatory behavior would allow for a greater flexibility in cellular needs by intermittently activating the energy-yielding processes to their maximum potential rates (114). It is unlikely that these EC_A transients can be detected b our current analytical methods, so we must be satisfied with time-weighted average values of cellular EC_A. Several thorough discussions of metabolic regulation by EC_A control have been published (10, 47, 48, 125, 194).

Unicellular eucaryotes. Table 4 summarizes selected data from the literature of intracellular ATP levels, EC_A values, and C/ATP

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ratios for a variety of unicellular eucaryotes. During exponential growth the mean range in ATP concentration is between 2.0 to 6.0 nmol of ATP per mg (dry weight) of cells. These intracellular concentrations are essentially indistinguishable from the levels presented above (Table 3) for procaryotic microorganisms. This suggests the occurrence of a similar, or perhaps common, physiological driving force behind the maintenance of these steady-state ATP pools.

Sakshaug (279) and B. L. Hunter (M.S. thesis, University of Hawaii, Honolulu, 1979) have independently reported a positive and significant correlation between cellular ATP levels (expressed as ATP per cell carbon) and growth rate for two species of marine diatoms under certain culture conditions (Table 4). Nevertheless, substantial interclass and interspecies differences were evident, with greater variations occurring at high growth rates (Hunter, M.S. thesis). Furthermore, the specific limiting nutrient was important in determining a correlation between growth rate and ATP concentrations. In the case of *Thalassiosira fluviatilis*, cellular ATP and growth rate were positively correlated during NO_3- and PO_4-limited chemostat growth conditions, but ATP was independent of growth rate in NH_4- and light-limited cultures (Hunter, M.S. thesis).

Even though the EC_A concept has been extended to considerations of metabolic regulation in eucaryotes, the theoretical assumptions are not as easily satisfied in eucaryotes as they are in the structurally simpler procaryotes. The theory specifies that adenylate kinase be present at all times and that it responds rapidly to disequilibrium conditions. Noda (247) has pointed out that adenylate kinase is notably absent from within the cellular mitochondrial complex. This requires that the adenine nucleotide translocase reaction be rapid and immediate so as to minimize differences between the intramitochondrial EC_A and the EC_A of the cytosol. Klingenberg (191) has indicated that the translocase reaction is specific for ATP and ADP only and that AMP is not affected. There also appears to be a rapid enzymatic transfer of ATP and ADP in plants from the chloroplast to the cytoplasm which is catalyzed by the phosphoglyceric acid-dihydroxyacetone phosphate shuttle (128). The AMP pools of the mitochondria, chloroplasts, and cytoplasm, however, have been shown to be distinct (191, 282). Nevertheless, it appears that the spatially averaged EC_A values in exponentially growing unicellular microorganisms fit within the prediction of the EC_A theory. Several notable exceptions have been reported, especially the extended viability of yeast and algal cells at EC_A values as low as 0.1 (22, 222). These differences may be due, at least in part, to the existence of separate adenine nucleotide pools within the highly compartmentalized eucaryotic cells.

Metazoa. Table 5 presents the few available data for ATP levels in microbial metazoans. On the average, the steady-state ATP pools are greater than those reported in unicellular microorganisms. The correct explanation for this difference is unknown. Karl et al. (177) have suggested that it may be related to differences in the distributions of structural versus living carbon or to apparent differences in the ATP/non-adenine NTP concentration ratios for these two heterogeneous groups of organisms. The presumably slower turnover rate (i.e., "metabolic response time") of the ATP pools of metazoa may also contribute to a general requirement for elevated steady-state pool levels.

The "whole body" EC_A ratios of metazoans also appear to fit into the framework of the EC_A theory, although it is difficult to rationalize these results. The conceptual problems of barrier compartmentation are magnified by the presence of individual tissues and organs. Significant differences undoubtedly exist between different portions of a multicellular organism as a result of specialized functions of individual groups of cells. Intertissue differences in EC_A have been shown to be significant for different types of muscle in both invertebrates and vertebrates (27). Therefore, it may be more meaningful to isolate a specific tissue or organ and to monitor changes in its ATP levels and EC_A values. This approach has been used for the analysis of tissues from higher plants, macroscopic invertebrates, and mammals, but difficulties in excising tissue or organ samples have precluded its application to the microbial metazoans.

Environmental Perturbations and Nutrient Limitations

Transient states. It is apparent that the steady-state ATP pools in exponentially growing unicellular microorganisms (procaryotes and unicellular eucaryotes) are poised at a level of approximately 2 to 6 nmol of ATP per mg (dry weight) of cell material (Tables 3 and 4). Transient states can be induced by rapid fluctuations in environmental conditions and by nutrient limitation or starvation. These transient decreases in ATP are generally accompanied by decreases in EC_A, and by a decrease in the total adenine nucleotide pool (i.e., removal of AMP) if the stress is severe. This latter effect is interpreted as a "strategy" used by the cells to maintain a high EC_A (10, 47). If the cells are maintained

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TABLE 4. *ATP levels, EC_A values, and C/ATP ratios in representative unicellular eucaryotes*

Species	Medium, growth conditions	ATP (nmol/mg of dry wt)	EC_A	C/ATP[a]	Reference
Algae					
Amphiprora paludosa	NO_3-limited, chemostat; mean for growth rates, 0.048–0.0066/h	2.76[b]		357 (±24%)	Hunter[c]
Cachonina niei	NH_4-grown, deep tank	4.44[b]		222	307
	NO_3-grown, deep tank	2.86[b]		345	
	Nutrient starved, deep tank	2.36[b]		417	
Chlorella fusca	Turbidostat culture	2.0		493[b]	220
Ditylum brightwellii	NH_4-grown, deep tank	4.83[b]		204	307
	NO_3-grown, deep tank	5.33[b]		185	
Dunaliella marina	N-deficient, batch	1.81[b]	0.64	545	274
	P-deficient, batch	2.86[b]	0.66	345	
Dunaliella tertiolecta	NO_3-limited, chemostat; mean for growth rates, 0.035–0.005/h	4.63[b]		213 (±20%)	Hunter[c]
Gonyaulax polyedra	NH_4-grown, deep tank	2.96[b]		333	307
Gonyaulax tamarensis	N-deficient, batch	2.02[b]	0.74	489	274
	P-deficient, batch	2.21[b]	0.83	447	
Pavlova lutheri	N-deficient, batch	3.08[b]		320	280
	P-deficient, batch	1.01[b]		1,000	
	Fe-deficient, batch	0.55[b]		1,800	
Scenedesmus quadricauda	Exponential growth	1.45		666	127
Skeletonema costatum	N/P ratio of medium				
	1.2 (N-deficient), batch	10.4 [b]		95	280
	20 (Fe-deficient), batch	3.29[b]		300	
	310 (P-deficient), batch	3.94[b]		250	
	830 (P-deficient), batch	2.01[b]		490	
Skeletonema costatum	Maximum batch	10.3 [b]		96	279
	Dialysis culture in Narragansett Bay	4.29[b]		230	
Skeletonema costatum	% of maximum growth rate:				
	95	3.94[b]		250	279
	80	1.97[b]		500	
	40	1.31[b]		750	
	20	1.0[b]		1,000	
	10	0.66[b]		1,500	
	5	0.5[b]		2,000	
Thalassiosira fluviatilis	NH_4-limited, chemostat; mean for growth rates, 0.039–0.007/h	3.15[b]		313 (±10%)	Hunter[c]
	P-limited, chemostat; mean for growth rates, 0.047–0.007/h	2.17[b]		454 (±75%)	
	Light-limited, chemostat; mean for growth rates, 0.037–0.0022/h	5.22[b]		189 (±14%)	

under these new environmental conditions and an energy source is available for ATP regeneration, the original steady-state ATP levels are restored, even though the ATP turnover rates and rates of biosynthesis may be significantly altered. These results again suggest that there is

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TABLE 4. *Continued*

Species	Medium, growth conditions	ATP (nmol/mg of dry wt)	EC_A	C/ATP[a]	Reference
Thalassiosira pseudonana	N-limited, chemostat, growth rate of:				
	0.041/h	7.09[b]		139	256
	0.017/h	5.48[b]		180	
	0.0085/h	3.19[b]		309	
6 Species of algae	Exponential growth, batch	6.9[b]		143 (±11%)	13
30 Species of marine algae	Exponential growth, batch	3.45[b]		286 (±14%)	140
7 Species of marine algae	Exponential growth, batch	3.20[b]		308 (±45%)	Laborde[d]
8 Species of marine algae	Continuous light, batch	1.45[b]		678 (±85%)	146
14 Species of freshwater algae	Batch	3.15[b]		313 (±35%)	257
Fungi					
Candida utilis	N-limited, chemostat	4.3	0.85	229[b]	316
	S-limited, chemostat	4.8	0.85	205[b]	
Dictyostelium discoideum	Light, 20°C	5.4		183[b]	166
	Light, 4°C	8.1		122[b]	
	Dark, 20°C	7.9		125[b]	
	Dark, 4°C	14.4		68[b]	
Neurospora crassa	Exponential growth	6.5		152[b]	295
Saccharomyces cerevisiae		9.0		110[b]	329
Schizosaccharomyces pombe	Exponential growth	3.85	0.87	256[b]	263
8 Species of fungi	Exponential growth	4.23[b]		233 (±9.3)	13

[a] Values in parentheses represent coefficients of variation.
[b] Assumed: organic carbon = 0.50 × dry weight (229).
[c] B. L. Hunter, M.S. thesis, University of Hawaii, Honolulu, 1979.
[d] P. Laborde, Ph.D. thesis, Université d'Aix-Marseille, Marseilles, France.

an apparent optimum intracellular ATP pool required for exponential growth.

In addition to these short-lived and readily reversible transient effects on cellular ATP pool levels, more permanent and predictable changes in ATP and EC_A may also result from (i) excessive nutrient limitation and starvation; (ii) toxicity induced by heavy metals, organic dyes, or biocides; (iii) antibiotics, (iv) the addition of uncouplers of oxidative phosphorylation, and (v) specific cell-cycle-related events.

Nutrient limitation and starvation. (i) Carbon. The effects of nutrient limitation on the steady-state ATP pools depend upon the nature of the limiting substance and on the specific culture conditions. For example, if chemoorganotrophs are cultured under C-limited batch culture conditions, the intracellular ATP concentrations and EC_A values both decrease upon exhaustion of the limiting organic substrate (48). By comparison, cells grown in C-limited chemostats maintain normal ATP pools and high EC_A values (81; Karl, unpublished data). Clearly, the chemostat-grown cells are not nutrient limited in the same metabolic or physiological sense as are the batch cultures, since the cells continue to grow exponentially and their intracellular nucleotide fingerprints are comparable to those of nutrient saturated batch cultures. One serious problem in conducting starvation experiments in batch cultures is that there is no mechanism for assessing the contribution of dead or senescent cells to the total ATP, EC_A, organic carbon, or dry weight determinations. Continuous culture techniques, on the other hand, select for growing organisms and

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TABLE 5. *ATP levels, EC_A values, and C/ATP ratios in microbial metazoans*

Species	Growth stage, wt	ATP (nmol/mg of dry wt)	EC_A	C/ATP	Reference
Adoncholaimus thalassophygas (nematode)	2–14 μg	26.4[a]		75[a]	112
Anopolstoma viviparum (nematode)	2–14 μg	26.6[a]		74[a]	112
Calanus finmarchicus (copepod)	0 Days, starvation	8.56[b]		115	21
	23 Days, starvation	8.95[b]		110	
Calanus helgolandicus (copepod)	Not given	4.93[b]		200	141
Calanus sp. (copepod)	130 μg of carbon	23.0[b]	0.93	43–66	177
Labidocera sp. (copepod)	32 μg of carbon	37.2[b]	0.89	26–27	177
Nippostrongylus brasiliensis (nematode)	Juvenile		0.78–0.85		23
Eight species of zooplankton	Variable	18[b]	0.70–0.89	43	Skjoldal[c]
Copepod	Adult female	10.4[b]		95	45
	Copepodite	7.8[b]		126	
Marine nematodes					
Station 1	2.19 μg	6.33		150	291
Station 2	2.83 μg	11.6		85	

[a] Assumed: dry weight = 0.20 × wet weight; organic carbon = 0.10 × wet weight.
[b] Assumed: organic carbon = 0.50 dry weight.
[c] H. R. Skjoldal, unpublished data.

minimize the potential contribution from dead or dying cells.

(ii) Nitrogen, sulfur, and iron. Contrary to the effects observed with carbon (energy) starvation, depletion of N from the medium either has no effect on the ATP pools and EC_A values of bacterial cells or results in a slight increase in the values (79, 80, 246, 326). This increase accompanying cessation of growth is presumably due to a decrease in the ATP demand for biosynthesis coupled with simultaneous ATP production from the catabolism of endogenous and exogenous reserves.

Thomas and Dawson (316) have investigated the independent effects of N, S, and Fe limitations on cultures of the yeast *Candida utilis*. Their results indicated that cultures grown under either N- or S-limited conditions had similar ATP pools (4.3 versus 4.8 nmol of ATP per mg of dry weight) and identical EC_A values (~0.85); however, in cells cultured under Fe-limited conditions, the ATP pool was reduced by approximately 70% (1.5 nmol of ATP per mg of dry weight) and the EC_A decreased to ~0.6. A reduction in the number of cytochromes was offered as a possible explanation for these results (316). Despite this low ATP pool and EC_A, the cells were able to grow. This result is in apparent conflict with a strict interpretation of the EC_A theory.

(iii) Phosphorus. The nucleotide data regarding P starvation and P-dependent microbial growth are extremely variable, with many apparent interspecies and interlaboratory inconsistencies. A summary is presented in Table 6. With the exception of a few reports (32, 226; B. Hunter and E. Laws, submitted for publication) P limitation has been found to be accompanied by a significant decrease in the cellular ATP pool, ranging from ~50% of the control steady-state level (209, 256, 324) to an extreme of >90% (45, 139, 280).

When microorganisms are limited for inorganic phosphate the synthesis of alkaline phosphatase is derepressed (induced) and rapidly accumulates in the cell. Torriani (323) and Garen and Levinthal (110) indicated that under inorganic phosphate limitation this enzyme alone may constitute as much as 6% of the total soluble protein. Alkaline phosphatase is a relatively nonspecific orthophosphoric-monoester phosphohydrolase and catalyzes the hydrolysis of many different organic phosphate compounds, including adenine nucleotides (AMP,

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TABLE 6. *Effects of phosphate concentrations on the intracellular ATP concentrations of microorganisms*

Species	Medium, growth conditions	ATP (nmol/mg of dry wt)	EC_A	C/ATP	Reference
Azotobacter vinelandii	P-rich, 12 h, batch	1.7	0.89	580[a]	324
	P-deficient, 12 h, batch	0.5	0.68	1,972[a]	
Dunaliella tertiolecta	N-starved, 7 days, batch	6.9[a]		143	139
	P-starved, 7 days, batch	1.48[a]		667	
	P-starved, 10 days, batch	1.0[a]		1,000	
Escherichia coli	Aerobic, batch				
	C-starved	2.6	0.78	379[a]	226
	N-starved	2.0	0.75	493[a]	
	P-starved	2.9	0.67	340[a]	
	Exponential growth	6.1	0.85	162[a]	
Monochrysis lutheri	N-starved, 7 days, batch	1.18[a]		833	139
	P-starved, 7 days, batch	0.5[a]		2,000	
	P-starved, 13 days, batch	0.3[a]		3,333	
Pavlova lutheri	P-saturated, exponential growth	3.08[a]		320	280
	P-starvation, batch	0.1[a]		9,500	
Peridinium sp.	P-saturated, batch	3.3[a]		297	45
	P-deficient, batch	0.42[a]		2,374	
Scenedesmus quadricauda	P-saturated, exponential growth	1.5[a]		667	127
	P-deficient, batch	0.3[a]		3,333	
Selenastrum capricornutum	P-rich, 4 days, batch	3.4		290[a]	209
	Balanced, 4 days, batch	3.1		318[a]	
	P-deficient, 4 days, batch	1.4		704[a]	
Skeletonema costatum	P-saturated, exponential growth	3.94[a]		250	280
	P-starvation, batch	0.25[a]		4,000	
Thalassiosira fluviatilis	P-limited, chemostat; growth rate of:				
	0.047/h	5.51[a]		179	Hunter[b]
	0.038/h	3.45[a]		286	
	0.032/h	3.25[a]		303	
	0.027/h	1.28[a]		769	
	0.022/h	1.87[a]		526	
	0.013/h	1.08[a]		909	
	0.011/h	0.70[a]		1,429	
	0.007/h	1.33[a]		740	
	$\bar{x}$ for all growth rates	2.3[a]	0.84	427	
	N-limited, chemostat	3.05[a]		323	
Thalassiosira pseudonana	P-limited, chemostat; growth rate of:				
	0.041/h	1.3[a]		780	256
	0.017/h	0.47[a]		2,088	

[a] Assumed: cellular carbon = 0.50 × dry weight (229).
[b] B. L. Hunter, M. S. thesis, University of Hawaii, Honolulu 1979.

ADP, and ATP). Moreover, the enzyme is extremely heat and acid stable and can easily survive most of the standard methods for extracting cellular ATP. This source of analytical interference has been shown recently to be the cause of at least one set of aberrant ATP data (186). The occurrence of alkaline phosphatase in environmental samples and its detrimental effects on measured microbial nucleotide levels have recently been discussed (176). One labora-

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tory investigation conducted in full awareness of this potential source of interference (Hunter, M. S. thesis) indicated that the ATP pools in P-deficient algal cells are also growth rate dependent (Table 6) and that the intracellular ATP pools of P-deficient cultures may actually exceed the pool levels in NO_3- and light-deficient cultures at high growth rates.

Nonadenine Nucleotide Triphosphates

Many important cellular reactions, notably those associated with microbial biosynthesis and growth, are coupled to energy derived from NTPs other than ATP. For example, GTP and uridine 5'-triphosphate are both required for the activation and interconversion of carbohydrate precursors for polysaccharide biosynthesis; cytidine 5'-triphosphate, GTP, and uridine 5'-triphosphate are required for RNA transcription; and the deoxyribose derivatives of cytidine 5'-triphosphate, GTP, ATP, and thymidine 5'-triphosphate are required for DNA replication. In addition, GTP is an obligate requirement for the initiation, the aminoacyl-transfer RNA binding and the translocation processes of protein synthesis. Previous calculations of the energy requirement for bacterial growth suggested that a significant proportion of the total energy flux may proceed through nonadenine nucleotide pools (214). Until recently, routine quantitative analyses of nonadenine NTPs in cell extracts have not been possible due to the lack of a rapid, sensitive, and specific assay comparable to the firefly bioluminescence assay of ATP. Consequently, there is a relative paucity of information concerning NTP pools in microorganisms.

Unlike the ATP pool, which is maintained at a relatively constant level independent of growth rate, the intracellular concentrations of nonadenine NTPs fluctuate in direct proportion to their requirements for biosynthesis (107, 169, 296, 297). In retrospect, this relationship might have been predicted, at least for cellular GTP levels, since (i) the number of sites for protein synthesis (i.e., the ribosomes) increases as a linear function of growth rate and (ii) the rate of protein synthesis does not appear to be limited by GTP concentrations under normal steady-state growth conditions (26, 64, 199). The intracellular GTP pool must increase as the growth rate increases in order to maintain saturating GTP concentrations at the ribosomes.

The possibility exists that each specific set of nucleotide bases (i.e., adenine, guanine, uracil, cytosine, etc.) maintains a characteristic EC independent of the others. Furthermore, the guanylate EC may regulate "GTP-powered" biosynthetic reactions (and likewise the cytidylate EC may affect "CTP-powered" reactions, etc.) in a manner analogous to the model discussed previously for EC_A control of "ATP-powered" reactions (10). Based on in vitro nucleosidediphosphate kinase reaction rate data, Thompson and Atkinson (317) have rejected this notion and concluded that there exists a functional cascade regulation between the individual charge ratios such that the overall cellular EC_A is maintained at the expense of the nonadenine NTPs. This predicts that the EC_A ultimately regulates the flux of phosphate into the nonadenine nucleotide pools. Any decrease in the EC_A would tend to sharply curtail the regeneration of nonadenine NTPs, and hence the EC values for guanylate, cytidylate, uridylate, etc., would decrease more rapidly than the EC_A under adverse conditions. Experimental observations, however, have indicated that there is no functional cascade regulatory step between EC_A and guanylate EC in the rat brain in vivo (76). More recently, Swedes et al. (310) have investigated the regulatory mechanism of protein synthesis during energy-limited growth of the yeast *Saccharomyces cerevisiae*. They found that the changes in EC_A and guanylate EC values paralleled each other, with no evidence for the existence of a regulatory cascade effect.

Diel Rhythms and Specific Cell-Cycle-Related Events

Most of the nucleotide measurements reported in the literature have been obtained with asynchronously dividing batch or continuous cultures, and consequently the majority of the experimental data represent time-averaged intracellular nucleotide pools and concentration ratios. Few studies have investigated transient states induced by specific cell-cycle-related metabolic events or controlled by diel metabolic rhythms.

When synchronously dividing cultures of microorganisms are compared with randomly dividing cells, many presumably cell-cycle-related oscillations are evident in the pool sizes of NTPs and deoxy-NTPs, EC_A values, and respiration rates (85, 223, 263, 316). Numerous studies have indicated a conspicuous correlation between the cellular levels of NTPs (especially the deoxy-NTPs) and the various stages of the mitotic cycle (84, 100, 154, 156, 189). Regardless of the mechanism(s) involved, variations in NTP pools associated with mitotic cycles are of a sufficient magnitude (up to fourfold) that they may represent important metabolic signals for the initiation of cell division (84).

Diel rhythms may be divided into two major categories: (i) those based on a passive response

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to environmental fluctuations during the 24-h cycle that cease when the organism is transferred to a constant environment (exogenous diel rhythms) and (ii) those that are entrained by environmental variations but ultimately result from physiological processes which persist under constant conditions (endogenous, or circadian, rhythms) (330). When microorganisms are exposed to light-dark cycles, diel rhythms occur in many physiological and biochemical processes. Plesner (261) and Jones (166) have suggested that cyclic fluctuations in intracellular ATP levels may be the underlying mechanism for the control of all biological clock phenomena. Several investigators have noted a diel periodicity in the ATP pool of algal cells entrained on a light-dark cycle (82, 330; Hunter, M.S. thesis). Weiler and Karl (330) have found that the cellular EC_A in *Ceratium furca* was maintained at a near-constant and high value (i.e., 0.8 to 0.9) throughout the 24-h period even though intracellular ATP and A_T decreased by ~50% during the dark period. Furthermore, when algal cells were placed in constant light (82) or constant darkness (330), the diel pattern continued, suggesting an endogenous rhythm in the rates of ATP production and utilization. The importance of these rhythms in environmental nucleotide determinations will be discussed in a subsequent section of this review (ATP and growth rates).

Nicotinamide and Flavin Nucleotides

The nicotinamide dinucleotides (NAD, NADH, NADP, and NADPH) and flavin nucleotides (FMN, reduced FMN, and flavin adenine dinucleotide) occur in all living cells. Collectively, they function as coenzymes for more than 300 different cellular reactions, and together with the cytochromes they are responsible for catalyzing the sequential oxidoreduction reactions of electron transport. A summary of our knowledge of NAD biosynthesis and pyridine nucleotide cycle metabolism in microorganisms has recently appeared (105).

Direct measurements of intracellular nicotinamide nucleotides reveal that their total concentration is ~10 mM, roughly equivalent to the internal pool of adenine nucleotides. During exponential growth in bacteria the pool of NAD(H) (i.e., [NAD] + [NADH]) is ~10- to 40-fold greater than the NADP(H) (i.e., [NADP] + ([NADPH]) concentration (4, 168, 224, 236). Significant variations exist in the nicotinamide coenzyme concentrations of bacteria exhibiting differences in their tolerance to, or requirements for, oxygen (168, 224, 336) and within a given strain as a function of medium composition (39, 70, 236) and growth conditions (236, 283). Representative data are presented in Table 7.

Anderson and von Meyenburg (4) have recently investigated the catabolic reduction charge ratio [i.e., NADH/NAD(H)] and the anabolic reduction charge ratio [i.e., NADPH/NADP(H)] in *E. coli*. Both ratios were found to be constant during exponential growth (catabolic charge, 0.05; anabolic charge, 0.45) and were independent of the growth rate (4).

In comparison with the data on nicotinamide nucleotides in microbial cells, relatively few data are available on the intracellular pools of flavin nucleotides. Chappelle (49) has reported that the "total" flavin [i.e., FMN, reduced FMN, and FAD] pool may range from 0.2×10^{-10} to 65×10^{-10} μg per cell in a variety of bacterial species. The highest concentrations were found in *Flavobacterium arborescens* and *Bacillus globigii*, and the lowest were found in *Spirillum serpens* and *Staphylococcus aureus* (49). Robrish et al. (272) found a much smaller range for representative oral microorganisms. Most of the total cellular flavin was associated with protein (49).

To date, neither pyridine nor flavin nucleotide measurements have been applied to ecological studies despite the ubiquitous occurrence of these nucleotides in living cells and their well-recognized and obligatory functions in cellular metabolism.

NUCLEOTIDE FINGERPRINTS IN NATURE

Detection of Life

Rapid and reliable detection of sparse populations of microorganisms is a requirement for many basic and applied investigations. Although the possibility of abiogenic synthesis of ATP cannot be discounted (262, 339), it is generally agreed that there is an obligate association between ATP and living organisms (152). Levin et al. (217) first described the use of the firefly bioluminescence assay of ATP for detecting the presence of viable microorganisms. Levin and Heim (218) and MacLoed et al. (230) subsequently proposed the use of ATP measurements for detecting extraterrestrial life.

The detection of life in extreme habitats, however, is not restricted to exobiological investigations. Azam et al. (14) have used ATP measurements to detect sparse populations of microorganisms living beneath the permanent Ross Ice Shelf in Antarctica. A recent report by Siegel et al. (290) revealed the presence of ATP and other life-associated compounds in Don Juan Pond, Antarctica, a previously suspected "abiotic" habitat. Karl et al. (187) have used ATP mea-

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TABLE 7. *Nicotinamide nucleotides in microorganisms*

Species	Medium, growth conditions	nmol/mg of dry wt		Reduced/oxidized		References
		NAD(H)	NADP-(H)	NADH/NAD	NADPH/NADP	
Bacillus cereus	Dormant spores	0.075	0.004	<0.02	<0.3	283
	6-min germination	0.198	0.104	0.26	2.2	
Bacillus cereus	Minimal, acetate, aerobic	1.66	1.42			224
	Minimal, glucose, aerobic	1.14	0.12			
Bacillus megaterium	Minimal, aerobic	0.56	0.11			224
	Complex, aerobic	1.52	0.24			
Bacillus megaterium	Dormant spores	0.108	0.018	<0.02	<0.05	283
	Log phase	2.31	0.958	0.18	1.2	
	Stationary phase	0.51	0.515	0.03	1.7	
Bacillus subtilis	Dormant spores	0.072	0.016	<0.04	<0.05	283
	65-min germination	0.026	0.060	0.36	1.1	
	120-min germination	0.043	0.073	0.26	1.2	
Clostridium kluyveri	Ethanol-acetate-bicarbonate, anaerobic	39.8	5.90	0.27	1.42	76
	Crotonate-bicarbonate, anaerobic	28.2	4.00	0.32	1.57	
Escherichia coli	Minimal, glucose, aerobic	2.44	0.24			224
	Minimal, glucose, anaerobic	2.67	0.58			
	Complete, glucose, aerobic	1.98	0.48			
Neurospora crassa	Minimal, glucose, aerobic	2.60	0.40	0.53	1.86	39
	Plus nicotinic acid	3.20	0.40	0.60	2.08	
	Plus tryptophan	3.65	0.55	0.62	2.23	
Pseudomonas fluorescens	Minimal, glucose, aerobic	0.19	0.04			224
	Minimal, succinate, aerobic	0.27	0.03			
Pseudomonas sp.	Lactate-limited, chemostat; growth rate of:					
	0.5/h	1.7	0.83			236
	0.25/h	3.75	0.97			
	0.08/h	5.6	0.83			
	NH_4-limited, chemostat; growth rate of:					
	0.5/h	3.5	0.76			
	0.25/h	3.6	1.24			
	0.08/h	3.8	0.82			
Saccharomyces cerevisiae	Complex, glucose, aerobic	8.26	0.37			224
	Complex, glucose, anaerobic	6.70	1.12			
Streptomyces griseus	Glucose-glycine-glutamate, aerobic	0.89	0.08			225

surements to support arguments for the production of organic carbon at deep-sea hydrothermal vents and have discussed the ecological significance of this primary production.

Microbial biofouling of introduced surfaces in aquatic ecosystems is a well-known phenomenon. Natural waters are used by many industries for cooling and other heat exchange purposes. The presence and succession of fouling microorganisms significantly reduce the efficiency and thermal exchange capacities of these systems. It has been estimated that a 25- to 30-μm-thick layer of microbial cells may reduce the efficiency of heat exchange in ocean thermal energy conversion plants to a point beyond which the generation of energy by temperature differentials is no longer economically feasible (63). Aftring and Taylor (2) have recently demonstrated the feasibility of ATP measurements for evaluating the presence and rate of increase of microbial biofouling.

ATP measurements have also been useful for detecting bacteria and other microorganisms in pharmaceuticals and domestic and industrial solutions and for the routine monitoring and quality control of presumed sterile products. Picciolo et al. (260) have described a semiautomated in-line flow system for ATP detection in wastewater effluents and potable water supplies. The ATP assay procedure has also been adopted by the food processing industry for assessing the effectiveness of various preservation techniques

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and storage conditions in addition to the routine monitoring of product contamination and spoilage (284).

Clinical detection of bacterial contamination of blood, urine, and other physiological fluids (75, 117, 318) and quantitative methods for evaluating the susceptibility of microorganisms to selected antibiotics (6, 117, 212) have both been advanced through the use of ATP measurements. In addition to the extremely high levels of sensitivity and reproducibility of the ATP assay, its application precludes the necessity for time-consuming culture techniques (usually 24 to 48 h) and therefore provides the means for more rapid diagnosis and treatment. Lee and Crispen (212) have demonstrated an excellent agreement (~100%) between the ATP index of drug susceptibility testing and conventional plate count methods, and Gutekunst et al. (117) have also reported a close agreement (>97%) between ATP concentrations and bacterial colony counts of 538 clinical urine specimens. ATP measurements have also been used to detect the presence of debilitated, but viable, coliform bacteria (69). The inability of stressed or otherwise injured cells to grow on diagnostic culture media emphasizes the importance and need for alternate methods for detecting the presence of viable and potentially infectious bacterial cells in clinical and applied ecological investigations.

Adenosine 5'-Triphosphate (ATP) as a Biomass Indicator

The necessity for quantitative determinations of total biomass (i.e., living) carbon is a primary concern in nearly all ecological studies. Quantitative assessment of the total standing stock of viable microorganisms in environmental samples, however, has proven to be "surprisingly difficult" (306). In 1966, Holm-Hansen and Booth (146) proposed the use of ATP measurements as a means of estimating total biomass carbon. Since that time more than 400 papers have appeared in the scientific literature describing specific ecological applications of the ATP-biomass methodology.

Four important assumptions are inherent in the application of this method: (i) all living organisms contain ATP, (ii) ATP is easily extracted from microbial assemblages and can be precisely measured, (iii) ATP is neither associated with dead cells not adsorbed onto detrital materials, and (iv) there exists a fairly constant ratio of ATP to total cell carbon for all microbial taxa independent of metabolic activity or environmental conditions. Of these assumptions, only the first is unequivocally acceptable. The second assumption is valid under certain experimental conditions which have been discussed above (Analytical Procedures). The third and fourth assumptions are discussed below.

Obligate association of ATP with living organisms. Several important analytical considerations have surfaced in recent years which have questioned the original assumptions of the ATP-biomass technique. Chappelle et al. (53, 54) have reported that viable cell counts decreased rapidly in batch cultures of *E. coli* cells which had entered the death phase, whereas total ATP decreased only gradually. They concluded that a portion of the total ATP must originate from "dead" (i.e., either nonviable or debilitated) cells. Furthermore, they suggested that the contribution of ATP from nonliving cells present in environmental samples will depend upon the specific cause of death or cell damage, as well as the chemical and physical nature of the fluid environment. This potential analytical interference of ATP associated with nonliving cellular materials should be considered as a possible source of error in environmental nucleotide determinations.

In 1973, Koenings and Hooper (197) examined the distribution and chemical nature of dissolved organic phosphorus compounds collected from the interstitial waters of a northern Michigan boglake. Their studies revealed the presence of hydrolysis products of RNA, as well as free mono-, di-, and triphosphate nucleotides (197). J. W. Deming, D. A. Nibley, and H. Okrend (Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, Q48, p. 198) have also reported the presence of soluble (i.e., cell-free) ATP at several stations in the Gulf of Mexico. Azam and Hodson (15) confirmed the ubiquitous occurrence of dissolved ATP (D-ATP) in the marine environment. From the results of carefully designed dialysis bag experiments, they demonstrated that D-ATP was not an artifact of sample preparation or filtration, but was in fact ATP which was freely dissolved in the seawater. B. Riemann (unpublished data) has subsequently reported this phenomenon in freshwater ecosystems and has additionally demonstrated significant seasonal variations in the steady-state concentration of D-ATP. Clearly, the original assumption concerning the obligate association of ATP with living organisms must be modified. The concentration of D-ATP (i.e., <0.2 μm) in aquatic environments may constitute up to 75% of the "total" ATP in freshwater lakes (Riemann, unpublished data) and over 90% of the total ATP in certain marine ecosystems (15). For these reasons, Karl (173) has cautioned investigators against the use of direct injection methods for extracting aqueous environmental samples.

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Although the presence of D-ATP in aquatic environments is now well documented, the origin of the soluble nucleotides is unknown. Laboratory studies with batch and continuous cultures of microorganisms indicate that cellular RNA is degraded to oligonucleotides, nucleotide monophosphates, nucleosides, and free bases under starvation conditions. Most of these degradation products are subsequently released by the cells into the medium; however, intracellular nucleotide diphosphates and NTPs are rarely, if ever, excreted (60, 86, 186, 216). Therefore, it is unlikely that excretion by viable cells is the source of D-ATP in natural waters. More likely, the intracellular ATP is released by feeding predators and also by the production and dissolution of feces. Lampert (201) has recently shown that up to 17% of the algal carbon which is ingested by the cladoceran *Daphnia pulex* is lost as dissolved organic carbon during grazing.

Although the pool of D-ATP in many marine and freshwater environments appears to be significant, it remains to be demonstrated whether or not this dissolved pool is adsorbed onto suspended particulate materials. Since the particulate materials from most environmental samples must be concentrated before extraction and analysis of the cellular nucleotides, the contribution from D-ATP should be minimal, even though it may be the most abundant form of ATP. However, if ATP is adsorbed to, or concentrated by, nonliving particulate organic materials, then the ATP concentrations which are presumed to be associated with living microbial cells will be grossly overestimated.

Holm-Hansen (141) dismissed the possibility that nonliving particulate materials contribute to the total ATP content of a filtered water sample on the basis of the following experimental data: (i) if suspensions of phytoplankton were heated to 60°C for a few minutes or frozen at −20°C for a few hours, no ATP could be detected in the cellular materials; (ii) after autolysis of the flexibacterium *Saprospira* sp., the total particulate ATP decreased to undetectable levels; and (iii) if ATP is added to a representative seawater sample which has been previously frozen and thawed, no significant amount of ATP is recovered in an extract of the particulate fraction. These results, in particular the data from the latter experiment, suggest that, under the laboratory conditions specified, D-ATP is not associated with organic detrital materials. Nevertheless, due to the ubiquitous occurrence and relatively high concentrations of D-ATP in many aquatic environments, additional experiments should be conducted with representative particulate materials found in a given habitat. Surface-active suspended materials (especially clay particles, soot, and ash), if present, may reversibly adsorb D-ATP and other soluble nucleotides, yielding overestimates of the concentrations associated with living cells.

C/ATP ratios. The most critical and most widely criticized assumption of the ATP-biomass assay is the validity of the application of a single C/ATP ratio for the purpose of extrapolating ATP measurements to estimates of total microbial biomass carbon. Without a doubt, the common practice of converting ATP to biomass carbon loses all the value of the analytical precision of the ATP measurement. From the data presented in Tables 3 through 6 it is apparent that the C/ATP ratios of microbial cells vary considerably, and somewhat predictably, between individual taxa and even within certain species as a function of culture conditions and growth rate. The most commonly used conversion factor for ecological studies is ATP × 250 to derive an estimate of total biomass carbon (146). This relationship has been established as the mean value from data obtained with seven strains of marine bacteria (121, 146) and 30 species of unicellular marine algae (139) and, for the most part, has been systematically applied to diverse ecosystem analyses without further verification or critical consideration of its general validity. Therefore, it is impossible to ascertain the accuracy of biomass estimates extrapolated from ATP data.

Rather than rely on a mean C/ATP ratio or even consider the range (or extremes) in possible C/ATP ratios that might occur in microbial cells, it would seem more logical to examine the coefficient of variation of the C/ATP ratio of a given species or group of organisms under a defined range of environmental and physiological conditions. This coefficient of variation will provide a more reasonable indication of the accuracy and reliability of the biomass carbon extrapolations. Skjoldal and Båmstedt (294) have compiled a table of coefficients of variation for ATP determinations of individual microbial taxa. The coefficient of variation ranged from ±10% for actinomycetes to ±129% for bacteria. They subsequently developed a model to predict the required minimum number of species (n) in a sample to have an accuracy of $\pm x\%$ in the ATP determination. The equation used was: $n = (t_{0.05} \times \text{coefficient of variation})/x^2$, where $t_{0.05}$ is the appropriate value from the t-table (294). Considering the species in a particular sample to be randomly selected, values for n at three different coefficients of variation and for various values of x are presented in Table 8. Assuming a value of 60% as the coefficient of variation of a mixed

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community of microorganisms, the population must be composed of at least 36 species before their mean ATP concentrations attain an accuracy of ±20% (294). According to this model, the greater the number of species present, the more accurate the ATP-biomass estimate. In practice, however, biomass estimates will be most accurate when the natural microbial assemblage approaches a monoculture of a known species and when ancillary data are also available on the growth rate and nutrient status of a population.

To further emphasize the probable magnitude and significance of reported variations in C/ATP ratios, let us examine a hypothetical environmental situation. Imagine that we wish to know the phytoplankton biomass at a particular station in the euphotic portion of the Pacific Ocean. Let us assume that the dominant microorganism is *T. fluviatilis,* thereby reducing the possibility of interspecies variations in the C/ATP ratio. Although the ocean is generally considered to be N limited with respect to phytoplankton growth, we cannot discount the existence of inorganic phosphate or light limitation, the latter occurring when the depth of the mixed layer exceeds the 1% light level. Under the conditions specified, the C/ATP ratio may range from 166 to 1,250 (Hunter and Laws, submitted for publication), depending upon the limiting nutrient and specific growth rate. If the growth-limiting nutrient was known, then the variability of the C/ATP ratio would be reduced to 257 to 286, 250 to 417, 179 to 1,250, or 167 to 238 for ammonium, nitrate, phosphate, or light limitations, respectively. Finally, if we further assume that the precise growth rate of the population is known, then the mean and coefficient of variation for the C/ATP ratio would be further reduced to 313 (±10%), 323 (±23%), 455 (±75%), or 187 (±14%) for a given growth rate limited by ammonium, nitrate, phosphate, or light, respectively (Hunter, M.S. thesis). The residual variability has been shown to be dependent upon a diel periodicity in the C/ATP ratio. Although the intent of this hypothetical example was to emphasize the importance of selecting the most appropriate C/ATP ratio for a particular environment, it has actually been an example in futility. If we actually knew the precise species composition, growth-limiting nutrient, and growth rate of any microbial assemblage in nature, it is unlikely that we would desire an estimate of the biomass carbon.

TABLE 8. *Minimum number of species constituting a sample required to have an accuracy of ±x% in the arithmetic mean ATP concentration*[a]

Degree of accuracy (mean ±x%)	No. of species required for a coefficient of variation of:		
	100%	60%	30%
5	1,600	576	144
10	400	144	36
20	100	36	9
30	45	16	4
40	25	9	3
50	16	6	2
70	8	3	1
100	4	2	1

[a] From Skjoldal and Båmstedt (294).

The original methods for environmental ATP determinations have been greatly modified and improved in recent years. Development of new analytical procedures has, for the most part, proceeded in parallel with acquisition of data. As refined procedures are adopted for general use in microbial ecology, there should be a reevaluation and, if necessary, a new calibration of the C/ATP ratio. Since most environmental studies have been reported by independent investigators using different methods of sample collection, extraction, and analysis, caution must be exercised in the comparison of biomass carbon estimates for different environments.

Despite these significant and predictable variations in cellular C/ATP ratios, indirect data from several independent field investigations have indicated that the proposed ratio of ~250 is a close approximation of the true in situ C/ATP ratio. This is especially true for phytoplankton-dominated portions of the marine environment where there is no evidence of inorganic nutrient limitation. Under such environmental conditions, the algal cells would be growing at or near their μ_{max}, and the C/ATP ratios would be expected to approach a value of 250 (55, 139; E. Sakshaug, Ph.D. thesis, University of Trondheim, Trondheim, Norway, 1978). Similarly, several field studies have made simultaneous measurements of ATP and total particulate organic carbon. For the euphotic zone of the ocean, an upper limit of 250 to 300 is derived for the in situ C/ATP ratio (138, 141, 146, 298).

Cavari (45) has reported a range of 200 to 5,000 for the C/ATP ratio of plankton populations from Lake Kinneret over an annual cycle, but for the major part of the year it conforms to a 250:1 ratio. The most prominent seasonal fluctuation results from a spring algal bloom dominated by the dinoflagellate *Peridinium.* Based on field and laboratory data, Cavari (45) suggested that the increase in C/ATP ratio is correlated with a phosphorus deficiency in the lake. He further proposed that measurements of the in situ C/ATP ratio may be useful as quantitative indicators of P limitation. This relationship, however, appears questionable in light of recent

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data obtained by Karl and Craven (176). Their results indicate that certain (presumed inorganic phosphate-limited) microbial habitats contain elevated quantities of heat- and acid-stable alkaline phosphatase activity which catalyzes the hydrolysis of the extracted ATP. This results in an underestimation of the true ATP concentrations, an overestimation of the true C/ATP ratios, and additional errors in EC_A determinations and GTP measurements.

Paerl and Williams (250) have examined the relationship between the cellular carbon contents of microorganisms as determined by autoradiography and scanning electron microscopy and ATP contents in several freshwater lakes. Their data indicated a consistent C/ATP ratio of 276 (±12), independent of nutrient conditions, species composition, degree of eutrophication, or water depth (250). The use of in situ dialysis cultures of representative microbial cells, as suggested by Sakshaug (279), may prove useful for determining reliable C/ATP ratios of individual components of the microbial community. It should be emphasized that microbial assemblages dominated by metazoans will require the application of a lower C/ATP ratio due to their higher concentrations of ATP per unit of carbon relative to unicellular microorganisms (Table 5).

Correlations of ATP with other measures of biomass and activity. Although the accuracy of the ATP-biomass technique has been questioned and numerous difficulties in its application have been cited, it is still by far the most convenient and reliable method for estimating total microbial biomass in most environmental samples. Biomass estimates based on chemical analyses of particulate organic carbon, particulate organic nitrogen, protein, or nucleic acids cannot be used in ecological studies due to the preponderance of nonliving organic materials. Direct microscopic enumeration and sizing of individual microbial taxa can be used to estimate total living carbon, provided there exists a previously determined empirical relationship between microbial cell volume and organic carbon. Particle volume may be determined via electronic particle counters; however, there is no means by which to distinguish living cells from nonliving detrital materials. Biochemical indices, such as chlorophyll *a* (chl *a*), lipopolysaccharide, and muramic acid, in addition to culture methods, exist for estimating individual components of the total microbial assemblage (i.e., either bacteria or algae).

Mel'nikov (240) has compared biomass estimates based on ATP with values obtained from direct microscopic examination of bacteria, phytoplankton, and microzooplankton at several stations in the Pacific Ocean. Although similar trends were observed in the spatial distributions of cellular biomass, there were significant quantitative differences between the two estimates. With the exception of 3 out of a total of 62 determinations, the biomass derived from ATP exceeded that calculated from microscopy, the latter representing from 3 to 27% of the total ATP biomass. It was concluded, and confirmed by electron microscopy, that the occurrence of ultraplankton not detected by light microscopy contributed to this discrepancy between methods.

Sorokin and Lyutsarev (298) conducted a similar field comparison of direct microscopic and ATP techniques for the estimation of total microbial biomass. An excellent agreement was observed between the two methods for euphotic zone samples from the southwest Pacific Ocean. The average ratio of ATP-biomass to direct microscopy biomass was 1.06 (298). They therefore concluded that the ATP assay can be used as a rapid measure of total microbial biomass in seawater samples.

J. E. Hobbie and S. W. Watson (Abstr. Annu. Meet. Am. Soc. Limnol. Oceanogr. 1978 p. 18) have compared three independent methods for estimating bacterial biomass in the deep Atlantic Ocean off northwest Africa. At one station 12 measurements between 150 and 1,800 m yielded mean biomass estimates of 2.08, 1.96, and 1.70 mg of C per m^3 for ATP, lipopolysaccharide, and acridine orange epifluorescence microscopic analyses, respectively. Although each technique has its inherent limitations, the excellent agreement among the three methods suggested that the majority (75 to 96%) of the microbial biomass in the aphotic portions of the world's oceans is bacterial and lends further credibility to the independent application of these biochemical indices of biomass in the environment.

Several studies have been conducted wherein comparisons of methods for estimating total phytoplankton carbon have been achieved. Investigations of coastal waters off southern California, as well as other geographical areas, have led to the conclusion that phytoplankton biomass estimates calculated from ATP and chl *a* concentrations (136, 141), total particulate organic carbon (140, 146), or cell counts (293) were all in close agreement for the euphotic zone. Sinclair et al. (293) have recently reported an excellent agreement between ATP-biomass estimates and phytoplankton carbon calculated from cell counts and volume measurements. They concluded that in high phytoplankton biomass areas (i.e., chl $a > 10$ μg/liter), ATP determinations can be used to estimate total

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phytoplankton carbon (293). However, Eppley et al. (90) found that the carbon estimates based on ATP concentrations were generally greater than those based on other indirect methods, and in 11 out of 17 groups of stations the ATP-biomass C estimate exceeded the measured value of total particulate organic carbon.

In other ecological studies, significant correlations have been observed between ATP concentrations and evolution of CO_2 from soils (13, 337), total dehydrogenase activity in marine sediments (252, 253), DNA synthesis in freshwater sediments (1), electron transport system activity in seawaters and lake sediments (164, 313), total lipid phosphate and rates of DNA synthesis in estuarine sediments (332), alkaline phosphatase and phosphodiesterase activities (242), potential for hydrocarbon oxidation in oil-polluted harbors (43), and biological oxygen demand (3, 66).

Selected Environmental ATP Applications

Partitioning of bacterial, algal, and metazoan carbon. The ubiquitous occurrence of ATP in all living organisms makes it a universal parameter for detecting life, but, ironically, this ubiquity poses a serious limitation on the ATP-biomass assay. For certain applications, bacteria may be considered to be the sole, or at least the dominant, microorganisms present (e.g., acid mine wastes, peat bogs, deep-sea habitats, and permanently anoxic environments); however, most naturally occurring microbial communities are composed of a complex assemblage of numerous taxa.

In many ecological studies, especially those concerned with energy transfer and organic productivity, it is essential to partition the community into individual trophic compartments. ATP measurements have proved useful for investigations of marine sediments (111, 291, 341) and microplankton communities (42, 150). Total ATP measurements and direct counts of sediment meiofauna were used by several investigators to compare the standing stocks of micro- and meiofauna and to estimate the contribution of meiofauna to the total community biomass. Yingst (341) has determined the numerical abundance of various meiofaunal taxa and has estimated the carbon contribution of each group in sediment samples from Long Island Sound. She reported that the top 2 cm of sediment contained ~70% of all of the meiofauna representatives,with 41% in the top 1 cm (341). Within the topmost layer of sediment the meiofauna constitute ~100% of the total ATP-biomass; however, the cumulative computational errors were substantial (±50%). Below this depth, ATP contributed by microorganisms (bacteria and protozoa) became more discernible. Ernst and Goerke (92) have likewise concluded that meiofauna may contribute a large percentage of the total ATP in surface sediments from the deep Atlantic Ocean (250 to 5,510 m). Sikora et al. (291) have described a method for the selective recovery of nematodes and other meiofaunal groups from heterogeneous sediment samples. Their results indicated that the proportion of total sedimentary ATP contained within the nematode population of littoral marine sediments can vary seasonally from 68% in the summer to >90% in the winter.

Gerlach (111) has recently constructed a trophic model for a hypothetical subtidal, silty, sand marine sediment ecosystem. He reported that the distribution of individual components of the total sediment biomass yields macroinfauna (10 g of carbon per m^2), bacteria (5 g of carbon per m^2), meiofauna (1 g of carbon per m^2), foraminiferans (0.5 g of carbon per m^2), and ciliates plus flagellates (<0.01 g of carbon per m^2). Clearly, the species composition and trophic organization of individual communities are extremely variable, and it is essential to select the most representative C/ATP ratios to evaluate the biomass of each biological compartment.

Before the extraction of ATP from water samples, prefiltration is generally used to exclude larger metazoans. However, the retention of chained or clumped unicells and individual cells attached to larger particulate materials is not precluded. Should an effective separation of the metazoa be achieved on the basis of size, one would still be faced with the task of distinguishing between the micrometazoans, protozoans, unicellular algae, and bacteria.

Holm-Hansen and Paerl (150) have proposed a method for estimating the relative contributions of bacteria and algae to the total biomass carbon in aquatic environments. In their study an attempt was made to estimate bacterial carbon by calculating the difference between total ATP-biomass carbon and total phytoplankton carbon obtained from direct microscopic identification, cell sizing, and enumeration. Their data from Lake Tahoe indicated that during the spring bloom, phytoplankton cells represent approximately 80 to 90% of the total biomass carbon, but in late summer their contribution had dropped to 20 to 50%.

This method of ecosystem analysis based on a two-component model (i.e., bacteria and algae) is not appropriate for all microbial communities due to the presence of protozoa and other microzooplankton. Jassby (159) has criticized the use of total ATP and phytoplankton counts to estimate bacterial carbon and has developed a sta-

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tistical model to evaluate the reliability of this approach. Disregarding systematic errors associated with the various analyses, he concluded that the propagation of errors is significant enough to render this method acceptable in only a very limited number of natural environments.

Sorokin and Lyutsarev (298) have proposed a method for the determination of bacterioplankton biomass in the marine environment. The method relied upon an empirically derived correction factor which must be applied to calculate the contribution of bacteria to total microbial biomass. The correction factor was equal to the inverse ratio of total ATP-biomass to the bacterial biomass determined by direct microscopy and varied with the nature of the habitat. The average values were 1.4 (range, 1 to 1.5) for the euphotic zone of upwelling waters, 2.3 (range, 2 to 3) for the euphotic zone of waters with moderate productivity, and 4.6 (range, 4 to 7) for deep waters below the euphotic zone (298). The accuracy and usefulness of this approach for general ecological applications are questionable. It may, however, provide a useful measure of bacterioplankton biomass in specialized habitats which do not experience significant temporal community changes (i.e., deep-sea habitats).

Several attempts have been made at a physical separation of individual microbial components based either on size, by differential filtration (16, 17, 42, 46, 123), or on density, by differential centrifugation (104). Burnison (42) first suggested that bacteria and algae may be separated by Nuclepore filtration techniques. This approach (i) implies nonoverlapping size spectra of the individual microbial groups, (ii) disregards the possible association of small microbial cells with larger particulate materials, (iii) assumes a nondestructive filtration procedure, and (iv) requires that the intracellular ATP pools remain constant throughout the experimental manipulations. It should be emphasized that none of the prerequisites is fully satisfied. For example, Burnison (42) found that ~18% of the total bacteria were retained by a 1-μm Nuclepore filter, whereas Azam and Hodson (16) reported the presence of microflagellates in the 1-μm Nuclepore filtrate. Azam and Hodson (16) reasoned that since ~90% of the total heterotrophic activity was associated with the <1-μm Nuclepore fraction in seawaters from various habitats, most bacteria must exist in the "free living" (i.e., unattached) state. However, other data exist which indicate that high rates of microheterotrophic activity may be often associated with particles ~3 μm in diameter (123, 124). Since the ratio of free-living to attached bacteria undoubtedly varies considerably in samples collected from different environments, the use of size fractionation of ATP to assess "bacterial" biomass is not fully justified. Nevertheless, ATP fractionation studies have yielded useful information concerning the size distribution of living organisms. These studies, when used in conjunction with measures of microbial activity and growth, have provided comparisons of the specific activities of various microbial populations (i.e., activity per unit biomass) (42, 123, 143, 234, 237, 288, 325).

Ferguson and Rublee (97) have combined acridine orange direct count estimates of bacterial cell numbers, dimensions, and frequencies with ATP biomass carbon measurements to estimate the contribution of bacteria to the total standing crop of coastal marine plankton. Their estimates indicated that the mean standing crop of bacteria for 19 coastal seawater samples was 5.5 ± 3.9 mg of C per m^3 and ranged from 4 to 25% of the total plankton carbon (mean, 9.8%). A noteworthy observation was that ~80% of the bacterial cells were free-living cocci but composed only ~40% of the total bacterial carbon. Moreover, the numerically less abundant (<15%) bacterial cells found in association with particulate materials contained a disproportionate percentage of larger rod-shaped cells and therefore accounted for nearly 45% of the total bacterial carbon. Palumbo and Ferguson (251) have also applied this approach in a study of the Newport River Estuary, N.C. They found that bacterial carbon represented 20 ± 9.5% (range, 7.5 to 41.6%, n = 13) of the total living carbon. In addition, total ATP was not correlated with bacterial numbers or to the ratio of bacterial C to total biomass C (251).

Several successful techniques have been developed to differentiate "bacterial" ATP from "nonbacterial" ATP in physiological fluids, such as urine (75, 117, 318) and blood (53, 75). However, none of these methods has been adapted for use with environmental samples. The most successful approach involves a selective chemical lysis of nonbacterial cells (i.e., eucaryotes) followed by enzymatic hydrolysis of soluble ATP in the extract and subsequent concentration and extraction of bacterial ATP. The method was developed for the clinical analysis of fluids containing high proportions of mammalian cells and D-ATP, so it is not known whether this approach would be useful for separating procaryotic and eucaryotic components of naturally occurring microbial assemblages. Although the diversity of eucaryotes present in environmental samples may frustrate attempts to devise a selective chemical lysing agent, chemical separation procedures for partitioning microbial com-

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ponents in natural aquatic environments deserve further attention and development.

ATP applications in oceanography. (i) Geographical and vertical distributions of ATP in the ocean. Our understanding of basic biological oceanographic processes, especially those regarding food chain dynamics and spatial and temporal distributions of living organisms, has been advanced substantially from ATP measurements. Since its first documentation (146), more than 100 papers have appeared concerning specific applications of ATP measurements in the ocean. Table 9 summarizes representative data from selected geographical locations.

A "typical" vertical profile of ATP concentration (versus depth) in the marine environment is presented in Fig. 2. Several recurrent and therefore characteristic features of the distribution of ATP in the ocean have been reported. (i) Elevated ATP concentrations are characteristic of the sea-surface interface (0 to 150 μm). This observed concentration of biomass may be the result of physical concentrating mechanisms or may result from microbial growth within this specialized habitat. (ii) High concentrations of ATP are present between 0 and 100 m, corresponding to the euphotic zone of the ocean. Frequently, a subsurface peak at or near the chl *a* maximum layer is also apparent. The absolute concentration of ATP in the euphotic zone varies regionally and is usually significantly correlated with phytoplankton biomass and rates of primary production. Typically, surface ATP concentrations range from >500 ng of ATP per liter for eutrophic waters to 100 to 500 ng of ATP per liter for regions with moderate productivity to <100 ng of ATP per liter for oligotrophic portions of the ocean. (iii) Below the euphotic zone, the concentration of ATP is more variable but usually exhibits a rapid decrease with increasing water depth to a value of <10% of the surface concentration by a depth of 200 to 400 m. (iv) Below 400 m there is a more gradual decrease in ATP concentration to minimum values of ~0.5 to 2 ng of ATP per liter in the deep-sea habitats examined thus far, regardless of the productivity of the surface waters (Table 9).

Although these typical vertical distributions of ATP have been observed by many investigators, it should be emphasized that intermediate-depth (400 to 1,000 m) ATP maxima occur (130, 146, 175, 180, 184; W. Campbell and W. Weibe, Abstr. Annu. Meet. Am. Soc. Limnol. Oceanogr. 1978, p. 10), occasionally with concentrations up to 30% of the surface maxima (184). Karl and coworkers (175, 180, 184) have stressed the importance of oceanic discontinuities (e.g., thermoclines, pycnoclines, ocean fronts, and water mass interfaces) for the production and maintenance of these subsurface zones of elevated biomass and activity. However, the organization and research objectives of most marine microbiological investigations conducted to date have been such as to preclude detailed sampling of ocean discontinuities, and therefore the few data that do exist have been serendipitous and incomplete. Future studies should concentrate on elucidating the roles of oceanic discontinuities in controlling the distribution, abundance, and metabolic activities of the autochthonous microorganisms.

There are several notable exceptions to the generalized vertical profile of ATP in the aphotic zone as described above. Microbiological investigations of specialized marine habitats in Antarctica, within permanently anoxic marine basins and at deep-sea hydrothermal vents, have revealed unique ATP distribution patterns. The waters under the Ross Ice Shelf, Antarctica, constitute a unique marine environment for living organisms due to the absence of an overlying euphotic zone resulting from a 400- to 600-m-thick layer of ice. Extremely low levels of ATP have been detected at all depths from 20 to 200 m beneath the ice cover, with typical concentrations ranging from 0.04 to 0.6 ng of ATP per liter (14). By comparison, ATP concentrations of seawater samples collected from similar depths in the adjacent ice-free Ross Sea were two to three orders of magnitude higher (145).

In most deep-sea environments circulatory processes supply dissolved molecular oxygen at rates that greatly exceed the biochemical oxygen demand. However, if these processes are impaired or if the input of organic matter is unusually high, microbial decomposition will result in depletion of dissolved oxygen. The Black Sea and the Cariaco Trench, Venezuela, are two well-studied anoxic marine environments. Measurements of the vertical distributions of ATP in these habitats have yielded several unusual, but consistent, features, including: (i) a large increase in ATP at the oxic-anoxic interface, (ii) elevated ATP concentrations (5- to 10-fold) within the anoxic zone relative to oxygenated oceanic environments of comparable depth, and (iii) a gradual increase in ATP concentration with increasing water depth within the anoxic zone. The factors controlling these atypical ATP distributions and the significance of elevated ATP levels in anoxic marine environments have been discussed by Karl and co-workers (171, 185). A similar increase in ATP has also been reported from the hypersaline, anoxic Orca Basin (Table 9) in the Gulf of Mexico (204).

One final exception to the generalized distribution of ATP in the aphotic zone is the recent

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TABLE 9. *ATP concentrations in neritic and oceanic seawaters*

Station	Coordinates	ATP (ng/liter) at:			Reference
		0–100 m	400–600 m	>1,000 m	
Pacific Ocean					
North of Hawaii	22°10′ N, 158°00′ W	60–90		0.2–1.5	150
San Diego Trough	32°41′ N, 117°35′ W	88–96	4–8		150
Southern California Bight	33°18.5′ N, 118°40′ W	125–350	35–70	0.5	146
Southern California Bight	30°40′ N, 120°02′ W	40–67	3–10	1–2	136
Southern California Bight	32°31.6′ N, 118°0.7′ W	100–150	5–8	1–2	180
Southern California Bight	(Several stations 1–10 km offshore)	500–1,500			90
North Pacific Gyre	31° N, 155° W	20–115			231
North Pacific Gyre	28°00° N, 155°00° W	35–110	8–12	1.5	147
East Pacific	10°32′ S, 79°58′ E	50–125	10–20	2	240
East Pacific	12°32′ S, 101°50′ E	8–21	1–5	0.4–1.5	
Costa Rica Dome			6–15	1.4–5	77
Peru coast	0.9°0.4′ S, 83°37′ W	55–400	4–6	1–2	138
Aleutian Trench		100–400	11–15	0.2–0.9	147
Galápagos Rift	00°47.0′ N, 86°08′ W	75–150	4–10	1–2	187
Galápagos Rift (hydrothermal vents)				500	
Cowichan Bay, British Columbia, Canada		300–1,800			287
Shimoda Bay, Japan		100–600			340
Gulf of Mexico					
West coast of Florida	10 km offshore	1,300			Deming[a]
	17 km offshore	750			
	30 km offshore	320			
	50 km offshore	150			
Orca Basin				2.5–15	204
Tampa Bay, Fl.	~27°40′ N, ~82°40′ W	50–300			203
Middle Grounds	~28°40′ N, ~84°20′ W	180–420			203
Destin Dome	~29°30′ N, ~86° W	90–230			203
Mobile Bay, Ala.	~30° N, ~88° W	350–2,000			203
Mississippi Bay	~30° N, ~88°40′ W	1,500–12,000			203
Caribbean Sea					
Cariaco Trench, Venezuela	10°32.2′ N, 64°43.4′ W	80–300	10–15	5–10	185
Columbian Basin	10°6.5′ N, 77°17.0′ W	10–30	4–6	1	175
Atlantic Ocean					
North Inlet Estuary, N.C.		500–2,500			57
Newport River Estuary, N.C.		500–2,400			251
Cape Lookout, N.C.	34°24′ N, 76°45′ W	125–182			97
Sapelo Island, Ga.		1,900			123
North of Cape Hatteras, N.C.	36°25′ N, 74°43′ W	500	16–25		132
Sargasso Sea	35°00′ N, 73°00′ W	40–80	2–5		132
Sargasso Sea	32°50′ N, 62°30′ W	20–30	1.2–7	0.4–1.2	115
Bedford Basin, Nova Scotia, Canada		85–680			312
Guinea Dome		25–600			130
Azores Plateau		20–40			288
Mid-Atlantic Ridge	26°18′ N, 44°44′ W	400	3–5	0.5–1	184
Nares Abyssal Plain	26°50′ N, 60°14′ W	190	40	0.5–1	184
Namibia Cape Basin		100–675	50–100	2–4	Watson et al.[b]
Georges Bank		120–400	60		41
Mediterranean Sea					
Near Toulon, France	42°55′ N, 05°54′ E	13–40	3	1–2	67
Marseilles Bay, France	43°18′ N, 05°17′ E	160–800			67
MEDIPROD-I	42°12′ N, 05°36′ E	40–120	10	~5	Laborde[c]

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TABLE 9. *Continued*

Station	Coordinates	ATP (ng/liter) at: 0–100 m	400–600 m	>1,000 m	Reference
Antarctica					
Heald Island	78°13′ S, 163°54′ E	100			145
Cape Chocolate	77°57′ S, 164°38′ E	200			145
New Harbor	77°38′ S, 163°50′ E	200–400			145
White Island	78°01′ S, 167°21′ E	300			145
Beneath Ross Ice Shelf Station J-9	82°22.5′ S, 168°37.5′ W	0.04–0.6			14, 144
Marginal seas					
Hudson Bay, Canada		90–190			213
Wadden Zee, The Netherlands		200–1,000			234
Black Sea	42°50′ N, 33°00′ E	60–250	10–15	5–15	171

[a] J. Deming, unpublished data.
[b] S. W. Watson, H. L. Quinby, F. W. Valois, J. B. Waterbury, J. E. Hobbie, and T. J. Novitsky, unpublished data.
[c] P. Laborde, Ph.D. thesis, Université d'Aix-Marseille, Marseilles, France, 1972.

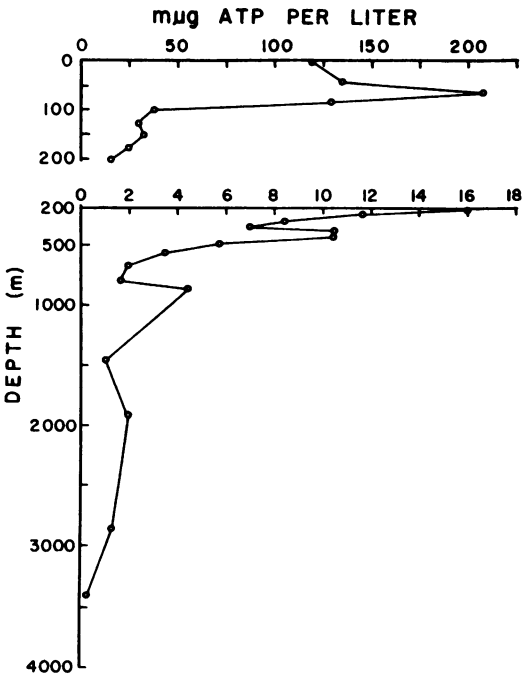


FIG. 2. *Distribution of ATP (in nanograms [mμg] per liter) with depth in the eastern Pacific Ocean (31°45′ N, 120°30′ W). Note that different scales are used for the intervals 0 to 200 and 200 to 4,000 m. From Holm-Hansen (140).*

discovery of deep-sea production at the Galápagos Rift hydrothermal vents. Karl et al. (187) have reported ATP concentrations in excess of 500 ng/liter for sulfide-rich vent waters, indicating elevated rates of microbial production in this specialized deep-sea habitat. It was suggested that energy in the form of geothermically reduced sulfur compounds is liberated during oxidation and is used for the reduction of CO_2 to organic matter by resident chemosynthetic bacteria (187).

A further application of ATP measurements in biological oceanography has been the estimation of biomass C relative to the total particulate organic carbon present in various marine habitats. Extensive studies have revealed that 50 to 100% of the total particulate organic carbon within the euphotic zone is living carbon. With increasing water depth, the proportion decreases to ~5 to 10% at 200 to 300 m and eventually to <1% at depths exceeding ~1,500 m. Representative data from samples collected off the coast of southern California are given in Fig. 3 and Table 10. The intermediate-depth ATP maximum at 386 m (Table 10) is again noteworthy in that a much higher percentage of living carbon is present relative to the surrounding contiguous waters. These data provide further evidence of elevated microbial activity and growth within isolated portions of the water column.

(ii) Sedimentary ATP distributions. A summary of selected data relating to the distribution of ATP in estuarine and marine sediments is presented in Table 11. Several generalized trends are evident, including: (i) ATP concentrations are highest in organic-rich coastal habitats (e.g., salt-marsh ecosystems and intertidal mud flats), substantially lower in sandy beaches and subtidal marine sediments, and even lower with increasing water depth and distance from shore; (ii) there is generally a decrease in ATP concentration with increasing sediment depth; and (iii) in temperate habitats, there is an annual fluctuation in sedimentary ATP concentrations which is most probably re-

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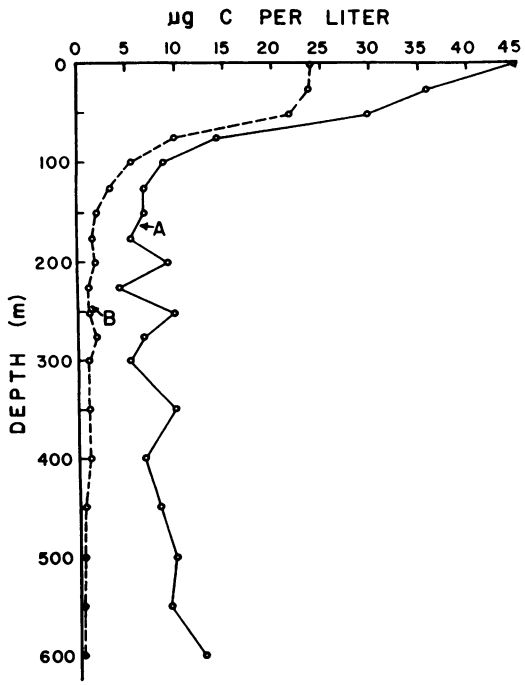


FIG. 3. *Distribution with depth of total particulate organic carbon (line A) and organic carbon in living cells, as estimated by ATP × 250 (line B). The station position was 32°41′ N, 117°35′ W. From Holm-Hansen (140).*

TABLE 10. *Distribution of ATP and particulate organic carbon (POC) at various depths at a hydrostation located off southern California (33°18.5′ N, 118°40′ W)*[a]

Depth (m)	ATP (ng/liter)	POC (μg/liter)	Biomass C[b] (μg/liter)	Biomass C/POC ratio
10	126.0	79	33.6	0.42
30	357.0	120	95.0	0.79
103	17.3	33	4.6	0.14
183	6.1	51	1.6	0.03
200	10.7	44	2.6	0.06
315	6.4	40	1.7	0.04
386	34.3	34	9.1	0.27
500	6.4	57	1.7	0.03
722	4.0	45	1.1	0.02
1,072	0.5	20	0.13	0.006

[a] From Holm-Hansen and Booth (146).
[b] Based on assumption that the C/ATP ratio is 266.

lated to temperature and overall ecosystem productivity.

(iii) ATP-based biochemical indices of physiological state. It is well known that the chemical composition of an assemblage of microorganisms is directly affected by the nature of the environment. The in situ metabolic state of individual cells depends upon the rate of supply of essential limiting nutrients. Deviations from exponential, nutrient-saturated, balanced growth are often reflected in the chemical composition of the cells. In theory, the chemical composition of natural microbial assemblages may yield information concerning their physiological state and nature of the limiting nutrient. Several ATP-based biochemical indices have been proposed and used in ecological studies. Physiological ratios based on the relative molar concentrations of the adenine nucleotides (e.g., EC_A) and on the concentrations of cellular GTP and ATP (i.e., GTP/ATP ratios) will be discussed below.

Laboratory and field studies have suggested that the physiological state of phytoplankton cells, especially in regard to limiting macronutrients, could be assessed by measurements of the chl *a*/ATP ratios (35, 256, 279, 280; Sakshaug, Ph.D. thesis). Perry (256) has reported that the chl *a*/ATP ratio in nutrient-deficient cultures of *Thalassiosira pseudonana* ranged from 3 to 5 during N limitation and from 26 to 27 during P limitation. Oceanic field observations have generally yielded ratios of <10, suggesting N limitation of phytoplankton growth in nature (213, 256, 279). Nevertheless, any attempt to identify the limiting nutrient from biochemical indices at our present level of knowledge should be approached with caution. For example, Sakshaug (279) has demonstrated a strong correlation between chl *a*/ATP ratios and temperature, and several investigators have noted fluctuations in the chl *a*/ATP ratios due to light intensity (93; Hunter, M.S. thesis). Moreover, Naiman and Sibert (243) have observed significant and disproportionate diel variations in both chl *a* and ATP in natural phytoplankton populations.

Lännergren and Skjoldal (202) have monitored chl *a*/ATP ratios during a plankton bloom in Lindåspollene Fjord, Norway. chl *a*/ATP ratios increased from <1 before the bloom to >20 during bloom conditions. They concluded that variations in chl *a*/ATP ratios were indicative of physiological changes in the phytoplankton related to the rate of growth. Benon et al. (28) have used chl *a*/ATP measurements to assess the mortality of phytoplankton entrained into freshwater intrusions in the Gulf of Fos, France. Increased chl *a*/ATP ratios were indicative of cell death and were found to correlate with decreases in percent living carbon and total number of phytoplankton cells.

Several studies have also used ATP-based biochemical indices to monitor heterotrophic processes. During an investigation of the decomposition of salt-marsh detritus, R. B. Hanson (unpublished data) used CO_2/A_T and O_2/A_T ra-

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TABLE 11. *ATP in estuarine and marine sediments*

Station location and description	ATP (ng/g of dry sediment)	Reference
Sapelo Island, Ga. (short *Spartina*) (0–1 cm)		
April	1,000[a]	56
July	3,570[a]	
Oct.	2,040[a]	
Dec.	2,720[a]	
Feb.	935[a]	
Newport River estuary, N.C.		
0–1 cm	762[a]	95
5 cm	190[a]	
15 cm	57[a]	
Newport River estuary, N.C.		
Winter (0–1 cm)	640[a]	96
(5 cm)	160[a]	
(15 cm)	40[a]	
Summer (0–1 cm)	1,180[a]	
(5 cm)	320[a]	
Lindaspollene Fjord, Norway		
Soft mud (0–1 cm)	3,800	252
(3–4 cm)	1,500	
Silty sand (0–1 cm)	3,800	
(3–4 cm)	1,400	
Fine sand (0–1 cm)	1,090	
(3–4 cm)	440	
Coarse sand (0–1 cm)	900	
(3–4 cm)	1,400	
Spanish Sahara		
21°18′ N, 17°22′ W (200 m) (0–1 cm)	550[a]	135
21°24′ N, 17°54′ W (1,000 m) (0–1 cm)	177[a]	
Northeastern Atlantic Ocean (0–1 cm, all stations)		
36°43.5′ N, 14°16.2′ W (252 m)	1,320	92
36°47.2′ N, 14°16.7′ W (408 m)	940	
36°42.8′ N, 14°29.9′ W (1,445 m)	180	
35°57.4′ N, 18°18.1′ W (4,897 m)	84	
Nares Abyssal Plain		
26°50.8′ N, 60°13.6′ W (6,011 m)		
(0–2 cm)	2.4	184
(4–6 cm)	0.2	
Black Sea		
41°21′ N, 30°14′ E (450 m) (5 cm)	350[a]	171
42°59′ N, 33°59′ E (2,200 m) (5 cm)	115[a]	
Long Island Sound		
April (0–1 cm)	2,873	341
(5 cm)	200	
(10 cm)	33	
(15 cm)	22	
June (0–1 cm)	6,356	
(5 cm)	269	
(10 cm)	239	
Oct. (0–1 cm)	1,093	
(10 cm)	349	
(20 cm)	97	
(50 cm)	2	
(150 cm)	1	
Southern California sandy beaches (low tide)		
Corona Del Mar		
(0–1 cm)	117	174
(5 cm)	126	
(10 cm)	77	
Catalina Island		
(0–1 cm)	452	
(5 cm)	47	
(10 cm)	12	
Ocean Beach		
(0–1 cm)	180	
(5 cm)	182	
(10 cm)	95	
Government Point		
(0–1 cm)	238	
(5 cm)	348	
(10 cm)	116	

[a] Values expressed as ATP per cm^3 of wet sediment.

tios to provide a broad measure of carbon oxidation and oxidative metabolism, respectively. When N was supplied to N-deficient organic detritus, the CO_2/A_T ratio increased substantially. Karl (171) suggested that particulate nucleic acid/ATP ratios may be used to compare relative growth rates of individual microbial communities in certain environments. In the anoxic waters of the Black Sea, the particulate nucleic acid/ATP ratios increased from 174 at 137 m to a maximum of 933 at 2,045 m, with an attendant and substantial increase in EC_A with increasing water depth (171).

Finally, ATP measurements have been used frequently in ecological studies as a means to normalize total production or activity estimates to a per biomass basis. The resultant "specific activity" indices are generally more meaningful measures of the metabolic response to in situ environmental conditions.

(iv) ATP and respiration rates. In several investigations, microbial biomass values estimated from ATP concentrations have been further extrapolated to obtain the total respiration rate (i.e., milliliters of O_2 utilized per liter per year) (132, 141, 142, 147). In ATP-based activity rate estimates the assumptions are made that: (i) the respiration rates of all microorganisms

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are identical when expressed in terms of equivalent biomass, (ii) respiration rates have approximately the same temperature coefficient as determined from laboratory studies, and (iii) none of the microorganisms in the sample are nutrient stressed to the extent that respiration is markedly affected (141). Hobbie et al. (132) have applied a respiration factor of 0.25 μl of O_2 per μg of organic C per day at 22°C (and a Q_{10} [increase in the rate of chemical reaction for each 10°C increase in temperature] of 2.0) to estimate total microbial O_2 consumption at a hydrostation in the North Atlantic Ocean. The ATP-derived rates of O_2 consumption in the euphotic zone compared favorably with rates determined by measurements of electron transport system activity and by direct O_2 respirometric methods, but the values were less comparable in deeper waters (132). Williams and Carlucci (334) have compiled a table of deep-sea respiration data estimated by several different methods. The data seem to converge on a value of ~1 μl of O_2 per liter per year, which is close to the value derived from ATP measurements. This positive correlation, however, may be fortuitous, and it should be emphasized that the extrapolation of respiration rates from total ATP biomass data is not justified under most environmental conditions. In theory, ATP measurements cannot be used to estimate metabolic activity, since such an application ignores the well-documented relationships between cell size and specific metabolic rate. In addition, several lines of evidence and extensive data have previously been presented to suggest that there is no a priori relationship between biomass and metabolic activity. Although the turnover rate of the ATP pool is positively correlated with metabolic activity (O_2 uptake) and growth, it has also been shown to be independent of the steady-state intracellular ATP concentrations.

(v) ATP and growth rates. Several investigators have attempted to estimate in situ growth rates of plankton populations in the sea by measuring the rate of increase of particulate ATP with time (41, 285, 288, 289).

Sheldon and Sutcliffe (285) have calculated growth rates (i.e., generation times) of near-surface Sargasso Sea microplankton populations to be 3.35 (±0.35) h in February and 2.91 (±0.21) h in July as determined by increases in ATP during a 4- to 7-h incubation period. These rates of production were approximately an order of magnitude greater than the rates measured previously by the standard ^{14}C primary production method. However, no mention was made of diel changes in ATP per cell, which may have partially contributed to their observed ATP changes with time. Therefore, despite the sound theoretical basis for using ATP to estimate the rate of net accumulation of biomass (i.e., net growth rate), the likelihood of temporal fluctuations in intracellular ATP concentrations suggests that this approach must be used with caution under natural field conditions.

Sieburth and co-workers (41, 288, 289) have estimated the growth rates of North Atlantic picoplankton (<3-μm fraction) by measuring changes in particulate ATP with time. Representative water samples were placed into diffusion culture chambers and were continuously nourished with seawaters pumped up from specific depths in the water column (e.g., oxygen minimum, chl *a* maximum). Their results indicated bacterial growth rates approaching two orders of magnitude greater than estimates based on [^{14}C]glucose and [^{14}C]glycolate uptake measurements (288). Periodic decreases in total particulate ATP (i.e., difference in ATP concentration/difference in time < 0) were concluded to be the result of fluctuations in cellular EC_A and not due to actual losses of particulate carbon. The diurnal pattern of growth was found to be discontinuous, with the greatest increase in total particulate ATP occurring in the late afternoon to early evening and in the midmorning hours (41). This diel growth pattern has been correlated with variations in the flux of dissolved carbohydrates and presumably with phytoplankton excretion.

(vi) Downward vertical flux of ATP in the ocean. It must be emphasized that our current understanding and interpretation of biological oceanographic processes are based primarily upon the results of static measurements of the spatial distributions of biologically important parameters and indices. Traditionally, the oceanic environment has been sampled using various types of water bottles. However, within recent years, the importance of numerically less abundant large particles has been recognized. These larger particles are not usually collected (in a statistical sense) by current sampling procedures, even though they may represent the dominant class of suspended particles in the sea. In situ particle collectors of various dimensions and configurations are currently being developed and deployed to assess the impact of these particles on the structure and trophic organization of communities in the marine environment. Preliminary measurements of the downward vertical flux of ATP in the northeast Pacific Ocean indicate that microbial cells associated with the sedimenting particles contribute from 1.4 to 15.4 (0 to 400 m) to >100 (1,550 m) times more biomass carbon than is measured by static

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sampling techniques (D. Fellows, D. Karl, and G. Knauer, submitted for publication). Furthermore, the sedimenting particles are very different from the "background" materials, both in terms of their chemical composition and the ratio of living to nonliving carbon. The rapidly sinking particles have lower C/N ratios (G. Knauer and J. Martin, unpublished data) and higher ratios of living to total carbon (D. Fellows, D. Karl, and G. Knauer, unpublished data). The potential metabolic significance of this downward vertical flux of viable microorganisms associated with large particles may require us to alter our present views concerning the production and cycling of carbon in the open ocean.

Terrestrial and aquatic decomposition studies. Plant litter in terrestrial ecosystems and particulate organic detritus in aquatic environments represent significant sources of organic carbon and therefore are important in the trophic organization and energy flow of the respective ecosystems. However, this detrital material is generally not directly grazed by higher organisms, but rather enters a microbe-based food web before becoming available to higher trophic levels (94). The decomposition rate and nutritive changes that occur during the growth and succession of the colonized microbial communities on detrital materials have been studied in attempts to assess the overall trophic role of detritus.

Most of the biochemical changes that occur during the decomposition process (e.g., changes in total dry weight, C/N ratios, and total protein) are the direct results of microbial colonization, growth, and succession. Many investigators believe that the "nutritive value" or "food quality" of the detritus is directly related to the quantity of associated microorganisms and their total community metabolism. Quantitative analyses of ATP associated with organic detritus offer a convenient measure of the standing stock of microbial cells.

E. B. Haines and R. Hanson (J. Exp. Mar. Biol. Ecol, in press) have examined the decomposition of detritus from three salt-marsh plants incubated in a P-enriched seawater microcosm. Of the various experimental conditions examined, the greatest rate of decrease in ash-free dry weight and increase in ATP occurred under N-supplemented aerobic conditions. An efficient conversion of detritus to microbial carbon was observed, ranging from 19% for decomposing *Salicornia* to 64% for *Spartina* detritus.

Knauer and Ayers (193) have monitored changes in C, N, chl *a*, and ATP during the decomposition of *Thalassia testudinum* leaves in a continuous-flow seawater microcosm. Throughout the 52-day experiment, there was a significant correlation between organic C and total ATP ($r = 0.97$), indicating that the transfer of plant C to microbial C was tightly coupled. They suggested that ATP measurements, when used in conjunction with C and N data, can yield a simple quantitative estimation of the nutritional value of the particle-microbe complex.

K. R. Tenore and R. B. Hanson (Limnol. Oceanogr., in press) have recently studied the qualities of several different sources of detritus at various stages of decomposition as food sources for the marine polychaete *Capitella*. In each case, both the microbial biomass (based on A_T measurements) and the total heterotrophic activity changed with the age of the detritus. They also reported that the maximum incorporation of detritus into *Capitella* biomass coincided with that stage of decomposition exhibiting maximum microbial biomass and metabolic activity as measured by oxygen consumption.

Morrison et al. (242) have followed the colonization, outgrowth, and succession of microbial communities associated with allochthonous plant litter in Apalachicola Bay, Fla. The initial colonization was dominated by bacteria as revealed by high muramic acid-to-ATP ratios (242). With increasing incubation time, more complex microorganisms became dominant, as evidenced by progressive decreases in the muramic acid/ATP ratios. These biochemical indices were confirmed by scanning electron microscopy of the plant litter (242) and by changes in the pattern of biosynthesis of characteristic microbial lipids (190).

Bobbie et al. (30) have compared the biodegradability of slash pine needles with that of morphologically similar polyvinyl chloride Christmas tree needles. Following a 14-week incubation in seawater, the slash pine detritus exhibited ATP levels, oxygen uptake, alkaline phosphatase and phosphodiesterase activities, and muramic acid concentration which were 2 to 10 times higher than those of the polyvinyl chloride controls. Furthermore, the polyvinyl chloride needles maintained higher muramic acid/ATP ratios, indicating a paucity of eucaryotic microorganisms. They concluded that biodegradable substrata support microbial communities that are three to five times more active than inert surfaces of comparable surface area.

Lopez et al. (225) have examined the assimilation of *Spartina* litter by adult amphipods of the genus *Orchestria* as a function of total microbial biomass. By assuming a C/ATP ratio of 285 and an N/ATP ratio of 63, they were able to calculate the percentages of C and N in the living and nonliving portions of the detritus. A

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chemical comparison of decomposing *Spartina* with the fecal pellets enabled an estimation of the relative degradabilities of the two components. Their results indicated decreases of 2.1, 15.9, and 60.5% for total C, N, and ATP, respectively. These data clearly demonstrate a preferential removal of living microorganisms from the detritus. It was also shown that the highest concentration of ATP per milligram of plant litter and the highest rates of microbial activity and growth all occurred during conditions of highest grazing intensity (225). This result indicates that grazing has a significant effect on the likelihood of microbial colonization and growth and thus on the rate of decomposition and the overall nutritional quality of the detritus.

Wastewater treatment, disinfectant control, and pollution assessment. The successful operation of municipal sewage and industrial wastewater treatment plants, the effective processing of potable water supplies, and the impact of thermal pollution and chemical pollution on natural ecosystems all require detailed microbiological investigations. Controlled treatment of wastewaters is one of the oldest, largest, and most applied aspects of environmental microbiology. In common with other applications in microbial ecology, wastewater treatment requires methods for rapid and reliable determinations of microbial biomass and activity. ATP measurements have recently and successfully been used in several activated sludge treatment plants (36, 155, 198, 219, 254, 277, 328). Kucnerowicz and Verstraete (198) have observed significant correlations between ATP concentration and substrate removal rate ($r = 0.96$, $p \leq 1\%$), both of which are important parameters for regulating the inflow of wastwater and return of sludge to maintain maximum plant efficiency.

Levin et al. (219) have used ATP measurements as the primary criterion for regulating the mixed liquor biomass in a 20×10^7-gallon/day (ca. 76-Ml/day)-flow sewage treatment plant and have concluded that "ATP control" provided a rapid and logical method for maintaining an optimum waste-to-microorganism ratio. Hysert et al. (155) arrived at a similar conclusion when they used ATP measurements to monitor the operation of a 3.5×10^5-gallon/day (ca. 1.3-Ml/day) brewery wastewater treatment plant. Patterson and co-workers (36, 254) have further supported the use of ATP measurements in the activated sludge process by demonstrating that ATP values were constant during normal operational conditions, but that ATP concentrations responded rapidly to changes in temperature, dissolved oxygen, substrate loading, pH, and heavy-metal concentrations. Therefore, in addition to providing an indication of biomass, ATP measurements may also be useful for monitoring changes in the metabolic activity of activated sludge.

The rate of disinfectant addition to wastewaters or potable water supplies is generally determined by specific bacterial culture enumeration techniques. Tifft and Spiegel (319) have proposed the use of ATP measurements for disinfection control. They observed a decrease in total particulate ATP with the addition of Cl_2 or ClO_2 which paralleled the results from the more traditional indices of total and fecal coliform bacteria. After disinfection treatment there was a significant correlation between ATP and total coliforms ($r = 0.84$) and between ATP and fecal coliforms ($r = 0.76$) for total ATP values in the range of 0 to 1.5 μg/liter. They concluded that decisions regarding sanitary water quality based on ATP measurements would be as sound as those based on current coliform enumeration criteria (319).

Several investigations have attempted to assess the impact of urbanization and industrialization on the quality of surrounding water masses (28, 43, 59, 89, 106, 206, 266, 327). ATP measurements have served as one of several criteria for evaluating the environmental response to pollutant discharge. ATP bioassays cannot yield absolute measures of water quality, per se, since the quality of the environment will depend upon species composition, diversity, and metabolic rates as well as total biomass. Eppley et al. (89) have found evidence of eutrophication in waters around the Point Loma and Whites Point sewage outfalls in southern California by discovering increased concentrations of chl *a*, ATP, particulate carbon, and rates of primary production. This response was attributed to the increased supply of inorganic nutrients (especially N) to the otherwise nutrient-depleted waters. However, no increased nutrient levels were ever observed in the outfall area, suggesting that the phytoplankton populations were efficient scavengers of sewage-derived N and P. Eppley et al. (89) found no toxic effects of the sewage effluent on phytoplankton growth, and, in fact, the sewage-enriched waters were regarded as stimulatory.

Laws and Redalje (206) have also evaluated the effects of sewage enrichment on the phytoplankton populations of Kaneohe Bay, Hawaii. Their data indicated a nearly eightfold decline in living particulate organic carbon between the outfall station and the extreme northwest sector of the bay. However, it was determined that the algal populations at all stations along the southeast (outfall)-to-northwest Kaneohe Bay tran-

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sect were nutrient saturated. Even at the outfall station, greater than 80% of the nitrogen required for phytoplankton growth was contributed by in situ recycling. They concluded that the decline in living particulate organic carbon along the transect (towards the northwest station) was due to dilution of bay water by offshore oligotrophic seawaters, and therefore the high concentrations of phytoplankton adjacent to the outfall could not be attributed to sewage enrichment. This study emphasized the necessity and importance of a thorough investigation of the environment before a valid assessment of pollutants can be achieved.

Environmental Adenylate Energy Charge

The well-established correlation between cellular EC_A and metabolic or growth potentials of individual organisms in theory provides a framework for estimating metabolic potentials of naturally occurring microbial populations. Since most microorganisms studied to date have control systems to maintain the relative molar concentrations of the adenine nucleotides (expressed as EC_A) within narrow limits during normal growth, marked decreases in EC_A may reflect environmental stress or other conditions which interfere with the normal functioning of the cells. In this regard, both the rate of protein synthesis and the potential for cellular biosynthesis have been shown to be much more closely correlated with changes in EC_A values than with fluctuations in the absolute concentrations of intracellular ATP, ADP, or AMP (311). The potential applications of EC_A measurements in experimental microbial ecology have been discussed by several investigators (28, 68, 148, 180, 333, 338).

There are, however, several limitations to the usefulness of the EC_A concept in general, and especially in its application to environmental studies. The first major concern is one of methodology. Since each adenine nucleotide has slightly different adsorption and reaction characteristics, three sets of internal standards must be included in each extract to assess and correct for individual nucleotide losses. The occurrence of soluble (i.e., cell-free) ADP and AMP must also be considered, especially when direct injection techniques are used or when sediment or soil samples are processed. Furthermore, difficulties have been reported in the determination of AMP, especially at concentrations typically encountered in most environmental extracts. Karl and Holm-Hansen (180), however, recently described a method to maximize the enzymatic conversion of AMP to ATP by increasing the ATP concentration in the sample extract.

Another concern is the validity of "community" EC_A measurements. Most microbial assemblages are undoubtedly composed of organisms in a variety of physiological and metabolic states. When the populations are extracted, a mass-weighted mean EC_A is measured. Consequently, it is extremely difficult, if not impossible, to interpret most community EC_A measurements. For example, if the measured EC_A is 0.6, the strict interpretation of the adenylate metabolic control hypothesis would lead one to conclude that the population was stressed, senescent, or incapable of cellular biosynthesis. However, a portion of the population may be growing at μ_{max} with an intracellular EC_A of ~0.8, and the remainder may be inactive (EC_A ~0.5). Undoubtedly, the most successful (and unequivocal) ecological applications of EC_A measurements are those in which the mean community EC_A values are $\geq$0.8, indicating that probably most of the microbial assemblage is potentially capable of cellular biosynthesis. Future studies combining microautoradiography with community EC_A measurements will help to determine the percentage of active cells and render this technique more useful.

Finally it must be reemphasized that the community EC_A cannot be used to estimate in situ growth rates or rates of cellular metabolism, since each parameter may vary significantly at fixed EC_A values. Attempts to compare the relative metabolic activities of different microbial assemblages will require additional estimates of respiration, carbon turnover, and growth.

Despite these shortcomings, environmental EC_A measurements have provided useful information concerning the relative growth potentials and probable metabolic statuses of several naturally occurring microbial populations. Karl and Holm-Hansen (180) have measured the concentrations of ATP, ADP, and AMP and have calculated the corresponding EC_A values for water samples collected in the Southern California Bight (Fig. 4). The EC_A values ranged from 0.5 to 0.89, with peak values corresponding to the subsurface maxima of ATP and chl *a* concentrations. Surface (1 to 10 m) water samples were found to have depressed EC_A values (~0.65 to 0.75) relative to the ratios measured at the chl *a* maximum. It has been suggested that this lower growth potential is the result of ultraviolet light stress or inorganic nutrient depletion or both. A similar effect was also noted in the Caribbean Sea, where reduced rates of stable RNA synthesis corroborated the interpretation of metabolically stressed cells within the surface layer of the ocean (175). An additional significant and recurring trend in seawater EC_A deter-

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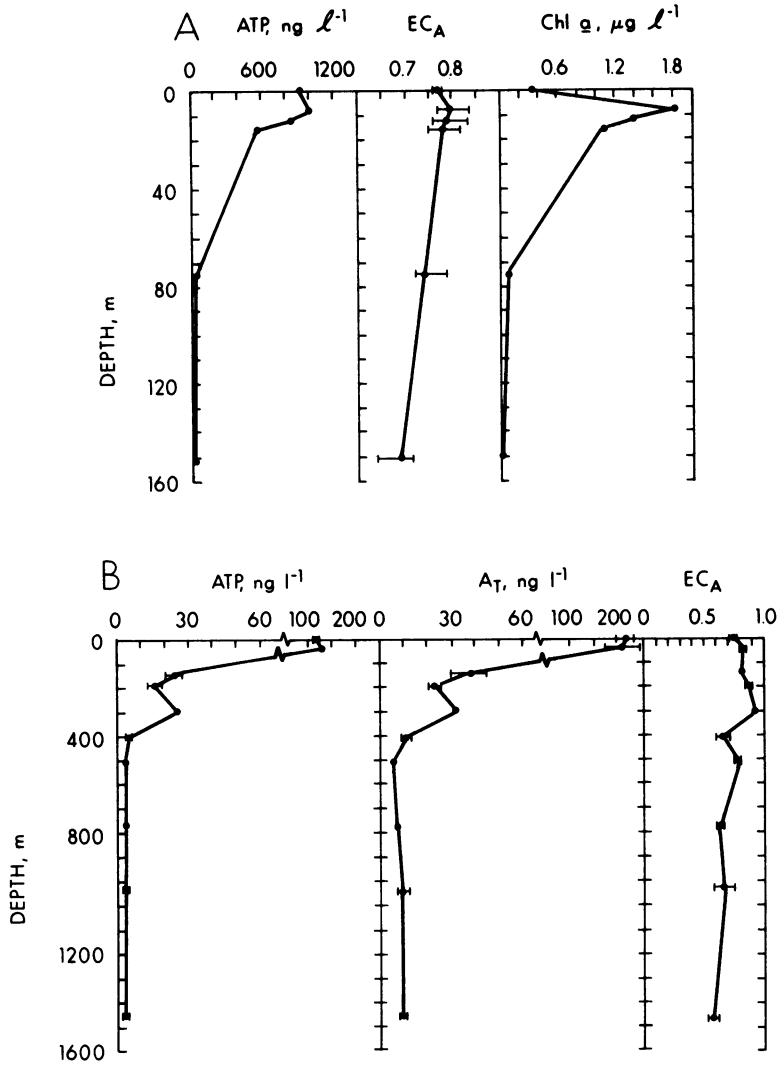


FIG. 4. *Vertical distribution of ATP, A_T, chl a, and EC_A in waters of the Southern California Bight. The data were obtained (A) at a station approximately 5 km offshore in water of 200 m (33°15' N, 117°41' W) and (B) at a station approximately 100 km offshore in water of 1,850 m. From Karl and Holm-Hansen (180).*

minations is the observed discontinuity in EC_A values at a depth of ~400 to 600 m. This discontinuity tends to sepaate microorganisms into communities with relatively high EC_A ratios (~0.7 to 0.9) above this depth and relatively low community ratios (~0.5 to 0.7) below this depth (175, 180, 333), suggesting a physiological or metabolic alteration in populations at depths of >500 m such that the community potential for cellular biosynthesis is reduced. Nonetheless, a community EC_A of 0.5 to 0.7 is indicative of either a senescent population or an association of actively growing cells ($EC_A > 0.8$) and dead or dying cells ($EC_A < 0.5$). The observation that in situ stable RNA synthesis continues in microbial assemblages within a portion of the water column where the EC_A is <0.8 (175), however, tends to support the latter hypothesis. Wiebe and Bancroft (333) have reported that environmental EC_A values and community densities vary independently, such that the potential for microbial growth in the ocean is not necessarily correlated with in situ biomass. Karl (175) has recently provided additional data by measuring rates of stable RNA synthesis to further support this contention.

The most extensive ecological investigation involving the use of EC_A measurements is a recent report by Witzel (338) wherein 356 water samples from several deep lakes in northern

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Germany were studied. His results indicated that the EC_A values ranged from 0.16 to 0.97. The highest EC_A values were measured in samples collected in the epilimnion. In deepwater samples (mostly from anoxic hypolimnia), EC_A values were generally <0.6. The relative frequency distribution showed two preferential groups, one with an EC_A of ~0.55 (0.4 to 0.65) and the other with an EC_A of ~0.75 (0.7 to 0.85). EC_A values of <0.3 or >0.85 were rare (338).

Karl (171) has measured the vertical distribution of ATP, A_T, and EC_A at several stations in the western basin of the Black Sea. Samples collected from the O_2-H_2S interface zone (~125 m) revealed a vertically restricted layer (~20 to 30 m)of cells with EC_A values of >0.8 (Fig. 5). These data suggest that the entire microbial population inhabiting the chemocline is capable of cellular biosynthesis, thereby supporting the Egunoff hypothesis for the existence of a metabolically active "bacterial plate" in the Black Sea. Within the anoxic portions of the water column, ATP concentrations were 5 to 10 times greater than in oxygenated oceanic environments of comparable depth, and both ATP and EC_A values increased with increasing water depth from ~600 m (Fig. 5). The deepwater EC_A data suggest that the increase in ATP below ~600 m is the result of in situ activity, implying a vertical gradient in specific organics required for growth and metabolism (171).

Measurement of EC_A values has also been applied to studies of marine sediments. H. R. Skjoldal (unpublsihed data) has reported a mean EC_A of 0.81 for marine sediments in western Norway. In general, the EC_A values decreased with increasing sediment depth, suggesting a lower potential for microbial community metabolism in the deeper sedimentary layers. The correlation between ATP concentration and EC_A was poor ($r = 0.28$), again supporting the argument that there is no a priori or obligatory relationship between biomass and in situ metabolic activity. R. Christensen and A. Devol (submitted for publication) have recently compared ATP and A_T concentrations and the corresponding EC_A values of continental shelf sediments off Washington to those of coastal Puget Sound. Although the magnitudes of the ATP and A_T values were similar at both locations, the mean EC_A values of the shelf microbial communities were lower than those calculated for communities inhabiting the sound. They concluded that the microorganisms of the shelf habitat were in a depressed physiological state relative to those in Puget Sound, and they have supported these conclusions with data on benthic respiration rates and excess interstitial alkalinity and NH_4^+ concentrations.

The most useful environmental application of the EC_A index may be in assessing the impact of pollution on selected components of an ecosys-

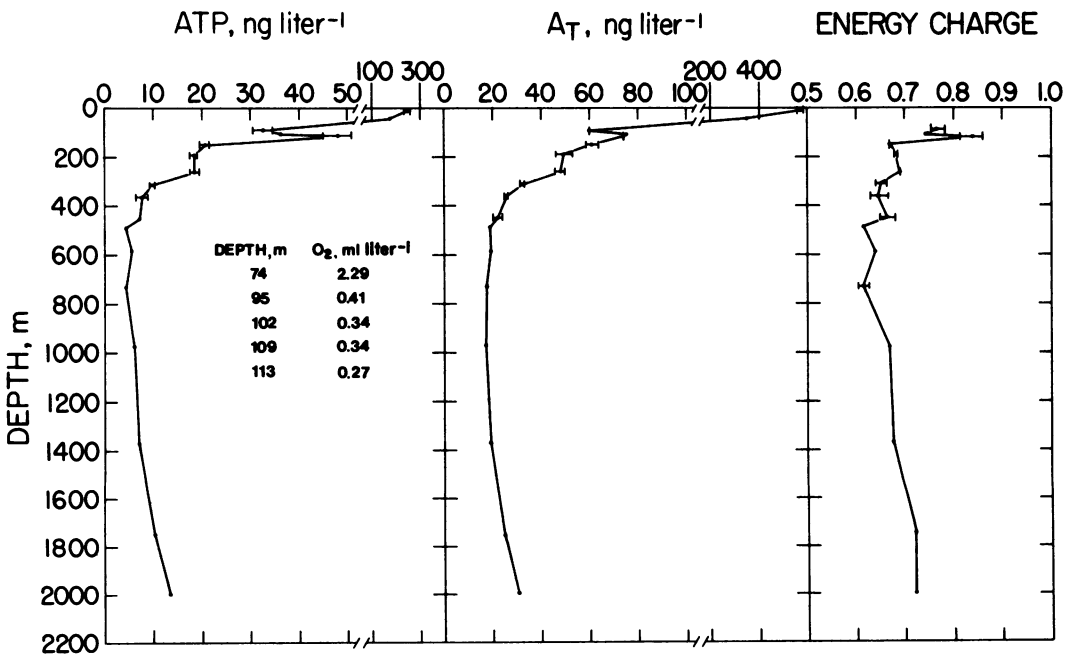


FIG. 5. *Vertical distribution of ATP, A_T, and EC_A for the water column at a station located in the western basin of the Black Sea (42°50' N, 33°00' E). Total water depth 2,160 m. From Karl (171).*

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tem. The method currently used for assessing the biological effects of environmental waste disposal is to conduct 50% lethal dose bioassays with key representatives from the natural assemblage of organisms. It has been suggested that a knowledge of the adenylate systems of key or indicator species with known response to particular environmental conditions may provide a convenient measure of the ecological "health" of a given ecosystem (267). Additionally, EC_A measurements may facilitate the detection of subtle sublethal effects of pollution or natural environmental stress on selected aquatic organisms, although additional laboratory and field data are needed before any recommendation of this approach can be made.

Guanosine 5'-Triphosphate and Cellular Biosynthesis

The guanine nucleotides, GTP and guanosine tetraphosphate, have been shown to exert a major influence on the rates of protein and RNA syntheses, respectively. As mentioned previously, GTP is required for the activation and interconversion of precursors for bacterial cell wall biosynthesis, for DNA replication (as deoxy-GTP) and RNA transcription, and for the initiation, the aminoacyl transfer RNA binding, and the translocation processes of protein synthesis. This indirect coupling of cellular energy during biosynthesis suggests that the total flux through the GTP pool may be correlated with cellular growth rates. Accordingly, it has been reported that the intracellular GTP pool sizes fluctuate in direct proportion to the demand for biosynthetic energy (107, 169, 174, 296, 297). This response is presumably a regulatory mechanism for maintaining saturating levels of GTP at all sites for protein biosynthesis (i.e., the ribosomes), with the number of ribosomes increasing in proportion to the growth rate (249). Karl (169, 174) has indicated that the GTP/ATP ratios of microorganisms were positively correlated with cellular growth rates. He found that the intracellular GTP/ATP ratios were much higher in growing bacteria (range, ~0.5 to 1.7) than in eucaryotes (range, ~0.1 to 0.35), presumably due to their innate potential for much higher specific rates of protein biosynthesis. Karl concluded that quantitative determinations of GTP/ATP ratios in environmental sample extracts may be useful for estimating and comparing community growth rates in naturally occurring microbial assemblages (169, 174).

The presence of GTP (and other nonadenine NTPs) in environmental extracts can be detected in one of several ways. Crude preparations of firefly luciferase will react with GTP, uridine 5'-triphosphate, inosine 5'-triphosphate, and, to a lesser extent, cytidine 5'-triphosphate to produce light. The reaction kinetics with these NTPs are different from the ATP-dependent kinetics, and therefore a semiquantitative assessment can be achieved by simply examining a plot of light emission versus time. In general, most environmental sample extracts that are assayed by integrated light flux determination yield "ATP" values that are higher than peak light emission data from the same sample. The difference (i.e., difference in [ATP] = integrated [ATP] − peak [ATP]), when normalized to the peak ATP concentration, may yield overestimates from 30 to 250% of the actual ATP concentrations when using crude luciferase preparations (169, 174). Moreover, it has been demonstrated that the difference in ATP concentration is significantly correlated with the GTP concentration ($r = 0.96$ for sediments [174], $r = 0.92$ for seawater samples [169]), suggesting that differences in ATP concentration may be used as relative estimates of the GTP concentrations in environmental nucleotide extracts. It should be emphasized, however, that the actual extent of NTP reactivity is extremely variable from one enzyme preparation to the next, which mitigates the possibility of a precise quantitative assessment of GTP by this approach. More recently, Karl (170) has devised a rapid and sensitive method for the measurement of guanine ribonucleotides (GTP, guanosine 5'-diphosphate, and guanosine 5'-monophosphate) present in mixed nucleotide extracts. The technique requires no special equipment or facilities beyond those already used for routine adenine nucleotide analyses and may be easily integrated into many ongoing research programs.

An inclusive summary of environmental GTP determinations is presented in Table 12. The vertical distribution of GTP at a hydrostation in the Southern California Bight exhibited vertical trends similar to those of ATP, with the highest concentrations at the surface, a rapid decrease with depth between 50 and 175 m, and a more gradual decrease in concentration with increasing water depth (169; Table 12). The GTP/ATP ratios ranged from 0.10 to 0.19. Throughout the water column, organisms of <10 μm had substantially higher GTP/ATP ratios (range, 0.35 to 0.40), indicating greater in situ rates of protein biosynthesis. The GTP/ATP ratios measured in the surface waters of the Black Sea and at a hydrostation located on the Galápagos Rift were identical to those obtained for surface waters off southern California (Table 12). However, the anoxic portion of the Black Sea (depths of >125 m), and the deep-sea hydrothermal vents on the

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TABLE 12. *GTP/ATP ratios in environmental samples*

Sample description	Concn[a]		GTP/ATP	Reference
	GTP	ATP		
Southern California seawater (32°43.2′ N, 117°30.1′ W)[b]				
10 m, total	55.7[c]	312	0.18	169
10 m, <10 μm	21.8[c]	58	0.32	
100 m, total	31.2[c]	22	0.14	
100 m, <10 μm	6.3[c]	16.5	0.38	
500 m, total	2.5[c]	11.8	0.22	
500 m, <10 μm	1.9[c]	4.5	0.42	
Southern California seawater (33°30.1′ N, 119°20.0′ W)				
5 m	82	550	0.15	169
50 m	68	523	0.13	
200 m	8.5	65	0.13	
500 m	5.0	26	0.19	
1,000 m	2.6	20	0.13	
1,550 m	2.5	20.8	0.12	
Black Sea seawater (42°50.0′ N, 33°00.0′ E)				
10 m	68	462	0.15	169
75 m	12.7	89	0.14	
123 m	16.6	41.4	0.40	
195 m	14.6	35.3	0.41	
489 m	3.6	9.1	0.40	
974 m	3.0	12.0	0.25	
1,992 m	6.4	26.2	0.24	
Galapagos Rift seawater (00°47.0′ N, 86°08′ W)				
50 m	46	286	0.16	187
500 m	<0.6	8	<0.075	
Hydrothermal vents (2,500 m)	832	968	0.86	
Catalina Island (Calif.) sediments (33°21′ N, 118°30′ W), low tide				
0–1 cm	620	892	0.70	174
5 cm	96	93	1.03	
10 cm	38	24	1.58	
Ocean Beach (Calif.) sediments (32°45′ N, 117°16′ W), low tide				
0–1 cm	208	355	0.59	174
5 cm	247	359	0.69	
10 cm	142	187	0.76	

[a] GTP and ATP concentrations are expressed as picomoles per liter for seawaters, and picomoles per cm^3 for sediments.

[b] Total, seawater filtered onto a Reeve Angel 984-H filter disk; <10 μm, 10-μm Nitex filtrate filtered onto a Reeve Angel 984-H filter disk.

[c] [GTP] = [GTP + uridine 5′-triphosphate] × 0.53 (169).

Galápagos Rift (~2,500 m) exhibited significantly higher GTP/ATP ratios, implying elevated rates of cellular biosynthesis and growth. At the hydrothermal vents, the GTP/ATP value was 0.86, indicative of extremely rapid in situ growth. By comparison, GTP/ATP ratios from a variety of southern California intertidal sediments ranged from 0.40 to 1.87, with an overall mean of 0.69 (187; Table 12).

Although GTP/ATP ratios in environmental extracts may be useful for detecting areas of elevated microbial growth and for comparing relative rates of community biosynthesis within natural microbial populations, it must be emphasized that these measurements reflect mean community estimates. After more extensive laboratory investigations have been conducted on the nucleotide levels in a wider range of representative microorganisms, it may be possible to translate GTP/ATP ratios directly into absolute rates of protein synthesis.

Cyclic Adenosine 3′,5′-Monophosphate

The ubiquity of cAMP in bacteria, phytoplankton, and metazoans has led to an extensive investigation of its biological function. The role of cAMP is regulatory, but not essential (270); the intracellular cAMP concentration varies

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with conditions of growth and with the energetic state of the cells. In bacteria, cAMP accumulates when cultures are limited by carbon (322), and in phytoplankton, cAMP levels have been correlated to inorganic nutrient availability (D. Francko and R. Wetzel, unpublished data). Tomkins (322) has designated cAMP an important metabolic symbol, and, consequently, measurements of cAMP concentrations in nature may contribute important information regarding the growth states of naturally occurring microbial populations.

J. W. Ammerman and F. Azam (Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, N62, p. 189) have reported the presence of dissolved cAMP in seawater at concentrations of up to 10 pmol/liter, and D. White (personal communication) has detected cAMP in nucleotide extracts from estuarine sediments. Francko and Wetzel (unpublished data) have recently investigated seasonal variations in particulate and dissolved cAMP in two lakes of differing trophic status. The concentrations of both cAMP fractions varied during the seasons, between the two lakes, and between the littoral and pelagic zones of a given lake (Francko and Wetzel, unpublished data). Dissolved cAMP levels were similar to those found in the media of phytoplankton cultures, although considerable seasonal variation was evident. Increased cellular cAMP levels coincided with increased phytoplankton population density, and a linear relationship was apparent between particulate cAMP and primary production in the hypereutrophic ecosystem. Seasonal changes in dissolved and particulate cAMP were correlated with phytoplankton community succession. Clearly, additional work is necessary to fully understand the metabolic and ecological significance of cAMP, but the preliminary data presented by Francko and Wetzel certainly warrant further investigations.

Nucleotide Metabolism and Growth

Dissolved nucleotides and microbial heterotrophy. The ubiquitous occurrence of D-ATP (and other nucleotides and nucleotide derivatives) in aquatic environments and constant steady-state production from grazing and autolysis pose questions concerning the ultimate fate(s) of these molecules in natural ecosystems. When radiolabeled ATP, AMP, adenosine, or adenine is added to a sample of seawater or freshwater, there is a rapid disappearance of radioactivity from solution. Azam and Hodson (15) considered three possible mechanisms for adenine nucleotide turnover in nature: (i) spontaneous hydrolysis, (ii) enzymatic hydrolysis, and (iii) microbial uptake and assimilation. Hulett (153) has calculated the half-life of ATP in artificial seawater at 21°C to be ~8 years, so it is unlikely that chemical hydrolysis is a significant pathway. The presence of extracellular adenosine triphosphatase, nucleotidase, acid and alkaline phosphatases, and additional nonspecific orthophosphoric-monoester phosphohydrolases in aquatic environments provides a mechanism for enzymatic degradation of soluble nucleotides. Finally, it is well known that many diverse groups of microorganisms can assimilate exogenous adenine supplies, in preference to de novo synthesis, if present in the growth medium. ATP uptake is competitively inhibited by ADP, AMP, adenosine, and adenine, suggesting a common transport system (134). Azam and Hodson (15) found that more than 95% of the radioactivity from [*U*-^{14}C]ATP was assimilated into cellular macromolecules with a negligible amount lost through respiration.

When freshwater or oceanic samples are incubated with [^{3}H]ATP (or other radiolabeled adenine derivatives) rapid turnover rates are observed for the dissolved adenine pools (15, 145, 175). Size fractionation studies have indicated that ~80% of the total uptake is due to microorganisms of <0.6 μm (15), suggesting that adenine may be an important molecule in the metabolism of aquatic bacteria. Rates of D-ATP and glucose uptakes and calculated turnover times for the steady-state soluble pools have been calculated to be of similar magnitude (15, Riemann, unpublished data). S. M. McGrath and C. W. Sullivan (unpublished data) have recently proposed the use of [^{3}H]AMP as a model compound to monitor the kinetics of uptake and to identify the compartments involved in the cycling of total adenylates by marine planktonic communities.

Nucleic acid biosynthesis. The uptake and incorporation of exogenous radiolabeled nucleosides have been used extensively in laboratory and field investigations to estimate the rates of nucleic acid biosynthesis (14, 37, 175, 204, 303, 315, 320). The observed correlations between RNA and DNA synthesis, protein syntheses, and cell growth are so universally acceptable that they lend themselves to the analysis of complex microbial populations, such as one finds in nature. Brock (37) pioneered the use of thymidine incorporation as an estimate of DNA synthesis and cell growth in natural assemblages of microorganisms. His experimental approach used the marine microbe *Leucothrix mucor* as a model organism. Data relating the rate of accumulation of radioactive cells (via autoradiography) to growth rate were obtained over a wide range of growth rates in order to establish a numerical constant (1% of the cells became radiolabeled in 0.002 generation) for estimating

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the growth rate in nature. In situ doubling times of *L. mucor* were then calculated to range from 660 to 685 min for a wide range of habitats (37).

Other investigators have used thymidine uptake, or occasionally uptake of uridine, as an indirect measure of microbial activity in soils (315), sediments (320), seawater (14, 204), and aerosol samples (303). Although thymidine uptake and ATP-biomass correlated well (320) and are expected to vary in direct proportion to metabolic activity and growth, the rate of labeling of cellular macromolecules cannot be assumed to be equal to the rate of nucleic acid biosynthesis. The actual rate will vary according to the specific transport mechanisms and dilution of the label by exogenous and endogenous pools before incorporation into nucleic acids. Therefore, quantitative measurements of the rates of DNA or RNA synthesis also require determinations of the specific radioactivities of the intracellular precursor pools. Even for the estimation of relative rates, failure to measure (and correct for) the intracellular specific radioactivities might lead to serious errors and gross misinterpretations of ecological data.

Karl (175) has recently devised a technique for measuring rates of stable RNA synthesis in naturally occurring populations of microorganisms. The procedure is based upon the uptake and incorporation of exogenous radiolabeled adenine into cellular RNA (Fig. 6). To calculate absolute rates of synthesis, measurements of the specific radioactivities of the intracellular ATP pools (precursor to adenine in RNA) and of the total amounts of radioactivity incorporated into RNA per unit time are required. The theoretical considerations and experimental details of this new approach to the study of microbial growth rates in nature have been presented and discussed in detail previously (175).

This technique has been successfully used to demonstrate that the microbial populations associated with the intermediate-depth oxygen minimum layer of the Columbian Basin (~450 m) maintain a higher specific rate of RNA synthesis (i.e., RNA synthesis per unit of biomass) than the assemblages found elsewhere in the entire water column (175). From production and standing stock measurements, a mean turnover rate (generation time) of 2.2 to 6.5 h was derived for this intermediate-depth microbial population, assuming steady-state conditions (i.e., change in biomass/change in time = 0). More recent, and as yet unpublished, data from this laboratory have expanded the application of this experimental approach to provide additional information pertaining to the rates of DNA synthesis (and, therefore, DNA/RNA synthesis rate ratios), adenine nucleotide pool turnover, production and interconversion of guanine ribonucleotides, and exogenous pool sizes of adenine-containing compounds in natural environments.

CONCLUDING STATEMENT

The growth of microorganisms is ultimately dependent upon innumerable enzyme reactions and the successful integration of many metabolic control systems. Consequently, the implementation of any unifying principles will undoubtedly yield numerous exceptions when applied to the study of microorganisms under a variety of growth regimes. This is especially true for extrapolating the results of laboratory studies to complex natural ecosystems where the various microbial assemblages have evolved to accept the diverse challenges of nature.

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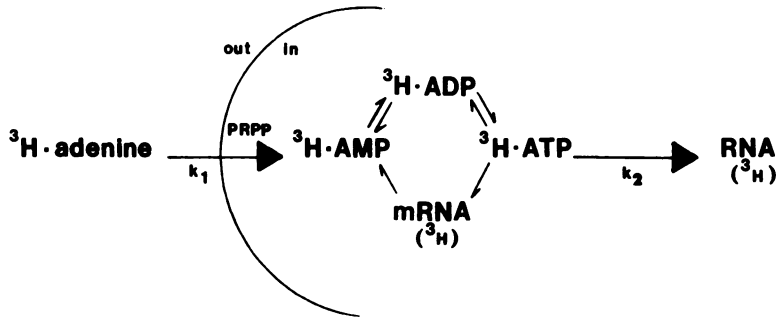


FIG. 6. *Schematic representation of the method presented by Karl (175) for quantitative determinations of rates of stable RNA synthesis in seawater samples. Exogenous [^{3}H]adenine is transported across the cell membrane at rate k_1 and is converted into [^{3}H]AMP, [^{3}H]ADP, and [^{3}H]ATP, the latter serving as the immediate precursor to stable RNA. By measuring total [^{3}H]RNA produced after a set period of incubation and by directly measuring the intracellular specific activity of the ATP pool, absolute rates of RNA synthesis can be calculated (i.e., picomoles of adenine incorporated into RNA per liter per hour). mRNA, Messenger RNA. From Karl (175).*

TABLE 13. *Environmental nucleotide fingerprinting*

Index	Rationale	Ecological information
ATP concn	1. Present in all cells 2. C/ATP ratio is fairly constant	Estimate of biomass
EC_A	1. EC_A is a linear measure of stored metabolic energy (range, 0.0–1.0) 2. EC_A appears to regulate cellular metabolism 3. Biosynthesis and growth are possible only at an EC_A of ≥ 0.8	Measure of community potential for growth (i.e., mean community metabolic status)
GTP/ATP ratio	1. GTP is required for protein synthesis 2. GTP/ATP ratio is positively correlated with growth rate	Estimate of community growth rate
[^{3}H]adenine assimilation and stable RNA synthesis	1. [^{3}H]adenine → [^{3}H]ATP → [^{3}H]-RNA 2. ^{3}H assimilation is proportional to rate of RNA synthesis 3. Rate of RNA synthesis is proportional to growth rate	Measure of community growth rate
DNA/RNA synthesis ratio	1. Intracellular DNA concentration is independent of growth rate 2. Stable RNA concentration is proportional to growth rate 3. Ratio of rate of DNA synthesis to rate of stable RNA synthesis provides indication of growth rate	Measure of community growth rate

In recent years, investigations in the field of experimental microbial ecology have returned to the fundamental principles of biochemistry, physiology, and cell biology. One area of significant progress has been in the study of cellular nucleotide concentrations and rates of nucleotide metabolism. The development of specific and sensitive techniques for quantitative measurements of intracellular nucleotides has enabled researchers to investigate the distribution of microbial biomass and to estimate the rates of protein and nucleic acid biosyntheses, nucleotide metabolism, metabolic activity, and growth within naturally occurring populations of microorganisms. This review has attempted to provide the physiological rationale and analytical procedures for considering measurements of cellular nucleotides in microbial ecology and to provide the theoretical framework and data base of laboratory results necessary for the proper interpretation of environmental nucleotide measurements. The approach of nucleotide fingerprinting (Table 13) is strongly recommended for field investigations in order to obtain corroborative data relating to the in situ physiological states of naturally occurring microbial assemblages. I hope that future ecological studies of microorganisms will adopt, refine, and ultimately expand the scope of the fingerprint to include additional nucleotide-based metabolic indices.

ACKNOWLEDGMENTS

I am most grateful to numerous colleagues for their helpful comments and overwhelming response to my plea for preprints, manuscripts, and unpublished data; without their assistance it would have been impossible to present an up-to-date account of this subject. I am indebted to D. Craven, R. Enos, C. Winn, and D. Wong for criticism, editorial appraisal, and helpful suggestions for the improvement of this manuscript. D. Wong also assisted in the preparation of the tables and was largely responsible for compiling the lengthy reference list; her enthusiasm and dedication to this project are deeply appreciated. I thank the various authors and publishers for their permission to reproduce previously published figures and tables. L. Agawa, J. Katsura, and V. Sen cheerfully and flawlessly typed several drafts of this review. Stimulating discussions with F. Azam, A. Carlucci, R. Eppley, O. Holm-Hansen, H. Jannasch, G. Knauer, P. LaRock, E. Laws, and K. Nealson have provided new ideas and insights into possible future applications of nucleotide measurements in microbial ecology. Finally, I wish to express my most sincere aloha to my former mentors, P. LaRock (Florida State University, Tallahassee) and O. Holm-Hansen (Scripps Institution of Oceanogra-

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phy, La Jolla, Calif.) for kindling my interests and supervising my earlier studies in the area of cellular nucleotides and nucleotide metabolism.

The original studies conducted in my laboratory were supported, in part, by research grants OCE 78-18926, OCE 78-20721, and OCE 78-25446 awarded through the Biological Oceanography section of the National Science Foundation.

LITERATURE CITED

1. **Afghan, B. K., R. S. Tobin, and J. F. Ryan.** 1977. An improved method for quantitative measurement of ATP and its application to measure microbial activity in natural waters, activated sludges and sediments, p. 349-389. *In* G. A. Borun (ed.), Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego, Calif.
2. **Aftring, R. P., and B. F. Taylor.** 1979. Assessment of microbial fouling in an ocean thermal energy conversion experiment. Appl. Environ. Microbiol. **38**:734-739.
3. **Allen, P. D., III.** 1973. Development of the luminescence biometer for microbial detection. Dev. Ind. Microbiol. **14**:67-73.
4. **Andersen, K. B., and K. von Meyenburg.** 1977. Changes of nicotinamide adenine nucleotides and adenylate energy charge as regulatory parameters of the metabolism in *Escherichia coli*. J. Biol. Chem. **252**:4151-4156.
5. **Anderson, J. M., K. Hori, and M. J. Cormier.** 1978. A bioluminescence assay for PAP (3',5'-diphosphoadenosine) and PAPS (3'-phosphoadenylyl sulfate). Methods Enzymol. **57**: 244-257.
6. **Ånséhn, S., S. Granström, H. Höjer, L. Nilsson, E. Åkesson, A. Lundin, and A. Thore.** 1976. *In vitro* effects on *Candida albicans* of amphotericin B combined with other antibiotics. Scand. J. Infect. Dis. Suppl. **9**:62-66.
7. **Atkinson, D. E.** 1969. Regulation of enzyme function. Annu. Rev. Microbiol. **23**:47-68.
8. **Atkinson, D. E.** 1971. Adenine nucleotides as universal stoichiometric metabolic coupling agents. Adv. Enzyme Regul. **9**:207-219.
9. **Atkinson, D. E.** 1977. Cellular energy control. Trends Biochem. Sci. **2**:N198-N200.
10. **Atkinson, D. E.** 1977. Cellular energy metabolism and its regulation. Academic Press, Inc., New York.
11. **Atkinson, D. E., P. J. Roach, and J. S. Schwedes.** 1975. Metabolite concentrations and concentration ratios in metabolic regulation. Adv. Enzyme Regul. **13**:393-411.
12. **Atkinson, D. E., and G. M. Walton.** 1967. Adenosine triphosphate conservation in metabolic regulation. J. Biol. Chem. **242**:3239-3241.
13. **Ausmus, B. S.** 1973. The use of the ATP assay in terrestrial decomposition studies. Bull. Ecol. Res. Comm. (Stockholm) **17**:223-234.
14. **Azam, F., J. R. Beers, L. Campbell, A. F. Carlucci, O. Holm-Hansen, F. M. H. Reid, and D. M. Karl.** 1979. Occurrence and metabolic activity of organisms under the Ross Ice Shelf, Antarctica, at Station J9. Science **203**: 451-453.
15. **Azam, F., and R. E. Hodson.** 1977. Dissolved ATP in the sea and its utilization by marine bacteria. Nature (London) **267**:696-698.
16. **Azam, F., and R. E. Hodson.** 1977. Size distribution and activity of marine microheterotrophs. Limnol. Oceanogr. **22**:492-501.
17. **Azam, F., and R. E. Hodson.** 1977. Use of ATP as an indicator of bacterial biomass in seawater, p. 109-125. *In* G. A. Borun (ed.), Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego, Calif.
18. **Bächi, B., and L. Ettlinger.** 1973. Influence of glucose on adenine nucleotide levels and energy charge in *Acetobacter aceti*. Arch. Mikrobiol. **93**:155-164.
19. **Bagnara, A. S., and L. R. Finch.** 1972. Quantitative extraction and estimation of intracellular nucleoside triphosphates of *Escherichia coli*. Anal. Biochem. **45**:24-34.
20. **Bagnara, A. S., and L. R. Finch.** 1973. Relationships between intracellular contents of nucleotides and 5-phosphoribosyl 1-pyrophosphate in *Escherichia coli*. Eur. J. Biochem. **36**: 422-427.
21. **Balch, N.** 1972. ATP content of *Calanus finmarchicus*. Limnol. Oceanogr. **17**:906-908.
22. **Ball, W. J., Jr., and D. E. Atkinson.** 1975. Adenylate energy charge in *Saccharomyces cerevisiae* during starvation. J. Bacteriol. **121**: 975-982.
23. **Ballantyne, A. J., M. J. Sharpe, and D. L. Lee.** 1978. Changes in the adenylate energy charge of *Nippostrongylus brasiliensis* and *Nematodirus battus* during the development of immunity to these nematodes in their hosts. Parasitology **76**:211-220.
24. **Bancroft, K., E. A. Paul, and W. J. Wiebe.** 1976. The extraction and measurement of adenosine triphosphate from marine sediments. Limnol. Oceanogr. **21**:473-480.
25. **Bauchop, T., and S. R. Elsden.** 1960. The growth of microorganisms in relation to their energy supply. J. Gen. Microbiol. **23**:457-469.
26. **Beck, C., J. Ingraham, O. Maaløe, and J. Neuhard.** 1973. Relationship between the concentration of nucleoside triphosphates and the rate of synthesis of RNA. J. Mol. Biol. **78**:117-121.
27. **Beis, I., and E. A. Newsholme.** 1975. The contents of adenine nucleotides, phosphagens and some glycolytic intermediates in resting muscles from vertebrates and invertebrates. Biochem. J. **152**:23-32.
28. **Benon, P., F. Blan, B. Bourgade, P. David, R. Kantin, M. Leveau, J. C. Romano, and D. Sautriot.** 1977. Impact de la pollution sur un écosystème méditerranéen côtier. II. Relations entre la composition spécifique des populations phytoplanctoniques, et les taux de pigments et de nucléotides adényliques (ATP, ADP, AMP). Int. Rev. Gesamten Hydrobiol. **62**:631-648.
29. **Bény, M., and M. Dolivo.** 1976. Separation of firefly luciferase using an anion exchanger. FEBS Lett. **70**:167-170.

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30. **Bobbie, R. J., S. J. Morrison, and D. C. White.** 1978. Effects of substrate biodegradability on the mass and activity of the associated estuarine microbiota. Appl. Environ. Microbiol. **35**:179–184.
31. **Booth, C.** 1975. Instrument developments and data processing for the ATP photometer, p. 104–129. *In* G. A. Borun (ed.), ATP Methodology Seminar. SAI Technology Co., San Diego, Calif.
32. **Bornefeld, T., and W. Simonis.** 1974. Effects of light, temperature, pH, and inhibitors on the ATP level of the blue-green alga *Anacystis nidulans*. Planta **115**:309–318.
33. **Borun, G. A. (ed.).** 1975. ATP Methodology Seminar. SAI Technology Co., San Diego, Calif.
34. **Borun, G. A. (ed.).** 1977. Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego, Calif.
35. **Bottomley, P. J., and W. D. P. Stewart.** 1976. The measurement and significance of ATP pools in filamentous blue-green algae. Br. Phycol. J. **11**:69–82.
36. **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.
37. **Brock, T. D.** 1967. Bacterial growth rate in the sea: direct analysis by thymidine autoradiography. Science **155**:81–83.
38. **Brock, T. D.** 1971. Microbial growth rates in nature. Bacteriol. Rev. **35**:39–58.
39. **Brody, S.** 1972. Regulation of pyridine nucleotide levels and ratios in *Neurospora crassa*. J. Biol. Chem. **247**:6013–6017.
40. **Bulleid, N. C.** 1978. An improved method for the extraction of adenosine triphosphate from marine sediment and seawater. Limnol. Oceanogr. **23**:174–178.
41. **Burney, C. M., K. M. Johnson, D. M. Lavoie, and J. McN. Sieburth.** 1979. Dissolved carbohydrate and microbial ATP in the North Atlantic: concentrations and interactions. Deep-Sea Res. **26A**:1267–1290.
42. **Burnison, K.** 1975. Microbial ATP studies. Int. Ver. Theor. Angew. Limnol. Ver. **19**:286–290.
43. **Caparello, D. M., and P. A. LaRock.** 1975. A radioisotope assay for the quantification of hydrocarbon biodegradation potential in environmental samples. Microb. Ecol. **2**:28–42.
44. **Cashel, M., and J. Gallant.** 1969. Two compounds implicated in the function of the *RC* gene of *Escherichia coli*. Nature (London) **221**:838–841.
45. **Cavari, B.** 1976. ATP in Lake Kinneret: indicator of microbial biomass or of phosphorus deficiency? Limnol. Oceanogr. **21**:231–236.
46. **Champiat, D., and J. P. Larpent.** 1978. Aquatic microflora enumeration by means of adenylic nucleotides dosage. Hydrobiologia **58**:37–42.
47. **Chapman, A. G., and D. E. Atkinson.** 1977. Adenine nucleotide concentrations and turnover rates. Their correlation with biological activity in bacteria and yeast. Adv. Microb. Physiol. **15**:253–306.
48. **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.
49. **Chappelle, E.** 1975. Determination of bacterial flavins by bacterial bioluminescence, p. 62–103. *In* G. A. Borun (ed.), ATP Methodology Seminar. SAI Technology Co., San Diego, Calif.
50. **Chappelle, E. W., and G. V. Levin.** 1968. Use of the firefly bioluminescent reaction for rapid detection and counting of bacteria. Biochem. Med. **2**:41–52.
51. **Chappelle, E. W., and G. L. Picciolo.** 1971. Assay of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) using the bacterial luciferase reaction. Methods Enzymol. **18**:381–385.
52. **Chappelle, E. W., and G. L. Picciolo.** 1975. Analytical applications of bioluminescence and chemiluminescence, NASA SP-388. National Aeronautics and Space Administration, Washington, D.C.
53. **Chappelle, E. W., G. L. Picciolo, and J. W. Deming.** 1978. Determination of bacterial content in fluids. Methods Enzymol. **57**:65–72.
54. **Chappelle, E. W., G. L. Picciolo, H. Okrend, R. R. Thomas, J. Deming, and D. A. Nibley.** 1977. Significance of luminescence assays for characterizing bacteria, p. 611–629. *In* G. A. Borun (ed.), Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego, Calif.
55. **Chervin, M. B.** 1978. Assimilation of particulate organic carbon by estuarine and coastal copepods. Mar. Biol. **49**:265–275.
56. **Christian, R. R., K. Bancroft, and W. J. Wiebe.** 1975. Distribution of microbial adenosine triphosphate in salt marsh sediments at Sapelo Island, Georgia. Soil Sci. **119**:89–97.
57. **Chrzanowski, T. H., L. H. Stevenson, and B. Kjerfve.** 1979. Adenosine 5'-triphosphate flux through the North Inlet marsh system. Appl. Environ. Microbiol. **37**:841–848.
58. **Clark, J. R., D. I. Messenger, K. L. Dickson, and J. Cairns, Jr.** 1978. Extraction of ATP from Aufwuchs communities. Limnol. Oceanogr. **23**:1055–1059.
59. **Clegg, T. J., and J. L. Koevenig.** 1974. The effect of four chlorinated hydrocarbon pesticides and one organophosphate pesticide on ATP levels in three species of photosynthesizing freshwater algae. Bot. Gaz. (Chicago) **135**: 368–372.
60. **Cohen, L., and R. Kaplan.** 1977. Accumulation of nucleotides by starved *Escherichia coli* cells as a probe for the involvement of ribonucleases in ribonucleic acid degradation. J. Bacteriol. **129**:651–657.
61. **Cole, H. A., J. W. T. 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.
62. **Conklin, A. R., Jr., and A. N. MacGregor.**

Downloaded from mmbr.asm.org at Arizona Health Sciences Library on May 20, 2019

1972. Soil adenosine triphosphate: extraction, recovery and half-life. Bull. Environ. Contam. Toxicol. 7:296–300.

63. **Conn, A. F., M. S. Rice, and D. Hagel.** 1977. Ultra clean heat exchanges—a critical OTEC requirement, p. VII-11–VII-14. *In* G. E. Ioup (ed.), Proceedings of the Fourth Annual Conference on Ocean Thermal Energy Conversion (OTEC). New Orleans University, New Orleans.
64. **Costantini, M. G., R. Zippel, and E. Sturani.** 1977. Levels of the ribonucleoside triphosphates and rate of RNA synthesis in *Neurospora crassa*. Biochim. Biophys. Acta **476:** 272–278.
65. **Cunningham, H. W., and R. G. Wetzel.** 1978. Fulvic acid interferences on ATP determinations in sediments. Limnol. Oceanogr. **23:**166–173.
66. **Daly, K. F.** 1974. The luminescence biometer in the assessment of water quality and wastewater analysis. Am. Lab. **6:**38–44.
67. **Daumas, R., and M. Fiala.** 1969. Evaluation de la matière organique vivante dans les eaux marines par la mesure de l'adénosine triphosphate. Mar. Biol. **3:**243–246.
68. **Daumas, R. A.** 1973. Évaluation de la teneur en adenosine triphosphate des organismes marins: possibilités actuelles et limites de la méthode. Tethys **5:**71–80.
69. **Dawe, L. L., and W. R. Penrose.** 1978. "Bactericidal" property of seawater: death or debilitation? Appl. Environ. Microbiol. **35:**829–833.
70. **Decker, K., and S. Pfitzer.** 1972. Determination of steady-state concentrations of adenine nucleotides in growing *C. kluyveri* cells by biosynthetic labeling. Anal. Biochem. **50:**529–539.
71. **DeLuca, M.** 1976. Firefly luciferase. Adv. Enzymol. Relat. Areas Mol. Biol. **44:**37–68.
72. **DeLuca, M., and W. D. McElroy.** 1978. Purification and properties of firefly luciferase. Methods Enzymol. **57:**3–15.
73. **DeLuca, M., J. Wannlund, and W. D. McElroy.** 1979. Factors affecting the kinetics of light emission from crude and purified firefly luciferase. Anal. Biochem. **95:**194–198.
74. **DeLuca, M. A. (ed.).** 1978. Methods in enzymology, vol. 57. Academic Press, Inc., New York.
75. **Deming, J. W., M. W. McGarry, E. W. Chappelle, and G. L. Picciolo.** 1977. Procedures for the quantitation of bacteria in fluids: firefly luciferase ATP assay used with concentration procedures, p. 465–490. *In* G. A. Borun (ed.), Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego, Calif.
76. **Derr, R. F., and L. Zieve.** 1972. Adenylate energy charge: relation to guanylate energy charge and the adenylate kinase equilibrium constant. Biochem. Biophys. Res. Commun. **49:**1385–1390.
77. **Devol, A. H., T. T. Packard, and O. Holm-Hansen.** 1976. Respiratory electron transport activity and adenosine triphosphate in the oxygen minimum of the eastern tropical North Pacific. Deep-Sea Res. **23:**963–973.
78. **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.
79. **Dietzler, D. N., C. J. Lais, and M. P. Leckie.** 1974. Simultaneous increases of the adenylate energy charge and the rate of glycogen synthesis in nitrogen-starved *Escherichia coli* W4597(K). Arch. Biochem. Biophys. **160:**14–25.
80. **Dietzler, D. N., M. P. Leckie, and C. J. Lais.** 1973. Rates of glycogen synthesis and the cellular levels of ATP and ADP during exponential growth and the nitrogen-limited stationary phase of *Escherichia coli* W4597(K). Arch. Biochem. Biophys. **156:**684–693.
81. **Dolezal, J., and F. Kaprálek.** 1976. Physiological characteristics of chemostatically grown *Citrobacter freundii* as a function of the specific growth rate and type of nutrient limitation. Folia Microbiol. (Prague) **21:**168–177.
82. **Driessche, T. V. D.** 1970. Circadian variation in ATP content in the chloroplasts of *Acetabularia mediterranea*. Biochim. Biophys. Acta **205:** 526–528.
83. **DuPont Instruments.** 1972. Bacteria in cutting oils and hydraulic fluids. Biometer Flashes no. 760BF3.
84. **Echetebu, C. O., and P. Plesner.** 1977. The pool of ribonucleoside triphosphates in synchronized *Tetrahymena pyriformis*. J. Gen. Microbiol. **103:**389–392.
85. **Edwards, C., M. Statham, and D. Lloyd.** 1975. The preparation of large-scale synchronous cultures of the trypanosomatid, *Crithidia fasciculata*, by cell-size selection: changes in respiration and adenylate charge through the cell-cycle. J. Gen. Microbiol. **88:**141–152.
86. **Edwards, S. W., and D. Lloyd.** 1977. Changes in oxygen uptake rates, enzyme activities, cytochrome amounts and adenine nucleotide pool levels during growth of *Acanthamoeba castellanii* in batch culture. J. Gen. Microbiol. **102:** 135–144.
87. **Eigener, U.** 1975. Adenine nucleotide pool variations in intact *Nitrobacter winogradskyi* cells. Arch. Microbiol. **102:**233–240.
88. **Eiland, F.** 1979. An improved method for determination of adenosine triphosphate (ATP) in soil. Soil. Biol. Biochem. **11:**31–35.
89. **Eppley, R. W., A. F. Carlucci, O. Holm-Hansen, D. Kiefer, J. J. McCarthy, and P. M. Williams.** 1972. Evidence for eutrophication in the sea near southern California coastal sewage outfalls—July; 1970. Calif. Coop. Oceanic Fish. Invest. Rep. **16:**74–83.
90. **Eppley, R. W., W. G. Harrison, S. W. Chisholm, and E. Stewart.** 1977. Particulate organic matter in surface waters off southern California and its relationship to phytoplankton. J. Mar. Res. **35:**671–696.
91. **Erkenbrecher, C. W., S. J. Crabtree, Jr., and L. H. Stevenson.** 1976. Computer-assisted

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analysis of adenosine triphosphate data. Appl. Environ. Microbiol. **32**:451–454.

92. **Ernst, W., and H. Goerke.** 1974. Adenosin-5'-triphosphat (ATP) in sedimenten und nematoden der nordostatlantischen Tiefsee. "Meteor" Forschungsergeb. Reihe C **18**:35–42.
93. **Falkowski, P. G., and D. P. Stone.** 1975. Nitrate uptake in marine phytoplankton: energy sources and the interaction with carbon fixation. Mar. Biol. **32**:77–84.
94. **Fenchel, T. M., and B. B. Jørgensen.** 1977. Detritus food chains of aquatic ecosystems: the role of bacteria. Adv. Microb. Ecol. **1**:1–58.
95. **Ferguson, R. L., and M. B. Murdoch.** 1973. Microbiology of the Newport River Estuary. Annual Report to Atomic Energy Commission by the Atlantic Estuarine Fisheries Center. U.S. Deptartment of Commerce, Beaufort, N.C.
96. **Ferguson, R. L., and M. B. Murdoch.** 1975. Microbial ATP and organic carbon in sediments of the Newport River Estuary, North Carolina. Estuarine Res. **1**:229–250.
97. **Ferguson, R. L., and P. Rublee.** 1976. Contribution of bacteria to standing crop of coastal plankton. Limnol. Oceanogr. **21**:141–145.
98. **Fertel, R., and B. Weiss.** 1978. Measurement of the activity of cyclic nucleotide phosphodiesterases with firefly luciferin-luciferase coupled assay systems. Methods Enzymol. **57**:94–106.
99. **Fiil, N. P., K. von Meyenburg, and J. D. Friesen.** 1972. Accumulation and turnover of guanosine tetraphosphate in *Escherichia coli*. J. Mol. Biol. **71**:769–783.
100. **Fink, K.** 1975. Fluctuations in deoxyribo- and ribonucleoside triphosphate pools during the mitotic cycle of *Physarum polycephalum*. Biochim. Biophys. Acta **414**:85–89.
101. **Forrest, W. W.** 1965. Adenosine triphosphate pool during the growth cycle in *Streptococcus faecalis*. J. Bacteriol. **90**:1013–1016.
102. **Forrest, W. W., and D. J. Walker.** 1965. Control of glycolysis in washed suspensions of *Streptococcus faecalis*. Nature (London) **207**: 46–48.
103. **Forrest, W. W., and D. J. Walker.** 1971. The generation and utilization of energy during growth. Adv. Microb. Physiol. **5**:213–274.
104. **Forsberg, C. W., and K. Lam.** 1977. Use of adenosine 5'-triphosphate as an indicator of the microbiota biomass in rumen contents. Appl. Environ. Microbiol. **33**:528–537.
105. **Foster, J. W., and A. G. Moat.** 1980. Nicotinamide adenine dinucleotide biosynthesis and pyridine nucleotide cycle metabolism in microbial systems. Microbiol. Rev. **44**:83–105.
106. **Fox, J. L., and M. S. Moyer.** 1973. Some effects of a power plant on marine microbiota. Chesapeake Sci. **14**:1–10.
107. **Franzen, J. S., and S. B. Binkley.** 1961. Comparison of the acid-soluble nucleotides in *Escherichia coli* at different growth rates. J. Biol. Chem. **236**:515–519.
108. **Fromm, H.** 1977. Cellular energy control. Trends. Biochem. Sci. **2**:N198–N200.
109. **Fynn, G. H., and J. A. Davison.** 1976. Adenine nucleotide pool and energy charge during growth of a tyrothricin-producing strain of *Bacillus brevis*. J. Gen. Microbiol. **94**:68–74.
110. **Garen, A., and C. Levinthal.** 1960. A fine-structure genetic and chemical study of the enzyme alkaline phosphatase of *E. coli*. I. Purification and characterization of alkaline phosphatase. Biochim. Biophys. Acta **38**:470–483.
111. **Gerlach, S. A.** 1978. Food-chain relationships in subtidal silty sand marine sediments and the role of meiofauna in stimulating bacterial productivity. Oecologia (Berlin) **33**:55–69.
112. **Goerke, H., and W. Ernst.** 1975. ATP content of estuarine nematodes: contribution to the determination of meiofauna biomass by ATP measurements, p. 683–691. *In* H. Barnes (ed.), Proceedings of the Ninth European Marine Biology Symposium. Aberdeen University Press, Aberdeen, Scotland.
113. **Goldberg, N. D., and M. K. Haddox.** 1977. Cyclic GMP metabolism and involvement in biological regulation. Annu. Rev. Biochem. **46**: 823–896.
114. **Goldbeter, A.** 1974. Modulation of the adenylate energy charge by sustained metabolic oscillations. FEBS Lett. **43**:327–330.
115. **Gordon, D. C., Jr., P. J. Wangersky, and R. W. Sheldon.** 1979. Detailed observations on the distribution and composition of particulate organic material at two stations in the Sargasso Sea. Deep-Sea Res. **26A**:1083–1092.
116. **Green, A. A., and W. D. McElroy.** 1956. Crystalline firefly luciferase. Biochim. Biophys. Acta **20**:170–176.
117. **Gutekunst, R. R., J. W. Deming, B. D. Jaffee, K. Marshall, G. L. Picciolo, E. W. Chappelle, and C. G. Schrock.** 1977. Luminescence assays for the determination of microbial levels: clinical results using centrifugation and filtration procedures with the firefly luciferase ATP assay-detection of bacteria in wine and blood and antibiotic susceptibility testing, p. 491–524. *In* G. A. Borun (ed.), Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego, Calif.
118. **Haddock, B. A., and W. A. Hamilton (ed.).** 1977. Microbial energetics. Twenty-Seventh Symposium of the Society for General Microbiology. Cambridge University Press, Cambridge.
119. **Haggerty, C., E. Jablonski, L. Stav, and M. DeLuca.** 1978. Continuous monitoring of reactions that produce NADH and NADPH using immobilized luciferase and oxido-reductases from *Beneckea harveyi*. Anal. Biochem. **88**:162–173.
120. **Hall, R. H.** 1971. The modified nucleosides in nucleic acids. Columbia University Press, New York.
121. **Hamilton, R. D., and O. Holm-Hansen.** 1967. Adenosine triphosphate content of marine bacteria. Limnol. Oceanogr. **12**:319–324.

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122. **Hammerstedt, R. H.** 1973. An automated method for ATP analysis utilizing the luciferin-luciferase reaction. Anal. Biochem. **52**:449–455.
123. **Hanson, R. B., and J. Snyder.** 1979. Microheterotrophic activity in a salt-marsh estuary, Sapelo Island, Georgia. Ecology **60**:99–107.
124. **Hanson, R. B., and W. J. Wiebe.** 1977. Heterotrophic activity associated with particulate size fractions in a *Spartina alterniflora* salt-marsh estuary, Sapelo Island, Georgia, USA, and the continental shelf waters. Mar. Biol. **42**: 321–330.
125. **Harrison, D. E. F.** 1976. The regulation of respiration rate in growing bacteria. Adv. Microb. Physiol. **14**:243–313.
126. **Harrison, D. E. F., and P. K. Maitra.** 1969. Control of respiration and metabolism in growing *Klebsiella aerogenes*. The role of adenine nucleotides. Biochem. J. **112**:647–656.
127. **Healey, F. P.** 1979. Short-term responses of nutrient-deficient algae to nutrient addition. J. Phycol. **15**:289–299.
128. **Heber, U.** 1974. Metabolite exchange between chloroplasts and cytoplasm. Annu. Rev. Plant Physiol. **25**:393–421.
129. **Henderson, J. F., and A. R. P. Paterson.** 1973. Nucleotide metabolism, an introduction. Academic Press, Inc., New York.
130. **Herbland, A., and J. Pages.** 1975. L'adénosine triphosphate (ATP) dans le dome de Guinée distribution verticale et signification écologique. Cah. ORSTOM (O.R.S.T.O.M.) Ser. Oceanogr. **13**:163–169.
131. **Herzberg, M. A., D. A. Klein, and D. C. Coleman.** 1978. Trophic interactions in soils as they affect energy and nutrient dynamics. II. Physiological responses of selected rhizosphere bacteria. Microb. Ecol. **4**:351–359.
132. **Hobbie, J. E., O. Holm-Hansen, T. T. Packard, L. R. Pomeroy, R. W. Sheldon, J. P. Thomas, and W. J. Wiebe.** 1972. A study of the distribution and activity of microorganisms in ocean water. Limnol. Oceanogr. **17**:544–555.
133. **Hobson, P. N., and R. Summers.** 1972. ATP pool and growth yield in *Selenomonas ruminantium*. J. Gen. Microbiol. **70**:351–360.
134. **Hodson, R. E., and F. Azam.** 1977. Determination and biological significance of dissolved ATP in seawater, p. 127–140. *In* G. A. Borun (ed.), Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego, Calif.
135. **Hodson, R. E., O. Holm-Hansen, and F. Azam.** 1976. Improved methodology for ATP determination in marine environments. Mar. Biol. **34**:143–149.
136. **Holm-Hansen, O.** 1969. Determination of microbial biomass in ocean profiles. Limnol. Oceanogr. **14**:740–747.
137. **Holm-Hansen, O.** 1970. Determination of microbial biomass in deep ocean water, p. 287–300. *In* D. W. Hood (ed.), Organic matter in natural waters. Institute of Marine Science, College, Alaska.
138. **Holm-Hansen, O.** 1970. Microbial distribution in ocean water relative to nutrients and food sources, p. 147–155. *In* G. B. Farguhar (ed.), Proceedings of an international symposium on biological sound scattering in the ocean. Maury Center for Ocean Science, Department of the Navy, Washington, D.C.
139. **Holm-Hansen, O.** 1970. ATP levels in algal cells as influenced by environmental conditions. Plant Cell Physiol. **11**:689–700.
140. **Holm-Hansen, O.** 1971. Determination of microbial biomass in deep ocean profiles, p. 197–207. *In* J. D. Costlow (ed.), Fertility of the sea. Gordon and Breech Science Publishers Ltd., London.
141. **Holm-Hansen, O.** 1973. Determination of total microbial biomass by measurement of adenosine triphosphate, p. 73–89. *In* L. H. Stevenson and R. R. Colwell (ed.), Estuarine microbial ecology. University of South Carolina Press, Columbia.
142. **Holm-Hansen, O.** 1973. The use of ATP determinations in ecological studies. Bull. Ecol. Res. Comm. (Stockholm) **17**:215–222
143. **Holm-Hansen, O.** 1974. Review and critique of primary production measurements. Calif. Coop. Oceanic Fish. Invest. Rep. **17**:53–56.
144. **Holm-Hansen, O., F. Azam, L. Campbell, A. F. Carlucci, and D. M. Karl.** 1978. Microbial life beneath the Ross Ice Shelf. Antarct. J. U. S. **13**:129–130.
145. **Holm-Hansen, O., F. Azam, A. F. Carlucci, R. E. Hodson, and D. M. Karl.** 1977. Microbial distribution and activity in and around McMurdo Sound. Antarct. J. U. S. **12**:29–32.
146. **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.
147. **Holm-Hansen, O., R. Hodson, and F. Azam.** 1975. Applications of adenine nucleotide measurements in oceanography, p. 75–87. *In* E. W. Chappelle and G. L. Picciolo (ed.), Analytical applications of bioluminescence and chemiluminescence. NASA SP-388. National Aeronautics and Space Administration, Washington, D.C.
148. **Holm-Hansen, O., and D. Karl.** 1978. Biomass and adenylate energy charge determination in microbial cell extracts and environmental samples. Methods Enzymol. **57**:73–85.
149. **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.
150. **Holm-Hansen, O., and H. W. Paerl.** 1972. The applicability of ATP determination for estimation of microbial biomass and metabolic activity. Mem. Ist. Ital. Idrobiol. Dott Marco de Marchi Pallanza Italy **29**(Suppl.):149–168.
151. **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 me-

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dia. Arch. Miccrobiol. **83**:95–109.
152. **Huennekens, F. M., and H. R. Whiteley.** 1960. Phosphoric acid anhydrides and other energy-rich compounds, p. 107–180. *In* M. Florkin and H. S. Mason (ed.), Comparative biochemistry, a comprehensive treatise. Academic Press, Inc., New York.
153. **Hulett, H. R.** 1970. Non-enzymatic hydrolysis of adenosine phosphates. Nature (London) **225**: 1248–1249.
154. **Huzyk, L., and D. J. Clark.** 1971. Nucleoside triphosphate pools in synchronous cultures of *Escherichia coli*. J. Bacteriol. **108**:74–81.
155. **Hysert, D. W., F. B. Knudsen, N. M. Morrison, and G. Van Gheluwe.** 1979. Application of a bioluminescence ATP assay in brewery wastewater treatment studies. Biotechnol. Bioeng. **21**:1301–1314.
156. **Iwamura, T., T. Kanazawa, and K. Kanazawa.** 1963. Nucleotide metabolism in *Chlorella*. II. Quantitative changes in component-nucleotides of the acid-soluble fraction of algal cells during their life-cycle, p. 587–596. *In* Japanese Society of Plant Physiologists (ed.), Microalgae and photosynthetic bacteria. University of Tokyo Press, Tokyo.
157. **Jablonski, E., and M. DeLuca.** 1976. Immobilization of bacterial luciferase and FMN reductase on glass rods. Proc. Natl. Acad. Sci. U.S.A. **73**:3848–3851
158. **Jablonski, E., and M. DeLuca.** 1978. Immobilization of bacterial luciferase and oxidoreductase and assays using immobilized enzymes. Methods Enzymol. **57**:202–214.
159. **Jassby, A. D.** 1975. An evaluation of ATP estimations of bacterial biomass in the presence of phytoplankton. Limnol. Oceanogr. **20**:646–648.
160. **Jeffers, E. L., R. R. Thomas, R. E. Taylor, G. L. Picciolo, E. W. Chappelle, and J. W. Deming.** 1977. Characteristics of various bacterial concentrator systems for use with luminescence assays, p. 601–610. *In* G. A. Borun (ed.), Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego, Calif.
161. **Jenkinson, D. S., and J. M. Oades.** 1979. A method for measuring adenosine triphosphate in soil. Soil Biol. Biochem. **11**:193–199.
162. **Johnson, R., J. H. Bentile, and S. Cheer.** 1974. Automatic sample injector, its application in the analysis of adenosine triphosphate. Anal. Biochem. **60**:115–121.
163. **Jones, C. W.** 1977. Aerobic respiratory systems in bacteria. Symp. Soc. Gen. Microbiol. **27**:23–59.
164. **Jones, J. G., M. J. L. G. Orlandi, and B. M. Simon.** 1979. A microbiological study of sediments from the Cumbrian lakes. J. Gen. Microbiol. **115**:37–48.
165. **Jones, J. G., and B. M. Simon.** 1977. Increased sensitivity in the measurement of ATP in freshwater samples with a comment on the adverse effect of membrane filtration. Freshwater Biol. **7**:253–260.
166. **Jones, P. C. T.** 1972. Central role for ATP in determining some aspects of animal and plant cell behavior. J. Theor. Biol. **34**:1–13.
167. **Jonsen, J., and S. Laland.** 1960. Bacterial nucleosides and nucleotides. Adv. Carbohydr. Chem. **15**:201–234.
168. **Kaplan, N. O.** 1960. The pyridine coenzymes, p. 105–169. *In* P. D. Boyer, H. Lardy, and K. Myrbäck (ed.), The enzymes, 2nd ed., vol. 3. Academic Press, Inc., New York.
169. **Karl, D. M.** 1978. Occurrence and ecological significance of GTP in the ocean and in microbial cells. Appl. Environ. Microbiol. **36**:349–355.
170. **Karl, D. M.** 1978. A rapid sensitive method for the measurement of guanine ribonucleotides in bacterial and environmental extracts. Anal. Biochem. **89**:581–595.
171. **Karl, D. M.** 1978. Distribution, abundance, and metabolic states of microorganisms in the water column and sediments of the Black Sea. Limnol. Oceanogr. **23**:936–949.
172. **Karl, D. M.** 1978. Determination of GTP, GDP, and GMP in cell and tissue extracts. Methods Enzymol. **57**:85–94.
173. **Karl, D. M.** 1979. Comments on a paper by J. G. Jones and B. M. Simon, "Increased sensitivity in the measurement of ATP in freshwater samples with a comment on the adverse effect of membrane filtration." Freshwater Biol. **9**:283–284.
174. **Karl, D. M.** 1979. Adenosine triphosphate and guanosine triphosphate determinations in intertidal sediments, p. 5–20. *In* C. D. Litchfield and P. L. Seyfried (ed.), Methodology for biomass determinations and microbial activities in sediments, ASTM STP 673. American Society for Testing and Materials, Philadelphia.
175. **Karl, D. M.** 1979. Measurement of microbial activity and growth in the ocean by rates of stable ribonucleic acid synthesis. Appl. Environ. Microbiol. **38**:850–860.
176. **Karl, D. M., and D. B. Craven.** 1980. Effects of alkaline phosphatase activity on nucleotide measurements in aquatic microbial communities. Appl. Environ. Microbiol. **40**:549–561.
177. **Karl, D. M., J. A. Haugsness, L. Campbell, and O. Holm-Hansen.** 1978. Adenine nucleotide extraction from multicellular organisms and beach sand: ATP recovery, energy charge ratios and determination of carbon/ATP ratios. J. Exp. Mar. Biol. Ecol. **34**:163–181.
178. **Karl, D. M., and O. Holm-Hansen.** 1976. Effects of luciferin concentration on the quantitative assay of ATP using crude luciferase preparations. Anal. Biochem. **75**:100–112.
179. **Karl, D. M., and O. Holm-Hansen.** 1977. Adenylate energy charge measurements in natural seawater and sediment samples, p. 141–169. *In* G. A. Borun (ed.), Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego, Calif.
180. **Karl, D. M., and O. Holm-Hansen.** 1978. Methodology and measurement of adenylate energy charge ratios in environmental samples. Mar. Biol. **48**:185–197.
181. **Karl, D. M., and O. Holm-Hansen.** 1978. ATP,

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ADP, and AMP determinations in water samples and algal cultures, p. 197-206. *In* J. A. Hellebust and J. S. Craigie (ed.), Handbook of phycological methods. Cambridge University Press, Cambridge.

182. **Karl, D. M., and P. A. LaRock.** 1975. Adenosine triphosphate measurements in soil and marine sediments. J. Fish. Res. Board Can. **32**:599-607.
183. **Karl, D. M., and P. A. LaRock.** 1975. Adenosine triphosphate measurements in marine sediments: some comments on extraction methodology and analysis, p. 360-393. *In* G. A. Borun (ed.), ATP Methodology Seminar. SAI Technology Co., San Diego, Calif.
184. **Karl, D. M., P. A. LaRock, J. W. Morse, and W. Sturges.** 1976. Adenosine triphosphate in the North Atlantic Ocean and its relationship to the oxygen minimum. Deep-Sea Res. **23**:81-88.
185. **Karl, D. M., P. A. LaRock, and D. J. Shultz.** 1977. Adenosine triphosphate and organic carbon in the Cariaco Trench. Deep-Sea Res. **24**: 105-113.
186. **Karl, D. M., and K. H. Nealson.** 1980. Regulation of cellular metabolism during synthesis and expression of the luminous system in *Beneckea* and *Photobacterium*. J. Gen. Microbiol. 117:357-368.
187. **Karl, D. M., C. O. Wirsen, and H. W. Jannasch.** 1980. Deep-sea primary production at the Galápagos hydrothermal vents. Science **207**:1345-1347.
188. **Kemp, C. W.** 1979. Adenylate energy charge; a method for the determination of viable cell mass in dental plaque samples. J. Dent. Res. **68**:2192-2197.
189. **Khachatourians, G. G., and L. Huzyk.** 1974. Relationship between nucleoside triphosphate pools and DNA replication in the cell cycle of *Escherichia coli*. Can. J. Microbiol. **20**:747-750.
190. **King, J. D., D. C. White, and C. W. Taylor.** 1977. Use of lipid composition and metabolism to examine structure and activity of estuarine detrital microflora. Appl. Environ. Microbiol. **33**:1177-1183.
191. **Klingenberg, M.** 1970. Metabolite transport in mitochondria: an example for intracellular membrane function. Essays Biochem. **6**:119-159.
192. **Klofat, W., G. Picciolo, E. W. Chappelle, and E. Freese.** 1969. Production of adenosine triphosphate in normal cells and sporulation mutants of *Bacillus subtilis*. J. Biol. Chem. **244**: 3270-3276.
193. **Knauer, G. A., and A. V. Ayers.** 1977. Changes in carbon, nitrogen, adenosine triphosphate, and chlorophyll *a* in decomposing *Thalassia testudinum* leaves. Limnol. Oceanogr. **22**:408-414.
194. **Knowles, C. J.** 1977. Microbial metabolic regulation by adenine nucleotide pools. Symp. Soc. Gen. Microbiol. **27**:241-283.
195. **Knowles, C. J., and L. Smith.** 1970. Measurements of ATP levels of intact *Azotobacter vinelandii* under different conditions. Biochim. Biophys. Acta **197**:152-160.
196. **Knust, E. A., E. W. Chappelle, and G. L. Picciolo.** 1975. A comparison of certain extracting agents for extraction of adenosine triphosphate (ATP) from microorganisms for use in the firefly luciferase ATP assay, p. 27-34. *In* E. W. Chappelle and G. L. Picciolo (ed.), Analytical applications of bioluminescence and chemiluminescence. NASA SP-388. National Aeronautics and Space Administration, Washington, D.C.
197. **Koenings, J. P., and F. F. Hooper.** 1973. Organic phosphorus compounds of a northern Michigan bog-lake system. Mich. Acad. **5**:295-310.
198. **Kucnerowicz, F., and W. Verstraete.** 1978. Direct measurement of microbial ATP in activated sludge samples. *In* E. Schram and P. Stanley (ed.), Proceedings of the International Symposium on Analytical Applications of Bioluminescence and Chemiluminescence. Vrije Universiteit, Brussels.
199. **Kudrna, R., and G. Edlin.** 1975. Nucleotide pools and regulation of ribonucleic acid synthesis in yeast. J. Bacteriol. **121**:740-742.
200. **Laffler, R., and J. A. Gallant.** 1974. Stringent control of protein synthesis in *E. coli*. Cell **3**: 47-49.
201. **Lampert, W.** 1978. Release of dissolved organic carbon by grazing zooplankton. Limnol. Oceanogr. **23**:831-834.
202. **Lännergren, C., and H. R. Skjoldal.** 1976. The spring phytoplankton bloom in Lindåspollene, a landlocked Norwegian fjord. Autotrophic and heterotrophic activities in relation to nutrients, p. 363-391. *In* G. Personne and E. Jaspers (ed.), Tenth European Symposium on Marine Biology, vol. 2. Universa Press, Wettern, Belgium.
203. **LaRock, P. A., and D. M. Karl.** 1975. Applications of ATP biomass estimates in several marine ecosystems, p. 563-583. *In* G. A. Borun (ed.), ATP Methodology Seminar. SAI Technology Co., San Diego, Calif.
204. **LaRock, P. A., R. D. Lauer, J. R. Schwarz, K. K. Watanabe, and D. A. Wiesenburg.** 1979. Microbial biomass and activity distribution in an anoxic, hypersaline basin. Appl. Environ. Microbiol. **37**:466-470.
205. **Larsson, C. M., and T. Olsson.** 1979. Firefly assay of adenine nucleotides from algae: comparison of extraction methods. Plant Cell Physiol. **20**:145-155.
206. **Laws, E. A., and D. G. Redalje.** 1979. Effect of sewage enrichment on the phytoplankton population of a subtropical estuary. Pac. Sci. **33**: 129-144.
207. **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.
208. **Lazzarini, R. A., M. Cashel, and J. Gallant.** 1971. On the regulation of guanosine tetra-

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phosphate levels in stringent and relaxed strains of *Escherichia coli*. J. Biol. Chem. **246**: 4381–4385.

209. **Lee, C. C., R. F. Harris, J. K. Syers, and D. E. Armstrong.** 1971. Adenosine triphosphate content of *Selenastrum capricornutum*. Appl. Microbiol. **21**:957–958.
210. **Lee, C. C., R. F. Harris, J. D. H. Williams, D. E. Armstrong, and J. K. Syers.** 1971. Adenosine triphosphate in lake sediments. I. Determination. Soil Sci. Soc. Am. Proc. **35**:82–86.
211. **Lee, Y., I. Jablonski, and M. DeLuca.** 1977. Immobilization of firefly luciferase on glass rods: properties of the immobilized enzyme. Anal. Biochem. **80**:496–501.
212. **Lee, Y. S., and R. G. Crispen.** 1977. Rapid quantitative measurement of drug susceptibility of mycobacteria, p. 219–235. *In* G. A. Borun (ed.), Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego, Calif.
213. **Legendre, L., and Y. Simard.** 1979. Océanographie biologique estivale et phytoplancton dans le sud-est de la baie d'Hudson. Mar. Biol. **52**: 11–22.
214. **Lehninger, A. L.** 1973. Bioenergetics, 2nd ed. W. A. Benjamin, Inc., Menlo Park, Calif.
215. **Leps, W. T., and J. C. Ensign.** 1979. Adenosine triphosphate pool levels and endogenous metabolism in *Arthrobacter crystallopoietes* during growth and starvation. Arch. Microbiol. **122**:61–67.
216. **Leps, W. T., and J. C. Ensign.** 1979. Adenylate nucleotide levels and energy charge in *Arthrobacter crystallopoietes* during growth and starvation. Arch. Microbiol. **122**:69–76.
217. **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. BioScience **14**:37–38.
218. **Levin, G. V., and A. H. Heim.** 1965. Gulliver and Diogenes—exobiological antitheses. Life Sci. Space Res. **3**:105–119.
219. **Levin, G. V., J. R. Schrot, and W. C. Hess.** 1975. Methodology for application of adenosine triphosphate determination in wastewater treatment. Environ. Sci. Technol. **9**:961–965.
220. **Lewenstein, A., and R. Bachofen.** 1978. CO_2-fixation and ATP synthesis in continuous cultures of *Chlorella fusca*. Arch. Microbiol. **116**: 169–173.
221. **Lipmann, F.** 1941. Metabolic generation and utilization of phosphate bond energy. Adv. Enzymol. Relat. Areas Mol. Biol. **1**:99–162.
222. **Lloyd, D., N. A. Morgan, L. John, and S. E. Venables.** 1978. Starvation of *Prototheca zopfii*. J. Gen. Microbiol. **105**:1–10.
223. **Lloyd, D., C. A. Phillips, and M. Statham.** 1978. Oscillations of respiration, adenine nucleotide levels and heat evolution in synchronous cultures of *Tetrahymena pyriformis* ST prepared by continuous-flow selection. J. Gen. Microbiol. **106**:19–26.
224. **London, J., and M. Knight.** 1966. Concentrations of nicotinamide nucleotide coenzymes in microorganisms. J. Gen. Microbiol. **44**:241–254.
225. **Lopez, G. R., J. S. Levinton, and L. B. Slobodkin.** 1977. The effect of grazing by the detritivore *Orchestia grillus* on *Spartina* litter and its associated microbial community. Oecologia (Berlin) **30**:111–127.
226. **Lowry, O. H., J. Carter, J. B. Ward, and L. Glaswer.** 1971. The effect of carbon and nitrogen sources on the level of metabolic intermediates in *Escherichia coli*. J. Biol. Chem. **246**: 6511–6521.
227. **Lundin, A., A. Rickardsson, and A. Thore.** 1977. Substrate and enzyme determinations by continuously monitoring the ATP level by a purified luciferase reagent, p. 205–218. *In* G. A. Borun (ed.), Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego, Calif.
228. **Lundin, A., and A. Thore.** 1975. Comparison of methods for extraction of bacterial adenine nucleotides determined by firefly assay. Appl. Microbiol. **30**:713–721.
229. **Luria, S. E.** 1960. The bacterial protoplasm: composition and organization, p. 1–34. *In* J. C. Gunsalus and R. Y. Stanier (ed.), The bacteria, vol. 1. Academic Press, Inc., New York.
230. **MacLeod, N. H., E. W. Chappelle, and A. M. Crawford.** 1969. ATP assay of terrestrial soils: a test of an exobiological experiment. Nature (London) **223**:267–268.
231. **Mague, T. H., N. M. Weare, and O. Holm-Hansen.** 1974. Nitrogen fixation in the North Pacific Ocean. Mar. Biol. **24**:109–119.
232. **Makman, R. S., and E. W. Sutherland.** 1965. Adenosine 3',5'-phosphate in *Escherichia coli*. J. Biol. Chem. **240**:1309–1314.
233. **Manandhar, M. S. P., and K. Van Dyke.** 1974. Guanosine triphosphate analysis: PEI-thin layer purification and luciferin-luciferase liquid scintillation quantitation. Anal. Biochem. **60**: 122–129.
234. **Manuels, M. W., and H. Postma.** 1974. Measurements of ATP and organic carbon in suspended matter of the Dutch Wadden Sea. Neth. J. Sea Res. **8**:292–311.
235. **Mathis, R. R., and O. R. Brown.** 1976. ATP concentration in *Escherichia coli* durig oxygen toxicity. Biochim. Biophys. Acta **440**:723–732.
236. **Matin, A., and J. C. Gottschal.** 1976. Influence of dilution rate on NAD(P) and NAD(P)H concentrations and ratios in a *Pseudomonas* sp. grown in continuous culture. J. Gen. Microbiol. **94**:333–341.
237. **Mayzaud, P., and S. Taguchi.** 1979. Size spectral and biochemical characteristics of the particulate matter in Bedford Basin. J. Fish. Res. Board Can. **36**:211–218.
238. **McElroy, W. D.** 1947. The energy source for bioluminescence in an isolated system. Proc. Natl. Acad. Sci. U. S. A. **33**:342–345.
239. **McGrew, S. B., and M. F. Mallette.** 1965. Maintenance of *Escherichia coli* and the assimila-

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tion of glucose. Nature (London) **208**:1096–1097.

240. **Mel'nikov, N. S.** 1977. Comparison of the magnitude of the microplankton biomass determined from ATP and by direct microscopy. Oceanology **16**:181–183.
241. **Miović, M. L., and J. Gibson.** 1971. Nucleotide pools in growing *Chromatium* strain D. J. Bacteriol. **108**:954–956.
242. **Morrison, S. J., J. D. King, R. J. Bobbie, R. E. Bechtold, and D. C. White.** 1977. Evidence for microfloral sucession on allochthonous plant litter in Apalachicola Bay, Florida, USA. Mar. Biol. **41**:229–240.
243. **Naiman, R. J., and J. Sibert.** 1977. Annual and diel variations in a small marine bay: interpretation of monitoring data. J. Exp. Mar. Biol. Ecol. **26**:27–40.
244. **Nazar, R. N., H. G. Lawford, and J. T. F. Wong.** 1970. An improved procedure for the extraction and analysis of cellular nucleotides. Anal. Biochem. **35**:305–313.
245. **Neidhardt, F. C., and D. G. Fraenkel.** 1961. Metabolic regulation of RNA synthesis in bacteria. Cold Spring Harbor Symp. Quant. Biol. **26**:63–74.
246. **Niven, D. F., P. A. Collins, and C. J. Knowles.** 1977. Adenylate energy charge during batch culture of *Beneckea natriegens*. J. Gen. Microbiol. **98**:95–108.
247. **Noda, L.** 1973. Adenylate kinase, p. 279–305. *In* P. D. Boyer (ed.), The enzymes, vol. 8. Academic Press, Inc., New York.
248. **O'Brien, R. W., and J. G. Morris.** 1971. Oxygen and the growth and metabolism of *Clostridium acetobutylicum*. J. Gen. Microbiol. **68**:307–318.
249. **Pace, N. R.** 1973. Structure and synthesis of the ribosomal ribonucleic acid of prokaryotes. Bacteriol. Rev. **37**:562–603.
250. **Paerl, H. W., and N. J. Williams.** 1976. The relation between adenosine triphosphate and microbial biomass in diverse aquatic ecosystems. Int. Rev. Gesamten Hydrobiol. **61**:659–664.
251. **Palumbo, A. V., and R. L. Ferguson.** 1978. Distribution of suspended bacteria in the Newport River Estuary, North Carolina. Estuarine Coastal Mar. Sci. **7**:521–529.
252. **Pamatmat, M. M., and H. R. Skjoldal.** 1974. Dehydrogenase activity and adenosine triphosphate concentration of marine sediments in Lindåspollene, Norway. Sarsia **56**:1–12.
253. **Pamatmat, M. M., and H. R. Skjoldal.** 1979. Metabolic activity, adenosine phosphates and energy charge of below ground biomass of *Juncus roemerianus* Schelle and *Spartina alterniflora* Loisel. Estuarine Coastal Mar. Sci. **9**: 79–90.
254. **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.
255. **Paul, E. A., and R. L. Johnson.** 1977. Microscopic counting and adenosine 5'-triphosphate measurement in determining microbial growth in soils. Appl. Environ. Microbiol. **34**:263–269.
256. **Perry, M. J.** 1976. Phosphate utilization by an oceanic diatom in phosphorus-limited chemostat culture and in the oligotrophic waters of the central North Pacific. Limnol. Oceanogr. **21**:88–107.
257. **Perry, W. B., J. T. Boswell, and J. A. Stanford.** 1979. Critical problems with extraction of ATP for bioluminescence assay of plankton biomass. Hydrobiologia **65**:155–163.
258. **Picciolo, G. L., E. W. Chappelle, J. W. Deming, M. A. McGarry, D. A. Nibley, H. Okrend, and R. R. Thomas.** 1976. Applications of luminescent systems to infectious disease methodology. X-726-76-212. Goddard Space Flight Center, Greenbelt, Md.
259. **Picciolo, G. L., E. W. Chappelle, R. R. Thomas, and M. A. McGarry.** 1977. Performance characteristics of a new photometer with a moving filter tape for luminescence assay. Appl. Environ. Microbiol. **34**:720–724.
260. **Picciolo, G. L., R. R. Thomas, J. W. Deming, and E. W. Chappelle.** 1977. Environmental applications of the firefly luciferase ATP assay: flow techniques for monitoring of waste water effluent and of drinking water supplies, p. 547–567. *In* G. A. Borun (ed.), Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego, Calif.
261. **Plesner, P.** 1964. Nucleotide metabolism during synchronized cell division in *Tetrahymena pyriformis*. C. R. Trav. Lab. Carlsberg **34**:1–76.
262. **Ponnamperuma, C., C. Sagan, and R. Mariner.** 1963. Synthesis of adenosine triphosphate under possible primitive earth conditions. Nature (London) **199**:222–226.
263. **Poole, P. K., and I. Salmon.** 1978. The pool sizes of adenine nucleotides in exponentially growing, stationary phase and 2'-deoxyadenosine-synchronized cultures of *Schizosaccharomyces pombe* 972h-. J. Gen. Microbiol. **106**: 153–164.
264. **Pradet, A.** 1967. Etude des adenosine-5'-mono, di et triphosphates dans les tissus vegetaux. I. Dosage enzymatique. Physiol. Veg. **5**:209–221.
265. **Purich, D. L., and H. J. Fromm.** 1972. Studies on factors influencing enzyme responses to adenylate energy charge. J. Biol. Chem. **247**:249–255.
266. **Quammen, M. L., P. A. LaRock, and J. A. Calder.** 1973. Environmental effects of pulp mill wastes, p. 329–343. *In* L. H. Stevenson and R. R. Colwell (ed.), Estuarine microbial ecology. University of South Carolina Press, Columbia.
267. **Rainer, S. F., A. M. Ivanovici, and V. A. Wadley.** 1979. Effect of reduced salinity on adenylate energy charge in three estuarine molluscs. Mar. Biol. **54**:91–99.
268. **Rasmussen, H., and R. Nielsen.** 1968. An improved analysis of adenosine triphosphate by the luciferase method. Acta Chem. Scand. **22**: 1745–1756.

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269. **Reed, E. B.** 1976. Coordination of adenylate energy charge and phosphorylation state during ischemia and under physiological conditions in rat liver and kidney. Life Sci. **19**:1307–1322.
270. **Rickenberg, H. V.** 1974. Cyclic AMP in prokaryotes. Annu. Rev. Microbiol. **28**:353–369.
271. **Riemann, B.** 1978. Differentiation between heterotrophic and photosynthetic plankton by size fractionation, glucose uptake, ATP and chlorophyll content. Oikos **31**:358–367.
272. **Robrish, S. A., C. W. Kemp, D. C. Adderly, and W. H. Bowen.** 1979. The flavin mononucleotide content of oral bacteria related to the dry weight of dental plaque obtained from monkeys. Curr. Microbiol. **2**:131–134.
273. **Robrish, S. A., C. W. Kemp, D. E. Chopp, and W. H. Bowen.** 1979. Viable and total cell masses in dental plaque as measured by bioluminescence methods. Clin. Chem. (Winston-Salem, N. C.) **25**:1649–1654.
274. **Romano, J.-C.** 1976. Contribution à l'étude de la phase de latence dans des cultures d'algues planctoniques. II. Modification dans les rapports entre les pigments, les nucléotides adényliques (ATP, ADP, AMP) et les acides ribonúcléiques. C. R. Seances Soc. Biol. Paris **170**: 1069–1074.
275. **Romano, J.-C., and P. Laborde.** 1976. Filtration et extraction "in situ" des adénosines-5′ phosphate (ATP, ADP, AMP) du matériel particulaire de l'eau de mer. Comparison avec les techniques classiques de prélèvement et d'extraction a bord d'un navire océanographique. Tethys **8**:197–202.
276. **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.
277. **Russell, J., and J. J. Gauthier.** 1978. Method for adenosine 5′-triphosphate measurement on coke waste activated sludge. Appl. Environ. Microbiol. **35**:450–452.
278. **Saglio, P. H. M., M. J. Daniels, and A. Pradet.** 1979. ATP and energy charge as criteria of growth and metabolic activity of mollicutes: application to *Spiroplasma citri*. J. Gen. Microbiol. **110**:13–20.
279. **Sakshaug, E.** 1977. Limiting nutrients and maximum growth rates for diatoms in Narragansett Bay. J. Exp. Mar. Biol. Ecol. **28**:109–123.
280. **Sakshaug, E., and O. Holm-Hansen.** 1977. Chemical composition of *Skeletonema costatum* (Grev.) Cleve and *Pavlova* (*Monochrysis*) *lutheri* (Droop) Green as function of nitrate-, phosphate-, and iron-limited growth. J. Exp. Mar. Biol. Ecol. **29**:1–34.
281. **Schram, E., and P. Stanley (ed.).** 1978. International Symposium on Analytical Applications of Bioluminescence and Chemiluminescence. Vrije Universiteit, Brussels.
282. **Sellami, A.** 1976. Évolution des adénosine phosphates et de la charge énergetique dans les compartiments chloroplastique et non-chloroplastique des feuilles de blé. Biochim. Biophys. Acta **423**:524–539.
283. **Setlow, B., and P. Setlow.** 1977. Levels of oxidized and reduced pyridine nucleotides in dormant spores and during growth, sporulation, and spore germination of *Bacillus megaterium*. J. Bacteriol. **129**:857–865.
284. **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.
285. **Sheldon, R. W., and W. H. Sutcliffe, Jr.** 1978. Generation times of 3 h for Sargasso Sea microplankton determined by ATP analysis. Limnol Oceanogr. **23**:1051–1055.
286. **Shoat, 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. Surv. **4**:241–245.
287. **Sibert, J., T. J. Brown, B. A. Kask, and J. D. Fulton.** 1976. Observations on the lower trophic levels of the Cowichan Estuary, Vancouver Island, B. C. Manuscript report series no. 1394. Fisheries Research Board of Canada, Pacific Biological Station, Nanaimo, British Columbia.
288. **Sieburth, J. McN., K. M. Johnson, C. M. Burney, and D. M. Lavoie.** 1977. Estimation of *in situ* rates of heterotrophy using diumal changes in dissolved organic matter and growth rates of picoplankton in diffusion culture. Helgol. Wiss. Meeresunters. **30**:565–574.
289. **Sieburth, J. McN., and D. M. Lavoie.** 1978. A non-standard approach to heterotrophy: ATP estimation of natural populations of selectively filtered bacterioplankton and their growth rates on *in situ* water in diffusion-culture, p. 77–86. *In* T. W. Duke and A. I. Simonov (ed.), First American-Soviet Symposium on the Biological Effects of Pollution on Marine Organisms. EPA-600/9-78-007. U. S. Environmental Protection Agency, Gulf Breeze, Fla.
290. **Siegel, B. Z., G. McMurtry, S. M. Siegel, J. Chen, and P. LaRock.** 1979. Life in the calcium chloride environment of Don Juan Pond, Antarctica. Nature (London) **280**:828–829.
291. **Sikora, J. P., W. B. Sikora, C. W. Erkenbrecher, and B. C. Coull.** 1977. Significance of ATP, carbon, and caloric content of meiobenthic nematodes in partitioning benthic biomass. Mar. Biol. **44**:7–14.
292. **Silverman, R. H., and A. G. Atherly.** 1979. The search for guanosine tetraphosphate (ppGpp) and other unusual nucleotides in eucaryotes. Microbiol. Rev. **43**:27–41.
293. **Sinclair, M., E. Keighan, and J. Jones.** 1979. ATP as a measure of living phytoplankton carbon in estuaries. J. Fish. Res. Board Can. **36**:180–186.
294. **Skjoldal, H. R., and U. Båmstedt.** 1977. Ecobiochemical studies on the deep-water pelagic community of Korsfjorden, Western Norway. Adenine nucleotides in zooplankton. Mar. Biol. **42**:197–211.
295. **Slayman, C. L.** 1973. Adenine nucleotide levels in *Neurospora*, as influenced by conditions of growth and by metabolic inhibitors. J. Bacteriol. **114**:752–766.

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296. **Smith, R. C., and O. Maaløe.** 1964. Effect of growth rate on the acid-soluble nucleotide composition of *Salmonella typhimurium*. Biochim. Biophys. Acta **86**:229–234.
297. **Smith, R. J.** 1979. Increasing guanosine 3′-diphosphate 5′-diphosphate concentration with decreasing growth rate in *Anacystis nidulans*. J. Gen. Microbiol. **113**:403–405.
298. **Sorokin, Y. I., and S. V. Lyutsarev.** 1978. A comparative evaluation of two methods for determining the biomass of planktonic microflora. Oceanology **18**:232–236.
299. **Stanley, P. E.** 1978. Quantitation of picomole amounts of NADH, NADPH, and FMN using bacterial luciferase. Methods Enzymol. **57**: 215–222.
300. **St. John, J. B.** 1970. Determination of ATP in *Chlorella* with the luciferin-luciferase enzyme system. Anal. Biochem. **37**:409–416.
301. **Stouthamer, A. H.** 1969. Determination and significance of molar growth yields, p. 629–663. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 1. Academic Press, London.
302. **Stouthamer, A. H.** 1977. Energetic aspects of the growth of micro-organisms. Symp. Soc. Gen. Microbiol. **27**:285–315.
303. **Straat, P. A., H. Wolochow, R. L. Dimmick, and M. A. Chatigny.** 1977. Evidence for incorporation of thymidine into deoxyribonucleic acid in airborne bacterial cells. Appl. Environ. Microbiol. **34**:292–296.
304. **Strehler, B. L.** 1968. Bioluminescence assay: principles and practice. Methods Biochem. Anal. **16**:99–181.
305. **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.
306. **Strickland, J. D. H.** 1971. Microbial activity in aquatic environments. Symp. Soc. Gen. Microbiol. **21**:231–253.
307. **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.
308. **Stubbs, M., R. L. Veech, and H. A. Krebs.** 1972. Control of the redox state of the nicotinamide-adenine dinucleotide couple in rat liver cytoplasm. Biochem. J. **126**:59–65.
309. **Sutcliffe, W. H., Jr., E. A. Orr, and O. Holm-Hansen.** 1976. Difficulties with ATP measurements in inshore waters. Limnol. Oceanogr. **21**: 145–149.
310. **Swedes, J. S., M. E. Dial, and C. S. McLaughlin.** 1979. Regulation of protein synthesis during energy limitation of *Saccharomyces cerevisiae*. J. Bacteriol. **138**:162–170.
311. **Swedes, J. S., R. J. Sedo, and D. E. Atkinson.** 1975. Relation of growth and protein synthesis to the adenylate energy charge in an adenine-requiring mutant of *Escherichia coli*. J. Biol. Chem. **250**:6930–6938.
312. **Taguchi, S., and T. Platt.** 1977. Assimilation of $^{14}CO_2$ in the dark compared to phytoplankton production in a small coastal inlet. Estuarine Coastal Mar. Sci. **5**:679–684.
313. **Takahashi, M., and A. B. Norton.** 1977. Seasonal changes in microbial biomass in the Fraser River estuary, British Columbia. Arch. Hydrobiol. **79**:133–143.
314. **Tempest, D. W.** 1978. The biochemical significance of microbial growth yields: a reassessment. Trends Biochem. Sci. **3**:180–184.
315. **Thomas, D. R., J. A. Richardson, and R. J. Dicker.** 1974. The incorporation of tritiated thymidine into DNA as a measure of the activity of soil micro-organisms. Soil Biol. Biochem. **6**:293–296.
316. **Thomas, K. C., and P. S. S. Dawson.** 1977. Variations in the adenylate energy charge during phased growth (cell cycle) of *Candida utilis* under energy excess and energy-limiting growth conditions. J. Bacteriol. **132**:36–43.
317. **Thompson, F. M., and D. E. Atkinson.** 1971. Response of nucleosidediphosphate kinase to the adenylate energy charge. Biochem. Biophys. Res. Commun. **45**:1581–1585.
318. **Thore, A.** 1977. An overview of the work of the Swedish bioluminescence group, p. 171–188. *In* G. A. Borun (ed.), Second Bi-Annual ATP Methodology Symposium. SAI Technology Co., San Diego., Calif.
319. **Tifft, E. C., Jr., and S. J. Spiegel.** 1976. Use of adenosine triphosphate assay in disinfection control. Environ. Sci. Technol. **10**:1268–1272.
320. **Tobin, R. S., and D. H. J. Anthony.** 1978. Tritiated thymidine incorporation as a measure of microbial activity in lake sediments. Limnol. Oceanogr. **23**:161–165.
321. **Tobin, R. S., J. F. Ryan, and B. K. Afghan.** 1978. An improved method for the determination of adenosine triphosphate in environmental samples. Water Res. **12**:783–792.
322. **Tomkins, G. M.** 1975. The metabolic code. Science **189**:760–763.
323. **Torriani, A.** 1960. Influence of inorganic phosphate in the formation of phosphatases by *Escherichia coli*. Biochim. Biophys. Acta **38**: 460–479.
324. **Tsai, J. C., S. L. Aladegbami, and G. R. Vela.** 1979. Phosphate-limited culture of *Azotobacter vinelandii*. J. Bacteriol. **139**:639–645.
325. **Venrick, E. L., J. R. Beers, and J. F. Heinbokel.** 1977. Possible consequences of containing microplankton for physiological rate measurements. J. Exp. Mar. Biol. Ecol. **26**:55–76.
326. **Villadsen, I. S., and O. Michelsen.** 1977. Regulation of PRPP and nucleoside tri- and tetraphosphate pools in *Escherichia coli* under conditions of nitrogen starvation. J. Bacteriol. **130**: 136–143.
327. **Weber, C. I.** 1973. Recent developments in the measurement of the response of plankton and periphyton to changes in their environment, p. 119–138. *In* G. Glass (ed.), Bioassay techniques and environmental chemistry. Ann Arbor Science Publishers, Inc., Ann. Arbor, Mich.
328. **Weddle, C. L., and D. Jenkins.** 1971. The viability and activity of activated sludge. Water

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Res. 5:621–640.

329. **Weibel, K. E., J.-R. Mor, and A. Fiechter.** 1974. Rapid sampling of yeast cells and automated assays of adenylate, citrate, pyruvate and glucose-6-phosphate pools. Anal. Biochem. **58**:208–216.
330. **Weiler, C. S., and D. M. Karl.** 1979. Diel changes in phased-dividing cultures of *Ceratium furca* (Dinophyceae): nucleotide triphosphates, adenylate energy charge, cell carbon, and patterns of vertical migration. J. Phycol. **15**:384–391.
331. **Welsch, F., and L. Smith.** 1969. Kinetics of synthesis and utilization of adenosine triphosphate by intact cells of *Rhodospirillum rubrum*. Biochemistry **8**:3403–3408.
332. **White, D. C., W. M. Davis, J. S. Nickels, J. D. King, and R. J. Bobbie.** 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia (Berlin) **40**:51–62.
333. **Wiebe, W. J., and K. Bancroft.** 1975. Use of adenylate energy charge ratio to measure growth rate of natural microbial communities. Proc. Natl. Acad. Sci. U. S. A. **72**:2112–2115.
334. **Williams, P. M., and A. F. Carlucci.** 1976. Bacterial utilization of organic matter in the deep sea. Nature (London) **262**:810–811.
335. **Wilson, D. F., J. Stubbs, R. L. Veech, M. Erecinska, and H. A. Krebs.** 1974. Equilibrium relations between the oxidation-reduction reactions and the adenosine triphosphate synthesis in suspensions of isolated liver cells. Biochem. J. **140**:57–64.
336. **Wimpenny, J. W. T., and A. Firth.** 1972. Levels of nicotinamide adenine dinucleotide and reduced nicotinamide adenine dinucleotide in facultative bacteria and the effect of oxygen. J. Bacteriol. **111**:24–32.
337. **Witkamp, M.** 1973. Compatibility of microbial measurements. Bull. Ecol. Res. Comm. (Stockholm) **17**:179–188.
338. **Witzel, K.-P.** 1979. The adenylate energy charge as a measure of microbial activities in aquatic habitats. Arch. Hydrobiol. **12**:146–165.
339. **Yamagata, Y., T. Matsukawa, T. Mohri, and K. Inomata.** 1979. Phosphorylation of adenosine in aqueous solution by electric discharges. Nature (London) **282**:284–286.
340. **Yamaguchi, Y., and Seki.** 1977. Microbial biomass in the eutrophic Bay of Shimoda as compared by estimations of several biomass parameters. J. Oceanogr. Soc. Jpn. **33**:38–44.
341. **Yingst, J. Y.** 1978. Patterns of micro- and meiofaunal abundance in marine sediments, measured with the adenosine triphosphate assay. Mar. Biol. **47**:41–54.

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