Adenosine Triphosphate Measurements in Soil and Marine Sediments

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A sulfuric acid–EDTA method for extracting ATP from soil and marine sediments was devised. The efficacy of this method is compared to the boiling Tris extraction procedure. Efficiency of ATP recovery from solid substrates typically ranged between 81–94% for the sulfuric acid–EDTA procedure in comparison to a recovery of 3–6% using boiling Tris buffer. The presence of various ions in the acid extract, however, inhibited light emission in the luciferin–luciferase measurement of ATP. Addition of EDTA to the extract reduced but did not eliminate the ionic interference. An internal ATP standard was thus used to determine the magnitude of the residual interference.

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Nous avons mis au point une méthode acide sulfurique–ATED pour l'extraction de l'ATP du sol et des sédiments marins. Nous comparons l'efficacité de cette méthode avec celle de l'extraction au Tris bouillant. L'efficacité de récupération de l'ATP des substrats solides varie typiquement de 81 à 94% par la méthode acide sulfurique–ATED, comparativement à 3–6% par celle du tampon Tris. La présence de divers ions dans l'extrait acide empêche toutefois l'émission de lumière lors de la mesure luciférine–luciférase de l'ATP. L'addition d'ATED à l'extrait réduit, mais n'élimine pas, l'interférence ionique. Nous avons donc utilisé un étalon ATP interne pour déterminer l'ordre de grandeur de l'interférence résiduaire.

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WITHIN recent years, adenosine triphosphate (ATP) has been shown to offer great promise for measuring microbial biomass. The reliability of ATP as a biomass indicator has been demonstrated by Holm-Hansen (1973a,b).

Extraction of ATP from the appropriate sample material is the first step in the ATP assay. Several ATP extraction procedures have been described and include the following: perchloric acid (Strange et al. 1963; Cole et al. 1967); trichloroacetic acid (Beutler and Mathai 1967); sulfuric acid (Lee et al. 1971); boiling glycine buffer (Lin and Cohen 1968); boiling water (Strehler 1968); dimethylsulfoxide (DMSO) in Tris buffer (Lee et al. 1971); *n*-butanol (Chappelle and Levin 1968); DMSO in sulfuric acid (Lee et al. 1971); *n*-bromo-succinimide and EDTA (MacLeod et al. 1969); boiling chloroform (Dhople and Hanks 1973); and boiling Tris-borate buffer (Beutler and Baluda 1964).

A method has been described for the quantitative extraction of ATP from seawater samples (Holm-Hansen and Booth 1966) using boiling Tris buffer. This technique has been successfully applied to water samples and bacterial suspensions (Holm-Hansen and Booth 1966; Hamilton and Holm-Hansen 1967). However, when applied to sediments, boiling Tris gives inconsistent results. Lee et al. (1971) and Ausmus (1973) have independently compiled a comparison of ATP extraction techniques for soil and sediments. In both studies it was concluded that nonheating acid extraction methods achieved better results in recovering ATP from solid substrates. In this publication we describe a nonheating ATP extraction method, the effect of anions and cations on inhibiting the luciferin–luciferase reaction, and how ionic inhibition may be overcome.

EXTRACTION OF ATP

Sediments for ATP analysis were subsampled from a dredge, piston core, or gravity core using sterile glass core tubes, 8 mm ID, and calibrated to contain 1 cc. Subsamples were taken in triplicate, transferred to 100 × 16 mm culture tubes, covered with aluminum foil, and stored on ice prior to extraction.

Extraction was done by adding 5 ml of 0.6 N

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H_2SO_4 (5 C) to each sediment sample, and mixing the tubes on a vortex mixer intermittently at 10-s intervals for a total of 60 s. Longer vortex mixing did not enhance recovery. The tubes were then allowed to settle for 10 min. An internal standard consisting of 1 ml of 100 ng/ml ATP was added to one of the triplicate samples, and 1 ml of Tris buffer to the remaining two samples. The sediments were vortex mixed for an additional 10 s and then centrifuged for 5 min at 1800 × *g*. After centrifugation, 4 ml of the supernatant were transferred to a 10-ml beaker, followed by addition of 1 ml of a 0.048 M (18 g/liter) solution of the disodium salt of ethylenedinitrilo-tetracetic acid (EDTA), made up in Tris. The pH of the acid extract was adjusted to 7.8 with NaOH using a Corning 476050 combination electrode. Tris has been reported to interfere with the accuracy of some electrodes (Sigma Chemical Company), but no effects were discernible at the molarity used. After neutralizing, the sample was quantitatively transferred to a calibrated test tube, the volume brought up to 10 ml with Tris and frozen for subsequent analysis.

In assaying the acid-extracted ATP samples, a standard curve prepared in Tris cannot be used to calculate final ATP concentrations. A series of ATP standards for acid-extracted samples was prepared at the same time the environmental samples were extracted. These standards were prepared by mixing 3 ml of 0.6 N H_2SO_4, 1 ml of 0.048 M EDTA solution and 1 ml of the desired ATP standard, neutralizing with NaOH and adding Tris for a final volume of 10 ml. The standards covered a range of 0.3–50 ng ATP/ml and were frozen along with the extracted samples to be assayed by the bioluminiscent reaction.

Boiling Tris extractions were used for comparative purposes in a number of experiments with *Escherichia coli* purchased from the American Type Culture Collection (ATCC 11775). The cells were either collected on a membrane filter (0.45 μm) under 10 psi vacuum or 0.1 ml of cell suspension was added directly to the Tris extractant. Sediments, sand, or bacteria-coated glass beads were put directly into the Tris. In all cases, the sample was added to 10 ml of boiling Tris buffer contained in a calibrated 25 mm OD screw cap tube. The Tris was maintained at 100 C by an oil bath at 120 C. The sample was boiled for 10 min in the Tris, cooled in an ice bath, and the volume restored to 10 ml with additional Tris. The extract was frozen until assayed. The Tris used in sample extraction and analysis was prepared as 0.1 M and stored in a refrigerator to prevent spoilage. Just before use, the Tris was diluted to 0.025 M.

In one experiment, glass beads were coated with bacteria to determine the effects of the solid phase on efficiency of ATP extraction. Resting cells of *E. coli* were harvested from nutrient agar plates and washed three times with sterile 0.85% NaCl. The suspension was adjusted to an optical density of 0.3 with sterile NaCl solution, yielding a final cell density of 2×10^8 cells/ml. One-half milliliter of the washed cell suspension was added to 2 g of 0.5 mm diameter glass beads, mixed, and allowed to air dry. The entire 2 g of bacteria-coated beads were then extracted using both the boiling Tris and the H_2SO_4 procedures.

The Bioluminescent Reaction and Its Measurement

Once extracted, ATP can readily be assayed by means of the firefly bioluminescent reaction (Strehler 1968). Firefly luciferin and luciferase react with ATP in the presence of Mg^{++} and molecular oxygen to yield a photon of light for each ATP molecule consumed at neutral or alkaline pH (McElroy et al. 1969). When the reactants are mixed, an initial burst of light occurs followed by a long decay period (Schram 1970). The addition of arsenate buffer to the reaction mixture retards the decay of luminescence after the initial light burst (Strehler and Totter 1954). Light emission in the luciferin–luciferase reaction is inhibited by the presence of both cations and anions (Aledort et al. 1966; Strehler 1968). Dilution of the enzyme mixture reduces the magnitude of the initial burst of light but does not affect the exponential relationship of the decay which lasts over a long period of time (Beutler and Baluda 1964; Rasmussen and Nielsen 1968; Schram 1970).

The luciferin–luciferase mixture used in our work was prepared by reconstituting each vial of lyophilized firefly lantern extract (Sigma Chemical Company, FLE-50) with 12.5 ml distilled water, 7.5 ml of 0.1 M arsenate buffer (Sigma Chemical Company, FF-AS-100), and 5 ml of 0.05 M magnesium sulfate. The enzyme was allowed to stand for between 6–7 h at 23–25 C in order to remove endogenous ATP. After aging, the preparation was filtered (Whatman #2) to remove residual debris. After clarification and during use, the enzyme was kept in an ice bath. One-half milliliter of enzyme was used for each ATP assay permitting about 45 assays at an approximate cost of 3–4 cents per assay.

A number of different light detecting instruments have been used for measuring the bioluminescent reaction. These generally can be divided into two types: those that measure the initial burst or peak intensity of the light emitted (Chappelle and Levin 1968; Rasmussen and Nielsen 1968; Allen 1973; Anderson and Davies 1973) and those that integrate the area under a portion of the decay curve that follows. A commercial instrument using peak intensity measurements has been developed but requires the use of specially prepared reagents (Allen 1973; E. I. DuPont Corp.). St. John (1970) has suggested that the peak intensity measurement might not be the most reliable means of determining ATP concentration.

The integral mode of counting has been used with a number of different light detecting instruments which include specially constructed photomultiplier assemblies (Holm-Hansen and Booth 1966), fluormeters (Beutler and Baluda 1964), a commercially available photometer (JRB Co., San Diego, Calif.), and the liquid scintillation counter (Strange et al.

1963; Lin and Cohen 1968; Schram 1970; Schram and Roosens 1972; Rudd and Hamilton 1973).

In our work the emitted light from the firefly reaction was measured on a manual liquid scintillation counter (LSC) (Nuclear Chicago model 4534). For ATP analysis, the scintillation counter was operated in a noncoincident mode using one photomultiplier tube (Schram 1970), and with a maximum window opening of 0.5–10.0 V. For analysis 1 ml of either the sample extract or the ATP standard was added to a chromic acid washed LSC vial, followed by addition of 0.5 ml of 0.025 M Tris buffer (pH 7.75). Next, the vial was closed and 0.5 ml of the enzyme preparation injected into the vial through a small hole drilled in the cap. The vial was immediately inserted into the LSC and exactly 1 min after enzyme injection, a 0.1 min count was taken. This count is an integral measurement of the area under the luminescent decay curve between 60 and 66 s after the start of the reaction. For each enzyme preparation, a standard curve was made by plotting the logarithm of the integral count rate versus the logarithm of the appropriate ATP concentration. Standard solutions containing 1 mg/liter disodium crystalline ATP (Sigma Chemical Company, A 3127) in 0.025 M Tris buffer, were prepared and stored frozen in 10 ml aliquots. When needed, the concentrated solution was diluted in Tris or the acid extracting menstrum to cover a range of 0.3–50 ng/ml ATP.

For some experiments the luminescent decay of the ATP-enzyme reaction mixture was followed by interfacing the LSC with a multichannel analyzer (Nuclear Data model 110) as described by Quammen et al. (1973). The ND-110 was operated in a multi-scaling mode so that each of the 128 channels was opened for data acquisition sequentially for a predetermined counting period over a range of one millisecond to 10 s/channel. A flow cell was used in place of LSC vials to permit mixing of reactants in the LSC detector.

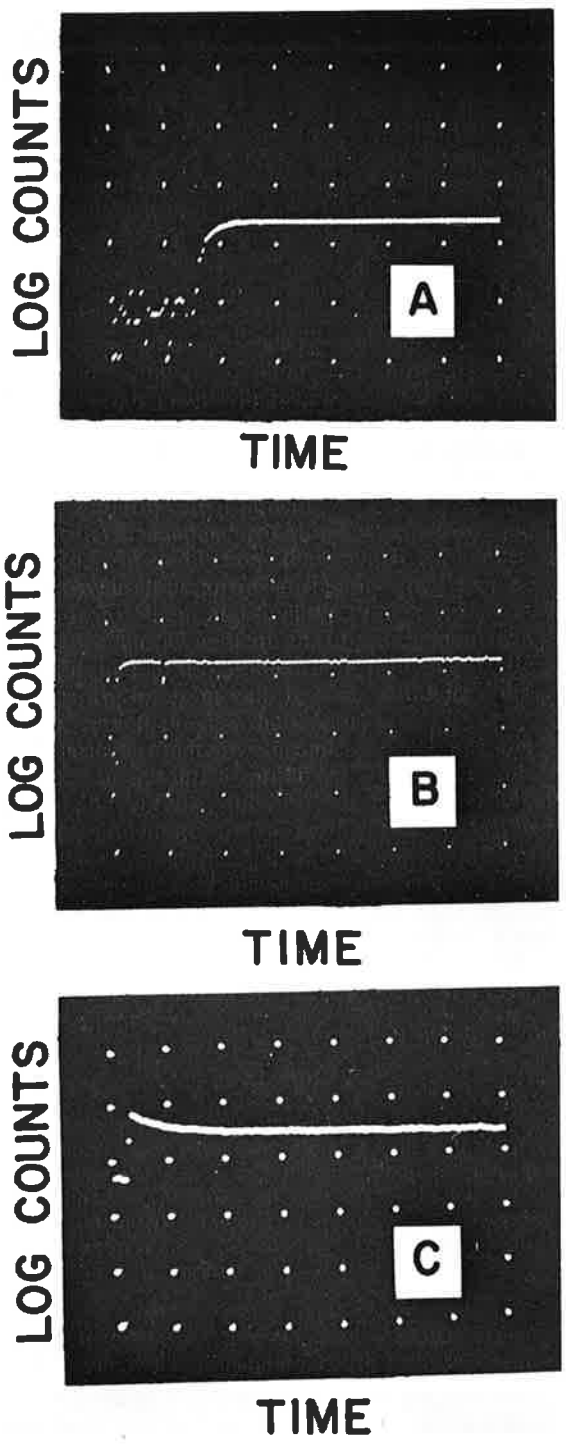
Results and Discussion

Many researchers have used crude, commercially available enzyme extracts reconstituted in 5 ml of distilled water as specified by one manufacturer (Sigma Chemical Company). In our enzyme preparation, however, each vial of lantern extract was reconstituted with magnesium and arsenate salt solutions to yield 25 ml of preparation. Reconstitution of the enzyme to 25 ml permits a greater number of assays to be made and dilutes the endogenous ATP in the enzyme (Schram 1970), thereby reducing the background count rate. The pertinent features of the bioluminescent decay curve using our enzyme preparation are shown in Fig. 1. The rise period, or time required for the light output to reach 60% of the peak intensity after mixing the reactants, was between 100–200 ms (Fig. 1A), and peak intensity count rate was reached within 1 s (Fig. 1B). The subsequent exponential decay period began after 24 s and lasted for up to 20 min (Fig. 1C).

The slopes of the decay curve 60 s after injection of the enzyme were parallel although they were vertically displaced as a function of their ATP content. By measuring the count rate, or more precisely the area under some portion of the decay curve over a fixed time interval, one can construct a standard curve based on an integral measurement. The time interval over which the ATP counts are collected is not critical and we used 0.1 min, the shortest preset time on our LSC. Standard curves were constructed for each enzyme preparation.

In our procedure, we found that the luminescent properties of some LSC vial caps and light exciting the photomultiplier tube as the sample was inserted into the detector greatly increased the apparent count rate and shape of the early portion of the luminescent curve (Quammen et al. 1973). These interferences decay to background levels within 50 s; thus, a 60-s delay period between injecting the enzyme and making the actual ATP measurement was incorporated into the assay procedure.

The lower limit of ATP detectability is influenced by the removal of endogenous ATP from the enzyme (Rasmussen and Nielsen 1968), photomultiplier tube noise, and in the case of an LSC, the use of a non-coincident counting mode (Schram 1970). For a given light detecting instrument the only practical means of altering the lower limit of detectability is to remove endogenous ATP. Gel filtration has been used to remove ATP from enzyme preparations (Rasmussen and Nielsen 1968; Schram and Roosens 1972) but such a purification procedure also removes luciferin which must be replaced prior to enzyme use (Schram and Roosens 1972). Lyman and DeVincenzo (1967) used apyrase to purify their enzyme preparation and reportedly detected ATP concentrations in the picogram levels. Aging the enzyme preparation, i.e. permitting the preparation to stand for some predetermined time before use, also reduces the background count rate. We found aging to be the most convenient method for general use. The background count rate of the enzyme decreased to a minimum level approximately 6 h after reconstitution (Fig. 2). Further aging at room temperature resulted in an increased count rate most probably a result of bacterial growth. Storing the enzyme in an ice bath maintained a lower background level for a



longer period of time. For our particular LSC the lower limit of ATP detection was 0.3 ng.

The maximum statistical variation of the extraction procedure and assay of ATP from environmental samples and bacterial suspensions was ±10% at the 95% confidence interval for samples containing 12 ng/ml of ATP. For a given operator, between-sample variation generally was ±5%.

Extraction Procedures I: Boiling Tris Buffer

The use of boiling Tris buffer for ATP extraction from water or other homogeneous aqueous samples is well documented (Beutler and Baluda 1964; Holm-Hansen and Booth 1966; Holm-Hansen 1969, 1970, 1973a,b; Patterson et al. 1971; Holm-Hansen and Paerl 1972; Quammen et al. 1973).

In our laboratory, attempts to use boiling Tris to extract ATP from beach sands and sediments yielded extremely variable results. The variability may be due to effects of thermal gradients established in a fluid–solid mixture. When solids are submersed in liquids, a transition or boundary layer of fluid is established around the solid that does not have the thermal characteristics of the bulk fluid. This boundary zone may be 0.025–0.25 mm thick. Heat transfer in the bulk fluid is by convection, but within the boundary layer adjacent to the solid heat transfer is by conduction and occurs at a very slow rate (Knudson and Katz 1954). When the fluid is heated, a sharp thermal gradient is established across the boundary layer. While the temperature of the bulk fluid may be near 100 C, at the solid surface it is considerably less. Bacteria adhering to the solid fall within this thermal gradient across the boundary layer, and thus the temperatures to which they are exposed may not be high enough to efficiently extract ATP and prevent its hydrolysis. Patterson et al. (1970) indicated that at

FIG. 1. Reaction kinetics of luciferin–luciferase enzyme preparation. A) Initial rise and slow approach to peak intensity after enzyme injection into 100 ng of ATP. Dwell period was 10 ms per channel; B) Maximum intensity and early decay of peak. Dwell period was 100 ms per channel and ATP concentration was 5ng; C) Initial peak and long lasting exponential decay period. Dwell period was 1 s per channel and ATP concentration was 1 ng. Elapsed time between reference markers in the horizontal direction is 160 ms, 1.6 s, and 16 s for figures A, B, and C, respectively. Reference markers in the vertical direction are the logarithm of counts per channel from $1–10^5$ for all figures.

TABLE 1. Recovery of ATP from *Escherichia coli* suspensions and *Escherichia coli* coated glass beads.

Sample description	No. cells added[a]	ATP added (ng)	ATP extracted (Tris)[b]		ATP extracted (H_2SO_4)[c]	
			ATP (ng)	% Recovery	ATP (ng)	% Recovery
Aqueous bacterial suspension	2×10^8	280	260	92.9	258	92.1
Aqueous bacterial suspension	2×10^8	280	270	96.4	239	85.3
Bacteria coated beads (2 g total)	10^8	140	9	6.4	122.2	87.3
Bacterial coated beads (2 g total)	10^8	140	–	–	131	96.3
Bacteria coated beads (2 g) and uncoated beads (2 g)	10^8	140	5	3.6	114.5	81.8
Bacteria coated beads (2 g) and uncoated beads (4 g)	10^8	140	4.5	3.2	127.5	90.9

[a]Theoretical ATP addition based on intracellular ATP measurement for *E. coli* of 1.4×10^{-15} g per cell.
[b]ATP values assayed using Tris standard curve.
[c]ATP values assayed using H_2SO_4 standard curve.

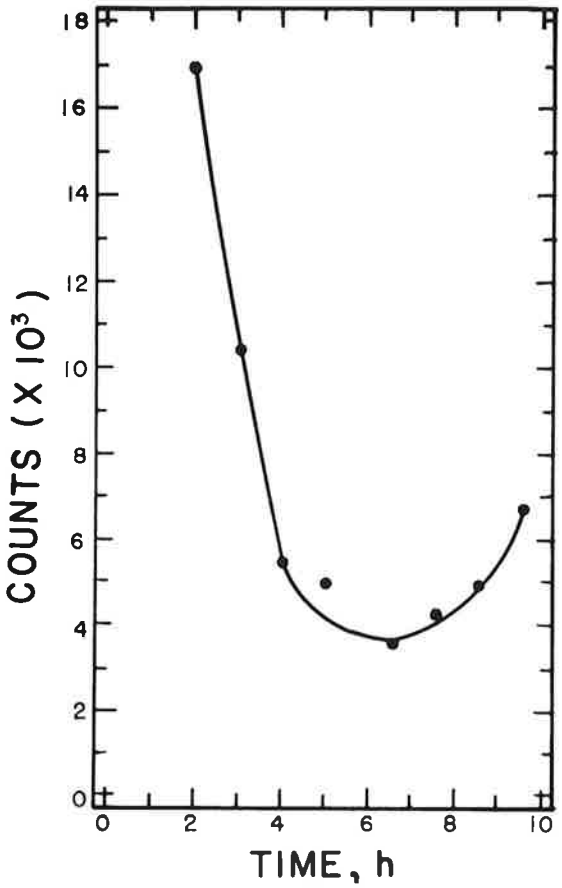


FIG. 2. Temporal changes in background count rate for the luciferin–luciferase enzyme preparation.

temperatures below 90 C the efficiency of ATP extraction with boiling Tris is greatly reduced.

EXTRACTION PROCEDURES II: H_2SO_4-EDTA

A comparison of ATP extraction procedures for soil and sediments has been independently compiled by Lee et al. (1971) and Ausmus (1973). Their results indicated that a cold acid procedure offered the greatest promise for extracting ATP from sediments. The problem of thermal gradients found in hot extraction procedures is avoided as well as the problem of adsorption of ATP by sediment components such as iron and aluminum oxides (Lee et al. 1971). Lee et al. (1971) compared sulfuric acid and a mixture of sulfuric acid and dimethyl sulfoxide (DMSO) and obtained comparable results with either extraction fluid. We found that the DMSO–sulfuric acid extract will not freeze at temperatures as low as –20 C, and that the ATP decayed with time. For this reason all work was done using sulfuric acid alone as the extracting medium for sediment samples.

A comparative experiment was performed to determine the relative efficiencies of boiling Tris and sulfuric acid in extracting ATP from bacterial cells coated on glass beads. The weight of coated beads was kept constant, but uncoated beads were added to determine if the quantity of solid substrate affected either extraction process. The results of this experiment are shown in Table 1. When 10^8 bacterial cells were coated on 2 g of glass beads, Tris extracted only 6.4% of the bacterial ATP in comparison to recoveries of

87 and 94% for the sulfuric acid. Increasing the quantity of glass beads, but maintaining the same bacterial density, further decreased ATP recovery by Tris extraction, but had relatively little effect in the sulfuric acid procedure. The data in Table 1 indicate that the sulfuric acid extraction procedure effectively removed bacterial ATP in the presence of a solid substrate, and in all cases the recovery was greater than 81%, regardless of the amount of solid present.

Attempts at using boiling Tris for extracting ATP from sediments in marine or estuarine environments have been reported (Ernst 1970; Ferguson and Murdoch 1973). In these works an internal ATP standard consisting of either a solution of an ATP salt or lyophilized bacteria was added to the sample to estimate extraction efficiency. In light of the previous discussion such additions will measure only ATP adsorption and ionic interference; they will not measure extraction efficiency of ATP from viable bacterial cells attached to sediment particles and within the fluid boundary layer. A more suitable standard would be bacterial cells coated on glass beads and added to the sample prior to extraction.

Aledort et al. (1966) indicated a marked drop in the light emission in the luciferin–luciferase reaction resulting from the addition of a small concentration of cations. They reported that a Ca^{++} concentration of 97 mM could completely inhibit the luminescent reaction, and that Na^{+} also greatly decreased light emission. These considerations are particularly critical when using an acid extraction procedure on marine sediments containing calcium carbonate.

A series of experiments was performed to determine the relative effects of selected anions and cations commonly found in sea water, using the sodium or chloride salts. Each cation or anion concentration was assayed in triplicate and the results were averaged. Maximum variation between any of the triplicates was never more than 5% of their average. The results of these experiments indicate: (1) At the highest concentrations tested (100 mM), all cations or anions tested inhibited light emission by 60 and 100% (Fig. 3, 4; Table 2). (2) Equimolar concentrations of anions inhibit in the order of $CO_3 = >PO_4 \equiv >SO_4 = >Cl-$ (Fig. 4). (3) Equimolar concentrations of cations inhibit in the order $Ca^{++} > K^{+} \geqslant Na^{+} > Mg^{++}$ (Fig. 3). (4) Light emission decreased linearly in relation to the logarithm of the cation or anion concentration with the exception of magnesium (Fig. 3, 4). (5) For a given molar concentration of the cation, the inhibition is more pronounced at lower ATP values (Table 2). For example, a Ca concentration of 50 mM permits only 5.9% light emission with an ATP concentration of 0.7 ng compared to 21% light emission with 70 ng of ATP (Table 2). Ionic inhibition in the luciferin–luciferase reaction, therefore, is a function of the nature of the anion or cation, its concentration, and the concentration of ATP in the sample. The net effect of these variables can best be determined using an internal ATP standard for each environmental sample.

Ionic inhibition by sulfate ions suggests that acid extracted samples will have a lower count rate than equivalent Tris extracts. A comparison of a series of ATP standards prepared in Tris buffer and sulfuric acid showed that for any given ATP concentration the count rate in sulfuric acid menstruum is less than that obtained with Tris

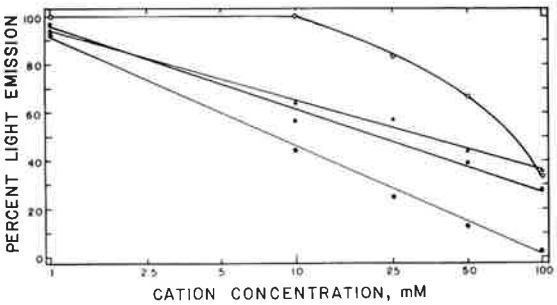


FIG. 3. Decrease in light emission in the luciferin–luciferase reaction as a function of cation concentration. All cations were added as chloride salts and the ATP concentration was 7 ng/ml. Symbols: open circle, Mg^{++}; solid circle, Ca^{++}; triangle, Na^{+}; square, K^{+}.

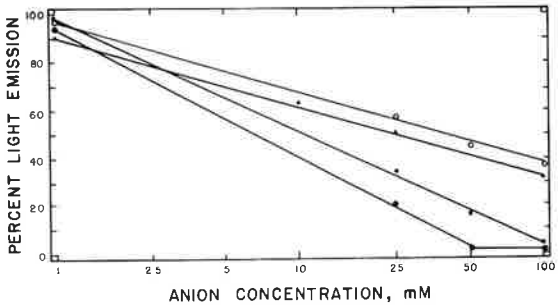


FIG. 4. Decrease in light emission in the luciferin–luciferase reactions as a function of anion concentration. All anions were added as sodium salts and the ATP concentration was 7 ng/ml. Symbols: open circle, Cl^{-}; solid circle, $CO_3^{=}$ triangle, $SO_4^{=}$; square, $PO_4^{\equiv}$.

TABLE 2. The interference of calcium and magnesium ions on light emission in the luciferin–luciferase reaction for ATP solutions of varying concentration.

Cation added[a]	Cation concentration (m*M*)	ATP concentration (ng)	% Light emission[b]
Ca	100	0.7	0
Ca	100	7	2.7
Ca	100	70	5.4
Ca	50	0.7	5.9
Ca	50	7	12.4
Ca	50	70	21
Ca	25	0.7	18.6
Ca	25	7	22.4
Ca	25	70	35.6
Ca	10	0.7	38
Ca	10	7	48
Ca	10	70	80.5
Ca	1	0.7	93
Ca	1	7	96
Ca	1	70	99.5
Mg	100	0.7	33
Mg	100	7	34
Mg	100	70	44.5
Mg	50	0.7	62
Mg	50	7	68
Mg	50	70	71.5
Mg	25	0.7	70.5
Mg	25	7	82
Mg	25	70	88
Mg	10	0.7	93
Mg	10	7	100
Mg	10	70	96
Mg	1	0.7	96
Mg	1	7	100
Mg	1	70	102

[a]Cations were added as Cl^- salts.
[b]ATP count rate with no exogenous cations was taken as 100% light emission.

buffer (Fig. 5). The difference between the two menstruums is not constant, however, and decreases with increasing ATP concentration. This observation is also consistent with the cation effects reported in Table 2. Because of the difference in count rate obtained with H_2SO_4 or Tris extractants, it is essential that ATP in a sulfuric acid menstruum be assayed and compared to a standard curve made up with sulfuric acid. Lee et al. (1971) used a Tris standard curve in evaluating the efficiency of the various extracting fluids they tested, leading to an underestimate of the ATP content.

Lee et al. (1971) suggested using a cation exchange resin to mitigate the overall ionic inhibition. The exchange of sodium for various metal ions on an equal charge basis would not

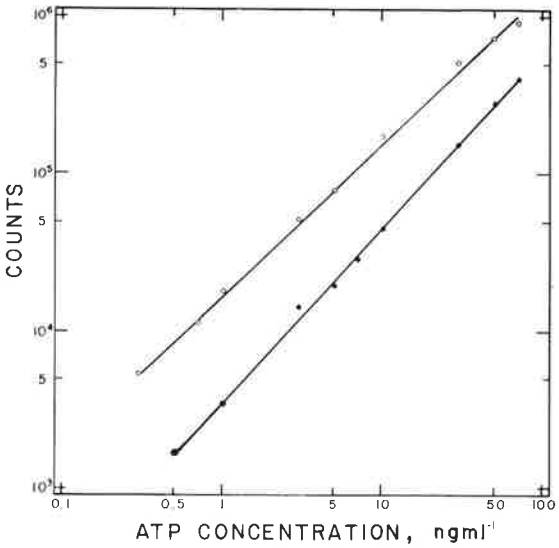


FIG. 5. Counts obtained for ATP standards in Tris and sulfuric acid menstruums, using the counting procedure outlined in Extraction Procedures II. Symbols: open circle, Tris buffer; closed circle, sulfuric acid.

appear to significantly reduce interference as one cation is exchanged for another that will also interfere.

Complex formation was investigated as a means of reducing the concentration of free metal ions in solution using EDTA, which has a high specificity for divalent cations, especially calcium (Perrin 1964). The addition of EDTA may be necessary to promote light emission in samples of low ATP content, where ionic inhibition is extensive and light emission otherwise totally inhibited. The effect of added EDTA to a sample containing 10 ng of ATP in H_2SO_4, is to first increase the count rate to a maximum with approximately 8 mg EDTA (Fig. 6). At greater concentrations of EDTA the count rate decreases and at 20 mg it is equivalent to the count rate obtained without EDTA. Further additions drastically suppress light output to levels below the enzyme background count rate. Presumably the decrease in light emission is a result of complexing magnesium ions which are essential for the luciferin–luciferase reaction.

A second experiment was performed in which EDTA was added to a solution containing 10 ng ATP and 10 mM Ca^{++}. The results of this experiment are shown in Fig. 7, indicating again an initial enhancement followed by suppression of light emission. The principal difference between Fig. 6 and 7 is that in the presence of interfering

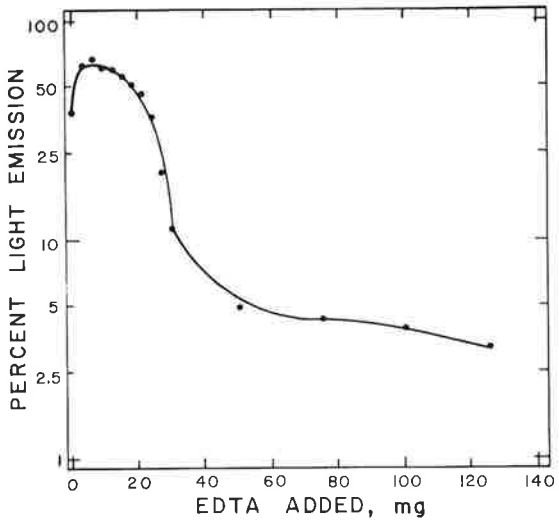


FIG. 6. The effect of EDTA additions on light emission in the luciferin–luciferase reaction. The ATP concentration was 10 ng/ml and the enzyme background count was 4.4%.

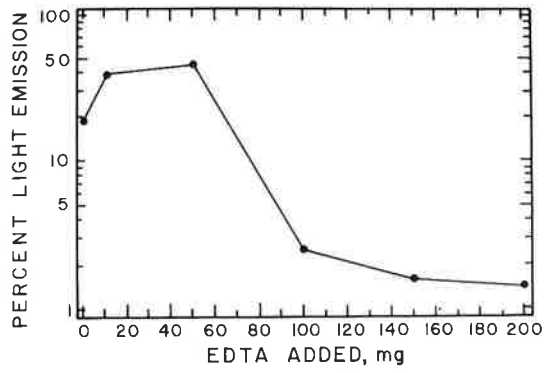


FIG. 7. The effect of EDTA additions on light emission in the luciferin–luciferase reaction in the presence of 10 mM Ca^{++}. The ATP concentration was 10 ng/ml and the enzyme background count was 2.5%.

cation, greater quantities of EDTA can be added before the light reaction is suppressed. The exact amount of EDTA to be added is thus dependent on the concentration of ionic species present in the sample. Based on the results of Fig. 6 and 7, a solution of 0.048 M EDTA was prepared and 1 ml of this solution added to each extracted sample. This corresponds to an EDTA addition of 18 mg/sample.

The hydrolysis of ATP in sulfuric acid–DMSO extracted samples raised the question whether extracted ATP would decay in an acid solution. An experiment was performed in which 3 and 10 ng/ml ATP standards were prepared in the H_2SO_4 extracting menstruum and distributed in a series of test tubes. At the desired time one tube of each ATP concentration was neutralized with NaOH and assayed. The results of this experiment indicated no loss of ATP occurred over 65 min as a result of hydrolysis in an acid solution. Once neutralized and frozen, acid extracted samples were stable for periods up to 1½ mo, the longest storage period we tested.

Conclusions

1) Reconstitution of lyophilized enzyme with arsenate buffer and magnesium sulfate to a total of 25 ml yielded a preparation that gave consistent results, reduced background count rate, and permitted a large number of samples to be assayed.

2) Cations and anions interfere with light emission in the luciferin–luciferase assay. In the presence of high concentrations of ions, light emission can be totally inhibited. The interference is dependent upon the specific ions encountered and the ATP concentration in the sample.

3) Boiling Tris buffer is a suitable menstruum for extracting samples concentrated on membrane filters but is unsatisfactory when applied to sediments. The concentration of detrital material on membrane filters along with the microbes, may necessitate the use of an internal ATP standard to determine losses resulting from ATP adsorption.

4) A sulfuric acid menstruum offered greater, more uniform ATP recovery from sediments than did the boiling Tris buffer. The addition of EDTA lessens the ionic effects and promotes light emission. The magnitude of all sources of interference can be determined with an internal ATP standard.

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