COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION of FISHERIES and OCEANOGRAPHY

Report No. 75

ADENOSINE TRIPHOSPHATE ANALYSIS IN MARINE ECOLOGY: A REVIEW AND MANUAL

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Reprint No. 973

Marine Laboratory
Cronulla, Sydney
1977

ISBN 0 643 02023 3

Printed by CSIRO, Melbourne

77.214-900

I. REVIEW

INTRODUCTION

Adenosine triphosphate (ATP) has long been recognised as one of the most important low molecular weight compounds in living organisms. Since its isolation from muscle in 1929 by Lohmann (1929) and Fiske and Subba Row, the role ATP plays in energy transfer in metabolic processes has become well documented, with substantial evidence that it is present in the cells of all animals, plants and microorganisms (Lehninger, 1965).

More recently, ATP has received interest as an estimator of living microbial biomass (Holm-Hansen and Booth, 1966), providing a valuable tool in ecological studies. Basing their method on that of Strehler and McElroy (1957), Holm-Hansen and Booth demonstrated that ATP can be used to estimate microbial biomass in the open ocean. Since then, the method has similarly been used in studies in freshwater (Rudd and Hamilton, 1973) and estuarine and coastal waters (Manuels and Postma, 1974). Concurrently the method was also adapted for use in studies of microbial biomass in soils (Doxtader, 1969; Ausmus, 1971 and 1973) and in sediment (Ernst, 1970; Lee *et al* 1971 a, b; Karl and LaRock, 1975; Bancroft *et al* 1976).

The suitability of ATP as an estimator of living biomass depends primarily on three factors, 1) its universality in living organisms, 2) its lack of association with dead material and 3) its occurrence in uniform concentration. The first condition is confidently accepted (Huennekens and Whitely, 1960; Lehninger, 1965; Atkinson, 1971). Secondly, the ease of degradation of the ATP molecule by cleavage of a pyrophosphate group by phosphatases makes its occurrence outside intact cells improbable. This is supported by the experimental evidence of Holm-Hansen and Booth (1966) who demonstrated that negligible ATP remained in algae and bacteria which had been killed by cyanide, repeated freezing or heat. They also found low levels of ATP in cultures which had been severely nutrient-depleted for some weeks. Similarly, Patterson *et al* (1970) showed that no ATP remained in activated sewage sludge which had been heated at 103°C for an hour. Thirdly. the concentration of ATP in microorganisms has received much attention and there are substantial data in terms of ATP per cell, per unit dry weight and per unit carbon which show that the ATP concentrations of different species of microorganisms in culture do not vary widely (see Fig. 1) provided that they are in a similar physiological state (Holm-Hansen, 1970). The intraspecific variation found between organisms growing at different rates or at different periods during synchronous division (Coombs *et al* 1967a, b) is somewhat wider. However, as natural communities of microorganisms are usually quite diverse and may generally be considered to be in a state of physiological youth, a useful relationship between biomass and ATP can be shown.

CHEMICAL NATURE OF ATP

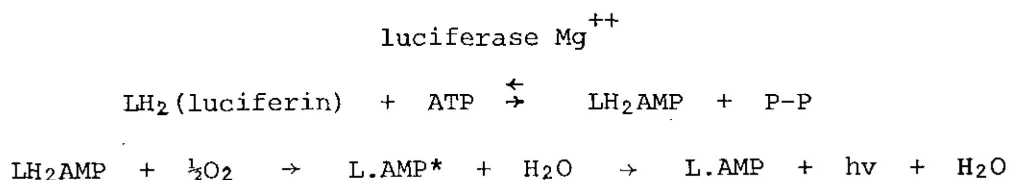
Adenosine-5'-triphosphate is a nucleotide, consisting of adenylic acid (adenosine-5'-monophosphate or AMP) linked by a high energy ester bond to a pyrophosphate group, (see Fig. 2). The latter is very susceptible to cleavage in acid solution, or by enzymatic activity, being dephosphorylated to ADP or AMP, with release of energy. For this reason, ATP is important as an energy source in many metabolic processes, including glycolysis, fatty acid synthesis and oxidation, protein and nucleic acid synthesis and in such organ functions as muscle contraction and nerve impulse generation. It is also responsible for energy transfer processes concerned with light production in bioluminescent organisms, as for example, the 'lantern' of *Photinus pyralis*, and it is this property which has resulted in a most effective method of analysis.

METHODS OF ANALYSIS

Early methods for determination of ATP were based on the colourimetric estimation of labile phosphate following acid hydrolysis, or conversion of ATP to AMP, the latter being estimated spectrophotometrically by its de-amination to inosine mono-phosphate (Munch-Petersen and Kalckar, 1957). However, the observation by McElroy (1947) that light production by the 'lantern' of the firefly *Photinus pyralis* was dependent on the presence of ATP led to the development of a more sensitive and specific analytical method (McElroy and Strehler, 1949; Strehler and Totter, 1952). With various refinements, this method has now been applied to the measurement of the ATP pool in red blood cells (Beutler and Baluda, 1964), bacteria (Hamilton and Holm-Hansen, 1967; D'Eustachio and Johnson, 1968; Chapelle and Levin, 1968; Dhople and Hanks, 1973) algae (Syrett, 1958; St. John, 1970; Holm-Hansen, 1970; Berland *et al* 1972) sediment nematodes (Ernst and Goerke, 1974) and zooplankton (Balch, 1972).

THE REACTION

The details of the light producing reaction were first described by McElroy and Strehler (1949). They were able to identify the compound luciferin which, in the presence of the enzyme luciferase, reacts with ATP to produce light. They described the reaction as proceeding in several stages: the cleavage of a pyrophosphate group and the formation of an activated complex which undergoes oxidation, producing light. The reaction also requires the presence of the magnesium ion, or certain other divalent metal ions.



$$LH_2\text{(luciferin)} + ATP \overset{\text{luciferase } Mg^{++}}{\rightleftarrows} LH_2AMP + P\text{-}P$$

$$LH_2AMP + \tfrac{1}{2}O_2 \rightarrow L.AMP^* + H_2O \rightarrow L.AMP + h\nu + H_2O$$

The optimum temperature of the reaction is about 25°C (Strehler, 1968) and the optimum pH about 7.7 (Fig. 3).

The progress of the reaction is complicated by the effects of the reaction products. The activated complex L.AMP*, being strongly bound to the enzyme luciferase, competitively inhibits formation of LH_2.AMP, although this effect is negligible at the concentrations usually adopted for biomass estimates. In addition, pyrophosphate in the presence of luciferase, can reform ATP by splitting adenyloxyluciferin. For a more detailed description, the reader is referred to McElroy and Strehler (1949), McElroy *et al* (1953) and McElroy and Green (1956a). The reviews of Strehler (1968) and Seitz and Neary (1976) cover the broader aspects of bioluminescence assay and its applications.

There are several other substances whose activity can affect the light producing reaction: nucleotides, phosphorylating enzymes and ionic material.

The presence of nucleotides other than ATP in the reaction mixture is not in itself of concern, as the luciferin-luciferase reaction is specific for ATP (McElroy and Green, 1956b). However, unpurified firefly lantern extract is a complex biological mixture, and some nucleotides in either the extract or sample, AMP and ADP in particular, may become converted to ATP by phosphorylating enzymes also present in the extract. The action of several nucleotides and high energy phosphate compounds has been tested (Holm-Hansen and Booth, 1966; St. John, 1970). Most of these showed little or no light production. Of those that did,

cytidine-5'-triphosphate and inosine-5'-triphosphate produced light levels similar to that of ATP, whereas with ADP the level was very low. The high concentrations of ATP in organisms compared with those of other nucleotides should ensure that interference is minimal. Besides, such errors, as Holm-Hansen and Booth (1966) suggest, should have little bearing on biomass determinations, as the quantity of ATP is eventually related to levels in other organisms. Should interference from other nucleotides be of concern, or if determinations at very low ATP levels are contemplated, the crude lantern extract may be purified.

It is well established that the presence of ionic material in the reaction mixture can have a severe quenching effect on light production. Strehler and Totter (1952) investigated the effect of sodium chloride solution added to the reaction mixture and found that 0.4 mg per ml of NaCl could reduce light emission by more than 50%. A detailed investigation of ionic interference by Aledort *et al* (1966) showed the effects of various anions and cations on light production. They found that the degree of inhibition by cations was in the order $Ca^{++} > K^{+} > Na^{+} > Rb^{+} > Li^{+}$ and for anions $I^{-} > H_2PO_4^{-} > Br^{-} > ClO_3^{-} > Cl^{-} > F^{-} > HCO_3^{-} > CH_3COO^{-}$ in equimolar strength. Several methods are available for reducing the extent of ionic interference, dilution being the most important. These are described in the section on sample preparations.

THE MEASUREMENT

On mixing a solution of ATP with firefly lantern extract, light production is almost instantaneous, the peak height being reached within a fraction of a second (Fig. 4). There then follows a fairly rapid, semi-exponential decay to a lower light level which slowly decreases over a period of several minutes. The height of the peak relative to the eventual lower light level depends on several factors mostly related to the purity of the lantern extract and the sample, as described above. If interference from other nucleotides is small, both the peak height and the total light production are proportional to the ATP concentration and both may be used in ATP assay, the choice depending on the instrumentation used. There seems to be, however, a substantial preference for integrated light measurements with counting periods typically lasting up to one minute, although light production may last for thirty minutes.

Various instruments have been used for measuring the light production of bioluminescent reactions (Strehler, 1968; Seitz and Neary, 1976). Of these the photomultiplier is the most widely used, in either individually constructed instruments, or liquid scintillation counters, or commercial instruments specifically designed for ATP analysis. 'Home-made" instruments frequently consist of a basic photomultiplier with specially designed sample injection and shutter mechanisms (Holm-Hansen and Booth, 1966; Chapelle and Levin, 1968; Rasmussen and Nielsen, 1968). This is of particular importance when peak height measurement is used, which necessitates the mixing of sample and enzyme in the counting chamber. The liquid scintillation counter is frequently used (Aledort *et al* 1966; Lee *et al* 1971a; Cheer *et al* 1974) owing to its ready availability at most laboratories. The method of sample addition depends on the construction of the particular instrument, as described, for example, by Johnson *et al* (1974) and Schram and Roosens (1971). Ausmus (1973) has used a photomultiplier with a specially designed centrifugal sample holder.

Instruments designed specifically for ATP analysis are now commercially available and one of these, the SAI (formerly JRB) ATP Photometer, is used in the method described below. This instrument integrates the light count over a 60 s period, commencing 15 s after sample and enzyme extract are mixed, and gives a digital readout. It may also be used to obtain measurements of peak height for enzyme kinetic studies, using a special sample injector.

THE EXTRACTION

Extraction of ATP into solution may be performed directly on sediment and some organisms (e.g. copepods, nematodes), by simply adding the sample to the extracting medium. Dense cultures of microorganisms may be pipetted directly into the extracting medium, or concentrated on a filter beforehand. Seawater samples require concentration on a filter, adopting the usual precautions for filtering such material (Riley, 1975). It is also important, when a boiling extracting agent is used, that the sample is not of sufficient mass to lower the temperature below 95°C. The freezing of unextracted material has proved unsatisfactory (Cheer *et al* 1974) with a loss of up to 80% of ATP. Some samples can be stored in liquid nitrogen without loss of ATP, although the choice of extraction methods then becomes limited, as boiling extractants cannot be used. Freeze clamping with liquid nitrogen remains the most effective method for extracting ATP from most animal tissue, where the bulk of the sample might render rapid extraction difficult (Wollenberger *et al* 1960).

The requirements of the extraction process are that 1) the living cells be killed instantly with rupture of the cell walls and release of intracellular matter, 2) all ATP be released from this material into the extracting medium without being adsorbed onto particulate matter, 3) no breakdown of ATP occurs in the extracting medium, 4) any phosphorylating enzymes present are denatured and 5) components of the extracting agent and materials released into it from the sample should not cause excessive interference with the bioluminescent reaction.

Extracting agents which satisfy these requirements fall roughly into three categories: organic solvents, acids and bases, and boiling, neutral buffers.

The organic solvents which have been tested are boiling ethanol, (St. John, 1970), n-butanol (Sharpe *et al* 1970), DMSO (Lee *et al* 1971a) and chloroform (Dhople and Hanks, 1973). These solvents act by dissolving or rupturing the cell walls and inactivating enzymes. The solvent, if water soluble, must be diluted out to reduce interference with the bioluminescent reaction, or the ATP must be transferred to an aqueous medium.

The acids and bases which have been tested are sulphuric acid (Lee *et al* 1971a; Karl and LaRock, 1975; Ausmus, 1973) TCA (Aledort *et al* 1966; Bagnara and Finch, 1972), formic acid (Klofat *et al* 1969), perchloric acid (Forrest, 1965; Knowles and Smith, 1970) and potassium hydroxide (Wettermark *et al* 1970). After extraction with acids and bases, the extracts must generally be neutralised to about pH 7.7 and diluted out before proceeding with the assay. Stanley and Williams (1969), however, were able to assay perchloric acid extracts without neutralisation, albeit with a shift of the emission spectrum and 10-fold loss of sensitivity. Acid extracts, of sediment in particular, contain relatively high concentrations of ionic material, although this interference can be reduced by chelation with EDTA (Karl and LaRock, 1975), by ion exchange with a cation exchange resin (Lee *et al* 1971a), by separation of the ATP by passage through an activated charcoal column (Hodson *et al* 1976) or, in the case of perchloric acid, by precipitation as potassium perchlorate.

Buffers which have been tested are Tris (tris (hydroxymethyl) amino methane), (Holm-Hansen and Booth, 1966; Ernst, 1970), bicarbonate (Bancroft *et al* 1976), n. bromosuccinimide (MacLeod *et al* 1969), Tris-phosphate, (Moriarty, 1975), Tris-EDTA (Patterson *et al* 1970) and McIlvaine buffer (Bulleid, in press).

With the exception of n.bromosuccinimide, these buffers are used at boiling point. The heat kills the cells releasing cell contents, and denatures enzymes. Care must be taken to maintain a high temperature (> 95°C) after sample addition. Karl and LaRock (1975) have suggested that boiling methods are unsatisfactory owing to thermal gradients set up around particles in the sample, preventing instantaneous temperature equilibrium and death of cells.

Lundin and Thore, (1975), Lee *et al* (1971a), Ausmus (1973), Bancroft *et al* 1976 and Bulleid (in press) have all published comparisons of the extracting ability of several of the above buffers.

Quite different extraction times have been suggested in the literature, related to different samples and extracting media. Holm-Hansen and Booth (1966) recommend 5 min for seawater and algal cultures extracted in boiling Tris buffer. One minute was adopted by St. John (1970) for *Chlorella* in boiling ethanol, by Karl and LaRock (1975) for sediment in cold sulphuric acid and by Bulleid (in press) for seawater and sediment in boiling McIlvaine buffer. Bancroft *et al* (1976) favour 30 s for sediment in boiling bicarbonate. There appears to be uncertainty about loss of ATP with prolonged extraction. Lee *et al* (1971a) found that extraction times between 5 s and 24 hr were satisfactory; Chapelle and Levin (1968) report that extraction is almost instantaneous and further periods of extraction are not critical; and Holm-Hansen and Booth (1966) found no loss of ATP over a 30 min extraction period. Bancroft *et al* (1976) however found that a 3 min extraction showed lower levels of ATP recovery than one of 30 s.

As a control on the extraction procedure, it is advisable to use internal standards. These may take the form of small volumes of a known ATP solution added to the extractant simultaneously with the sample, or of a portion of a bacterial or algal suspension, whose ATP content has been determined. ATP solution may also be used as a check on the degree of inhibition encountered by the bioluminescent reaction, by addition of a known quantity to the sample extract just prior to analysis. Bancroft *et al* (1976) used stationary phase *Enterobacter aerogenes*, Lee *et al* (1971a) *Aerobacter aerogenes* and Sutcliffe *et al* (1976) *Dunaliella tertiolecta*. Ausmus (1973) added ATP solution directly to his soil core samples, while St. John (1970) and Patterson *et al* (1970) both added ATP to their extracts before analysis. The use of microorganisms as internal standards at the time of extraction is probably the most realistic method, as ATP in added cellular material more closely resembles the sample ATP than would an ATP solution.

Lundin and Thore (1975) and Bancroft *et al* (1976) have investigated the effect of varying the ratio of standard added and ATP in the sample, finding that degree of ionic interference and recovery of standard ATP varied with this ratio. The latter suggest that a ratio of 1:1 gives maximum recovery.

Extraction of samples of marine origin may yield ionic material both from the sample itself and from seawater carried over with the sample. In addition, the extracting agent itself frequently contains ionic material. To alleviate quenching of light production during analysis, the concentration of ionic material may be reduced by diluting the sample until no further apparent increase in ATP concentration occurs, after correcting for the extent of dilution. The sensitivity of the method is such that quite extensive dilution may be performed, unless very small quantities of material have been extracted. Dilution is generally performed immediately prior to analysis.

Ionic interference may be greater when an acidic extraction medium is used, as neutralisation of the extract will introduce further ionic material. Furthermore, at low pH, heavy metal and calcium ions become mobilised from sediment samples. However, Karl and LaRock (1975) were able to overcome such interference by addition of EDTA and by dilution.

An alternative method of reducing interference is available, enabling complete separation of nucleotides from ionic material (Hodson *et al* 1976). This involves activated carbon column chromatography of the sample extract, followed by elution of nucleotides with ammoniacal ethanol. This has the advantages of avoiding the need for dilution and of allowing concentration of ATP from dilute samples, enabling very low levels of biomass to be determined.

THE ENZYME EXTRACT PREPARATION

Firefly lanterns are available commercially as either whole desiccated fireflies or firefly tails, or as dried, powdered extract, which, when hydrated according to the manufacturer's instructions, contains the required concentration of luciferin, luciferase and Mg^{++} buffered with arsenate. As these preparations contain residual ATP, it is wise to 'age' the extract after hydration. Karl and LaRock (1975) recommend about 6 hr at $24^{o}C$ and Holm-Hansen and Booth (1966) 4 hr at room temperature, whereas Patterson *et al* (1970) store the extract, after hydration and filtration, for 24 hr in an ice bath.

The overall sensitivity of the method is ultimately determined by the purity of the enzyme preparation as, at very low ATP concentrations the effects of residual ATP, other nucleotides and phosphorylating enzymes become appreciable. Using unpurified, 'aged' enzyme extract, ATP levels of about 10^{-10} g can be measured with accuracy. The extract may be further purified by the method of Chappelle and Levin (1968), who obtained a luciferase preparation with very low residual nucleotide content by passing the crude extract through a Sephadex G-100 column. Luciferin and Mg^{++} are also removed by this process and must therefore be added after purification. Use of this purified extract is reported to enable detection of 10^{-14} g ATP.

II. MANUAL

The following section describes the methods of extraction and analysis currently in use at this laboratory.

I. REAGENTS

A. Buffers

1. McIlvaine buffer

The extracting medium used is dilute McIlvaine buffer, the basis for its selection being described by Bulleid (in press). It is made up as follows:

10.72 g of $Na_2HPO_4 . 7H_2O$ are made up to one litre with distilled water to give an 0.04 M solution. 0.210 g of citric acid are dissolved in 50 ml of distilled water to give an 0.02 M solution. The phosphate solution is adjusted to pH 7.70 by adding citric acid solution in a ratio of approximately 19:1 (phosphate : citrate).

2. Tris buffer

This is used as the diluting and buffering medium during the bioluminescence assay. An 0.02 M solution of Tris is made up by diluting 2.42 g

of tris (hydroxymethyl)-aminomethane to one litre with distilled water and adjusting to pH 7.70 with 1N. hydrochloric acid.

3. Tris/McIlvaine Buffer

During the course of the extraction and analysis, the samples become diluted by a factor of 5 and consequently the standard solutions with which they are to be compared should be of similar composition. A mixture is therefore made of 80% Tris buffer and 20% McIlvaine buffer, made up as above.

These three buffers should be stored frozen in convenient volumes.

B. Standards

ATP is kept as a concentrated stock solution, dispensed into vials, and frozen at $-50^{o}C$. No loss of activity has been detected over a period of about a year. A stock solution of 20 µg per ml in Tris buffer is convenient. For use as an internal standard, this stock solution should be further diluted with Tris buffer to a working strength of between 1 and 10 µg per ml, depending on the expected ATP content of the sample.

The standards used during the assay are also made up from this stock solution. A range of 8-80 ng per ml for sediment and 1-40 per ml for seawater samples is usually adequate. After preparation, these working strength standards should be stored in an ice bath.

C. Enzyme

Commercially available vials of lantern extract (Sigma Chemical Co. FLE. 50; Calbiochem, 341862) typically contain 50 mg of crude firefly lantern extract, Mg^{++} and arsenate buffer at pH 7.4, at which pH the enzyme is most stable. These vials are stored frozen. The required number of vials is thawed - each will be sufficient for about 50 analyses - the contents of each vial hydrated with 5 ml of distilled water, then left for 24 hr at $4^{o}C$ to 'age'. The hydrated extract is then filtered through a glass fibre filter (Whatman, GF/C), made up to 10 ml with Tris buffer pH 7.70 and stored in an ice bath.

II. PROCEDURES

A. Extraction

The lability of the ATP molecule in complex biological samples dictates that care must be taken from the time of sampling until the ATP can be stored as a frozen extract. For this reason, the first 'disturbance' to the sample should be the extraction itself, where possible.

1. Sediment

The sediment sample should, where possible, remain undisturbed before extraction, without mixing of aerobic and anaerobic zones. It should not be allowed to drain.

The sample is first given any preparatory treatment required, e.g. sieving through a 1 mm mesh. An 8 ml portion of McIlvaine buffer is dispensed into each of nine 20 ml beakers, which are covered by watch glasses and placed on a recessed aluminium block at about $130^{o}C$. The sediment is stirred thoroughly and a portion of about 0.2 ml is added to each of the first three beakers. Further portions are added to the next three beakers simultaneously

with a small volume of internal standard, either a suspension of bacteria or algae, or an ATP solution. Lastly three portions of the standard alone are dispensed into the remaining beakers. The quantity of internal standard used can be selected either on the basis of an analysis carried out beforehand, or from previous experience of the sample type. The ATP content of the sediment extracted and the standard should be approximately equal. With a little experience, the moment of sample addition can be judged just before the buffer begins to boil, when it is superheated. The temperature then barely falls below 100°C. One minute after addition of the sample, the beaker is removed to an ice bath. After cooling, the contents are added to a centrifuge tube with 2 washings of distilled water and centrifuged at about 1500 g for 5 minutes. The supernatant is then decanted into a graduated test tube, made up to 10 ml with distilled water and immediately frozen and stored at -20°C until analysis. The sediment pellet is dried in an oven to constant weight and the weight recorded.

2. Seawater

Seawater samples should be extracted as soon after sampling as possible and without undergoing any extremes of light and temperature in the intervening period.

The water is prefiltered through a nylon screen to remove large, infrequent organisms and through a 25 mm diameter filter under 100 mm Hg vacuum. Either membrane or glass fibre filters are suitable, although the membrane filter possesses better retention characteristics, particularly for bacteria. However, if particulate organic carbon analyses are also being performed on the water sample, glass fibre filters should be used so that the POC and ATP results are comparable. In coastal and estuarine waters, 250 ml of seawater is sufficient, but a litre or more may be necessary for oligotrophic areas or for depths below 200 m. Sutcliffe *et al* (1976) have found that the ATP they extracted from inshore waters was linearly related to the volumes filtered only below about 500 ml, with an apparent loss of ATP with larger volumes. They suggested that this lower recovery with larger volumes filtered was related to increased quantities of suspended matter in inshore waters and recommend that linearity should be checked by comparing concentrations after filtering a range of volumes.

Meanwhile, several 20 ml glass beakers containing 8 ml of McIlvaine buffer, covered by a watch glass, are placed on a recessed, heated aluminium block. Shortly before filtration has finished, the vacuum is released and, when the filter has drained but still remains wet, it is transferred to the boiling buffer. It is essential that the filter is not allowed to dry out. A further portion of the water sample is filtered and the filter added to the boiling buffer simultaneously with a portion of bacteria, algae or ATP solution as an internal standard. A further extraction is performed on the standard material itself. One minute after addition of the filter, the beaker is transferred to an ice bath. After cooling, the liquid is decanted off the filter into a graduated test tube, made up to 10 ml with several washings of distilled water and immediately frozen and stored at -20°C until analysis.

The difficulties of recovering ATP from seawater are somewhat less than from sediment, since there are fewer sites to bind the ATP molecule and less interfering ionic material is released. Also, the problems of representative subsampling are less, owing to the finely divided, homogeneous nature of the living material. In many cases, then, it will be necessary to perform only one extraction, rather than extracting in triplicate. Care should be taken with water samples containing a high particulate mineral load.

3. Cultures

Cultures of bacteria and algae may be extracted either by pipetting a portion of the culture directly into boiling buffer or by filtering onto a membrane or glass fibre filter and proceeding as for a seawater extraction. The volume of culture required, 1-10 ml depending on density, precludes the direct extraction of dilute cultures unless greater sensitivity is achieved by further purification of the enzyme preparation (see above). However, bacterial cultures denser than 10^6 cells per ml and algae (depending on size) denser than 10^4 per ml can generally be analysed by extracting 1 ml of culture directly. A filtration step will be necessary for cultures of lower density.

These methods are also applicable to yeasts and fungi in culture.

Care should be taken to ensure that cultures are well mixed during sub-sampling as heterogeneity due to clumping of cells and vortex formation from a circular stirring motion can seriously affect sampling accuracy. Extractions should be performed in triplicate.

B. Enzymatic Assay

The method of analysis described below is applicable in detail only for assays performed with the SAI* ATP Photometer. It is, however, adaptable to other systems providing that a new protocol is chosen suited to the light detector used.

The instrument should be switched on and allowed to warm up for an hour or so, during which period the standard solutions and enzyme extract may be prepared, as above. The sample extracts are thawed and placed in an ice bath with the standards. The sensitivity and zero settings of the instrument are adjusted according to the manufacturer's instruction manual, after which the instrument is ready for use.

1. Enzyme blank

A blank determination is first performed to check on the residual activity of the lantern extract. With a crude lantern extract, the count obtained will have an equivalent ATP concentration in the range 0.1 - 1.0 ng/ml ATP, depending on batch variation and 'ageing'. Apart from a slow drift with time, this count is quite steady and satisfactory analyses can be performed at around the 0.5 - 1.0 ng/ml level.

Tris/McIlvaine buffer (0.2 ml) is dispensed into a scintillation vial. (An automatic pipette with disposable tips will be found convenient for this and other dispensing). The vial is then spun on a vortex stirrer and, during stirring, 0.2 ml of enzyme preparation is added and the instrument's foot switch depressed simultaneously. The vial is then loaded into the counting chamber of the photometer, the chamber's cap replaced and the shutter opened. Fifteen seconds after the footswitch is depressed, the instrument starts its 60 second counting period at the end of which the total count is displayed. If ATP determinations are being made at levels near the enzyme blank, the blank determination should be repeated to establish an accurate value.

*SAI Technology (formerly JRB Inc.) 4060 Sorrento Valley Blvd, San Diego, Calif.

If the standard curve is to be plotted graphically, it is unnecessary to substract the enzyme blank count from that of the samples and standards. This may be necessary, however, if a programmable calculator is used, depending on the curve that is fitted.

2. Standard determination

The procedure used for determination of the enzyme blank is repeated, with 0.2 ml of each standard in turn being dispensed into a vial and 0.2 ml of lantern extract being added.

3. Sample determination

Before the sample extract is analysed, it must first be diluted to reduce the concentration of interfering ionic material, as described above. For both seawater and sediment extracts, a four-fold dilution has been found adequate, although when unfamiliar samples are being extracted, it would be wise to check this by diluting a sample extract and an ATP standard in identical buffer solutions side by side. The sample may conveniently be diluted by dispensing 0.2 ml of extract and 0.6 ml of Tris buffer into a small test tube and stirring briefly, 0.2 ml of the resultant mix being used in the analysis, as above.

Following analysis, it is advisable to rinse the used vials with seawater to prevent coagulation of proteinaceous material in the residual lantern extract and its adsorption onto the glass surface. It is essential that the vials be perfectly clean when re-used and a chromic acid or ethanolic KOH wash may be introduced to ensure this. Thorough washing followed by several distilled water rinses and draining upside down will probably prove adequate, however.

ACKNOWLEDGMENTS

I gratefully acknowledge the permission granted by Dr O. Holm-Hansen and the University of South Carolina Press to reproduce Figure 1. Professor W.J. Wiebe offered useful advice during the early stages of development of the extraction method. Drs D.F. Smith and O.A. Woodward and Mr D.J. Tranter read the manuscript and made helpful suggestions.

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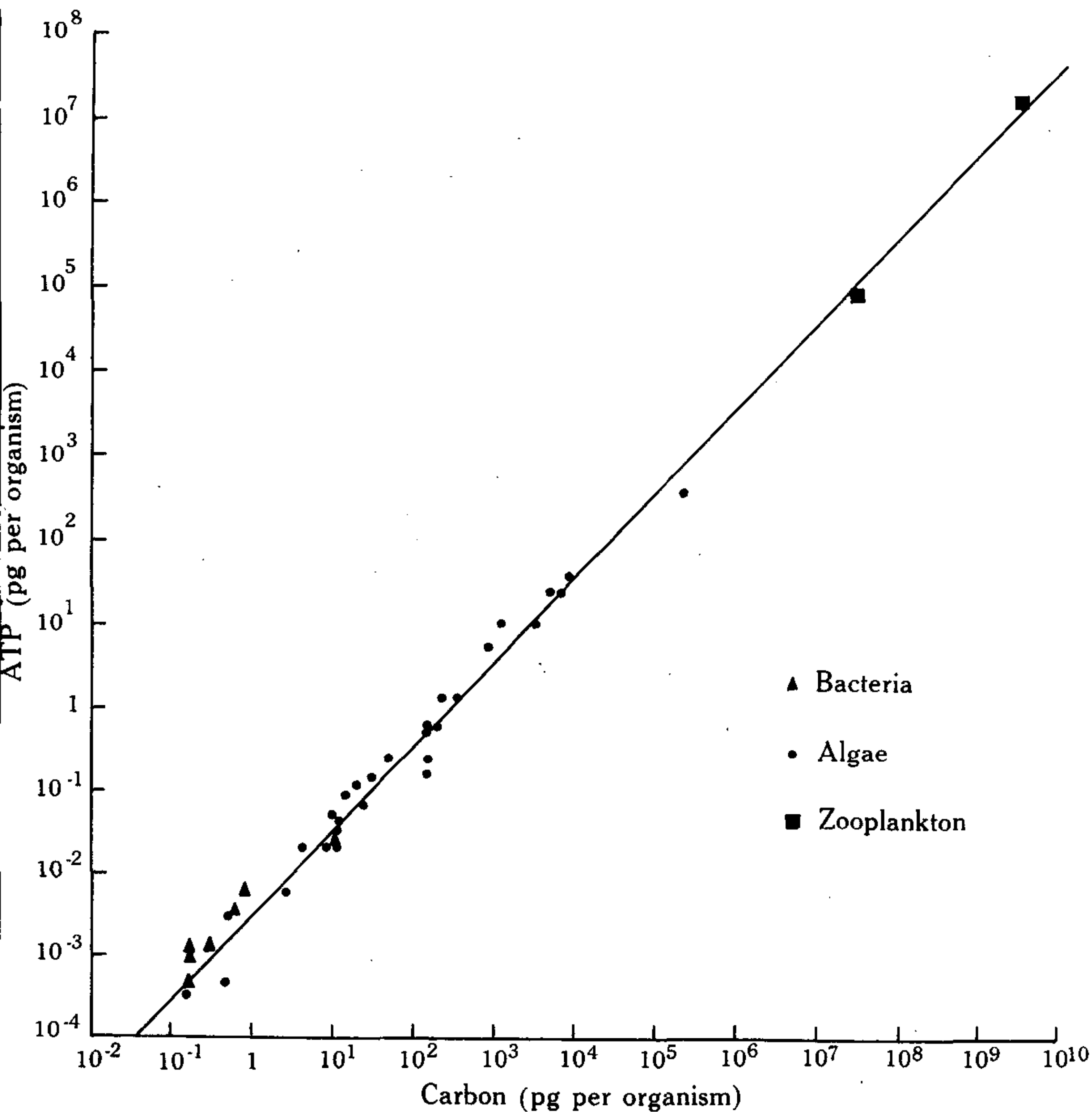
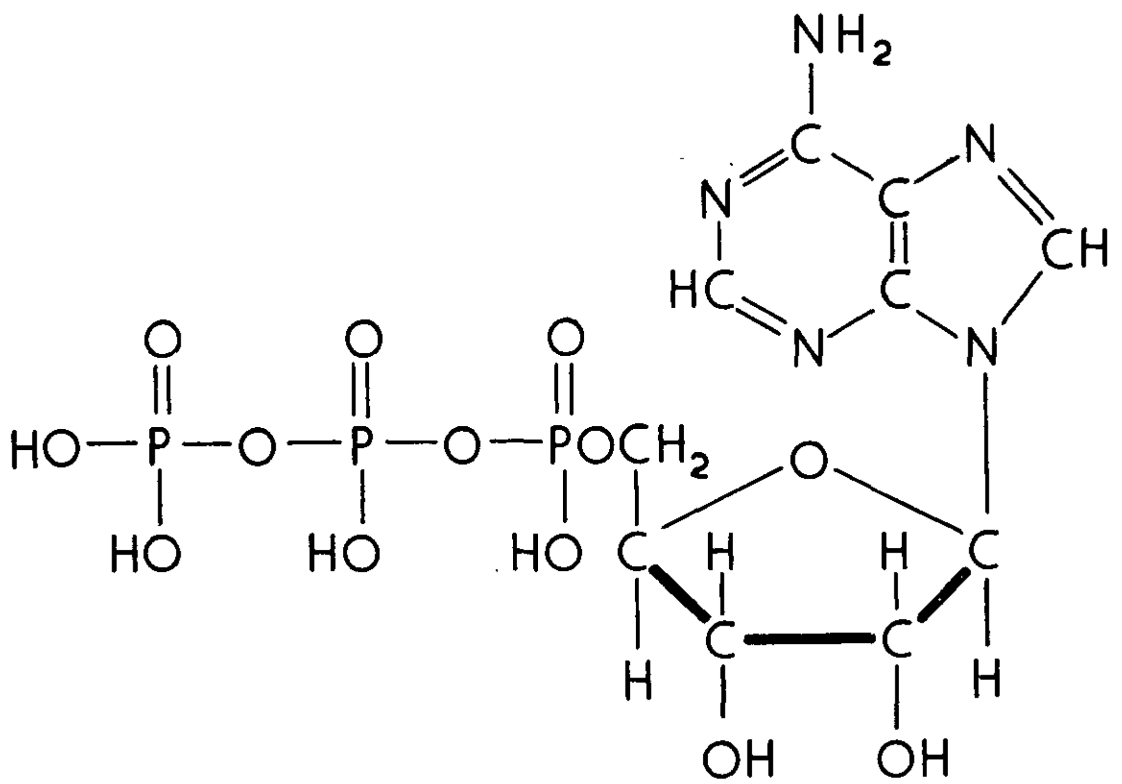


Figure 1. The relationship between ATP and cellular organic carbon (after Holm-Hansen, 1973). †Reprinted from Estuarine Microbial Ecology, edited by L. Harold Stevenson and R.R. Colwell, page 75, figure 1 by permission of the University of South Carolina Press. Copyright © by the University of South Carolina Press.



Adenosine-5′-triphosphate (ATP)

Figure 2. The molecular structure of ATP.

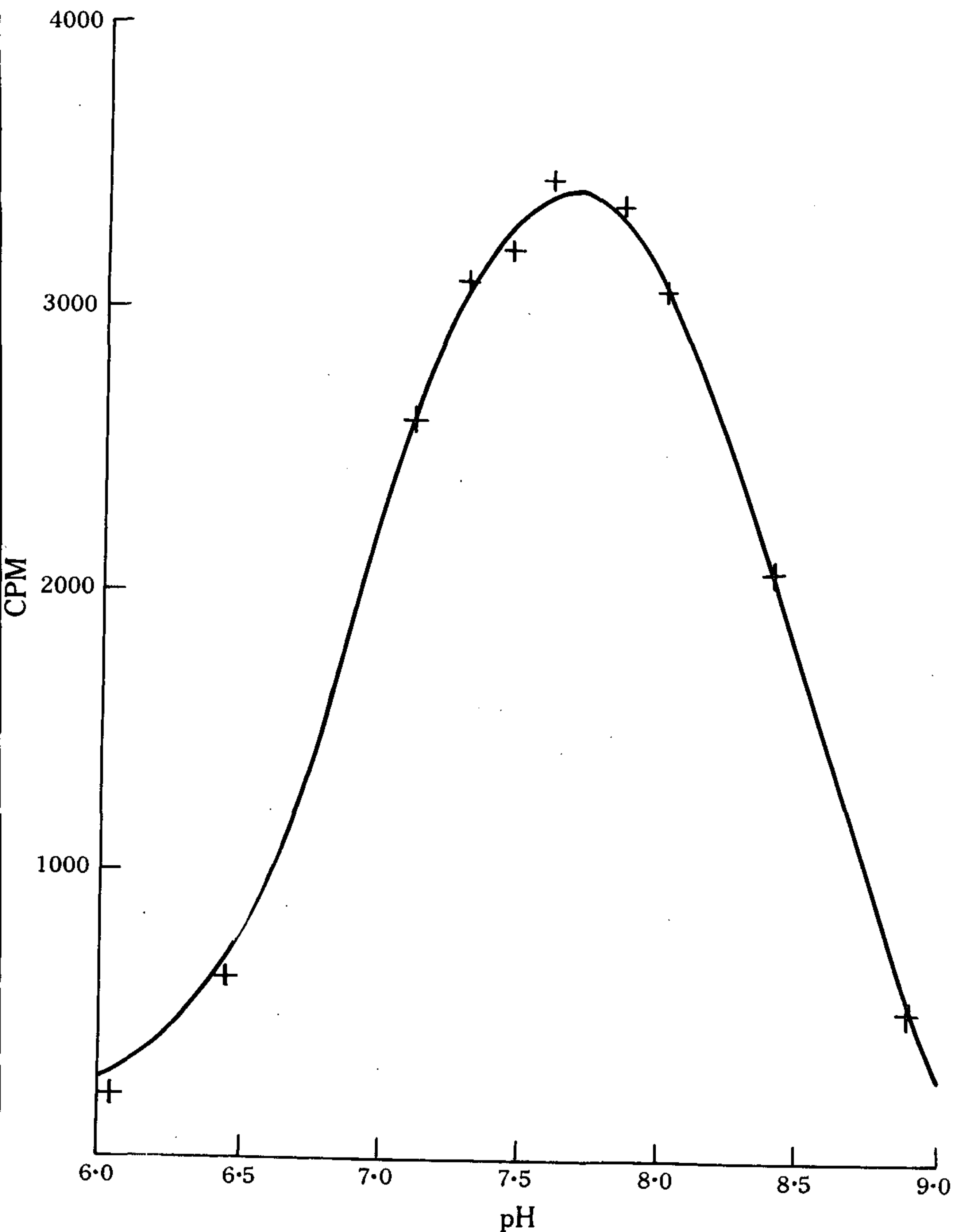


Figure 3. The variation of intensity of light emission with pH. ATP concentration was 50 ng/ml in Tris/McIlvaine buffers, as described in text.

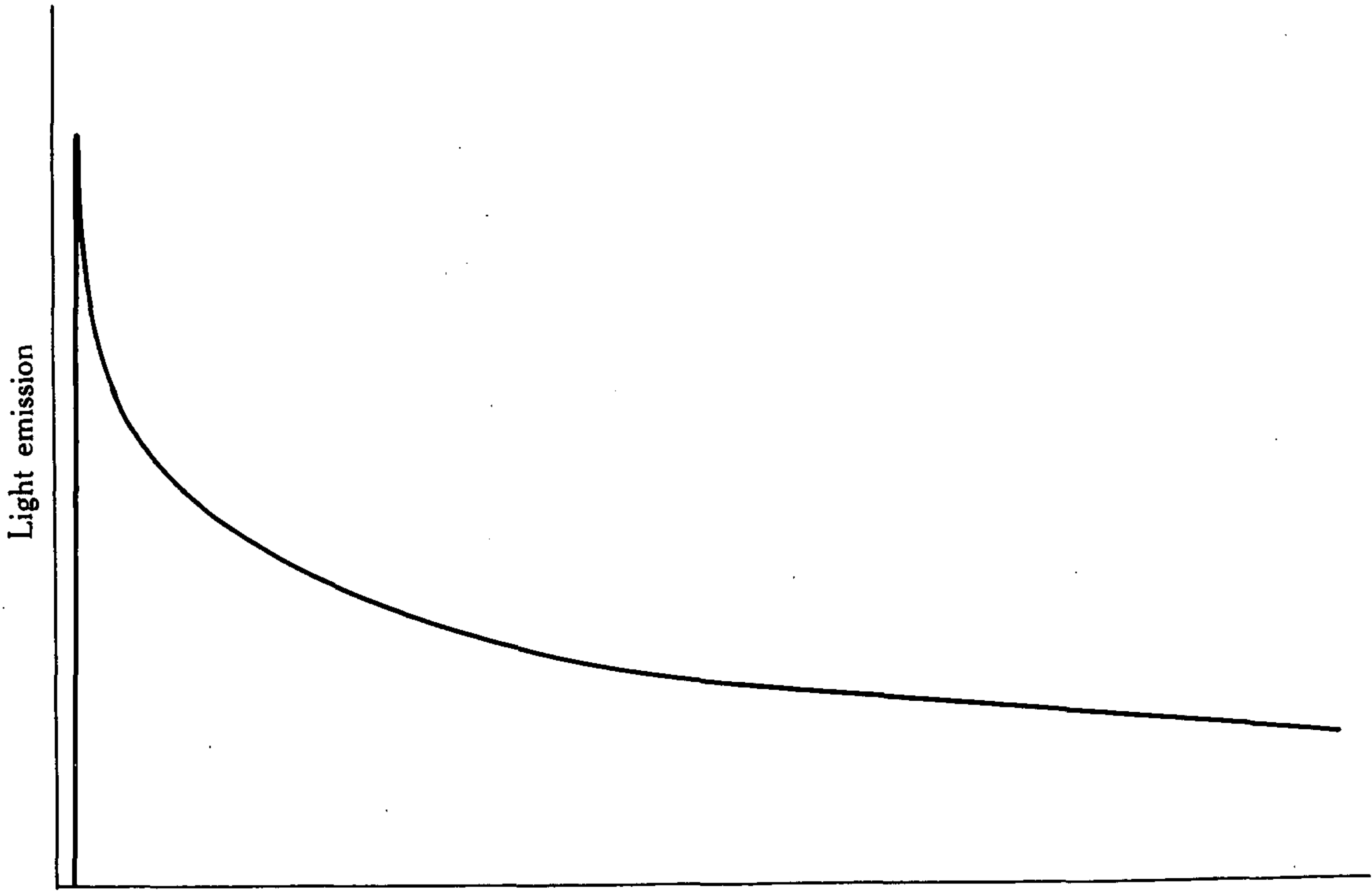


Figure 4. Schematic diagram showing the variation of light emission with time after mixing ATP and firefly extract.