

BIOLUMINESCENCE MEASUREMENTS : FUNDAMENTAL ASPECTS,
ANALYTICAL APPLICATIONS AND PROSPECTS

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INTRODUCTION

Bioluminescence, more especially firefly bioluminescence, has been used for analytical purposes in several laboratories for about 20 years. During the initial period measurements were but seldom performed with quantum counters and the sensitivity of the method was therefore limited by that of the available photometers. After it had become clear that scintillation counters could adequately be used for this purpose (1) the method has been investigated further and its sensitivity for routine applications increased by several orders of magnitude. Modern scintillation counters, as used for the counting of soft beta emitters (carbon-14 and tritium), undoubtedly possess features that are useless for the mere measurement of bioluminescence : e.g. multiple counting channels, coincidence circuitry, external radioactivity standard etc. Their availability in most biochemical laboratories has nevertheless made their use quite popular for this purpose. On the other hand, it may be anticipated that simplified instruments, based on the same technology, will become more popular in the future. One should also realize that bioluminescence is only a special case of chemiluminescence and some of the conclusions mentioned here might therefore be extended to other fields than biochemistry. Such luminescence results from chemical reactions at room temperature, without absorption of light, whenever the free energy of reaction equals or exceeds that of electronic transition in one of the product molecules. However, even when this energy is sufficient to bring an electron in its lowest excited state, no luminescence will often be observed because of internal

quenching or external quenching by the surrounding medium, more especially in polar media. This difficulty was circumvented by the living cell through the mediation of enzymes which show an extraordinary efficiency in the fulfillment of the most delicate and intricate chemical reactions. In firefly bioluminescence this efficiency is exceptionally high and the quantum yield even approaches unity, which means that all excited product molecules return to the ground state with the emission of light.

Chemiluminescence is a very widespread phenomenon encountered among all kinds of chemical substances : inorganic (phosphorus), organic (luminol, lucigenin) and biological (luciferins). Bioluminescence offers undoubtedly the most sophisticated aspects, be it only because of the existence of the enzymatic machinery. Its use as an analytical tool requires some familiarity with its reaction mechanisms and this problem will therefore be dealt with briefly in the first part of this paper in the case of the two best known systems : firefly and bacterial bioluminescence.

The literature on bioluminescence is quite abundant and has been reviewed by several authors : McElroy and Seliger (2), Cormier and Totter (3 and 4), Seliger and McElroy (5), Hastings (6 and 7), Strehler (8), Seliger and Morton (9), Chase (13). Further information is also to be found in the proceedings of symposia devoted to the same subject : McElroy and Glass (10), Johnson and Haneda (11), Hercules, Lee and Cormier (12) (only the publications that have appeared since 1961 are mentioned here). In the next paragraphs the accent will be laid on some important features of firefly and bacterial luminescence, more especially on the latest findings in this field.

Beside its biological significance bioluminescence offers two important aspects :

1° Biochemical aspects :

The following problems deserve special attention:

- Identification and structure of the excited molecule : some progress has been made in recent years but many difficulties were encountered because of the tiny quantities of material involved and the fact that the actual end-product may further react before it can be isolated. Sensitized fluorescence might also have to be taken into account.
- Reaction mechanism : bioluminescent reactions occur in

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several steps which are not always easy to dissociate and more than one substrate may be involved; the enzyme plays a fundamental role not only in catalyzing the chemical reaction but also in creating the necessary micro-environment around the active site, in order to reduce quenching and stabilize the light-emitting species.

2° Physical aspects :

Bioluminescence is directly related to fluorescence phenomena and has therefore also retained the attention of physicists (see Seliger and Morton, 9).

From a thermodynamic point of view the emission of a photon requires about 50-60 Kcal per einstein, which is far more than the energy released in all known biochemical reactions, at least in a single step. Bioluminescent reactions must therefore differ from the other reactions usually encountered in the living cell.

In recent years bioluminescence has developed into an ultrasensitive tool for the measurement of biochemical substrates and enzymes. To understand this one should consider the sensitivity of modern quantum counters such as those used in most biochemical laboratories for the measurement of radioactivity by means of scintillating solutions. Taking into account the background of photomultipliers (10-100 pulses/sec), their efficiency for single photons (10-30%) and the quantum yield of bioluminescent reactions (e.g. 1%), it is theoretically possible to assay quantities of material down to 10^{-18} moles/sec (this evaluation is of course very rough). This sensitivity is lowered by a few orders of magnitude by the finite rate of luminescent reactions but on the whole the present method remains one of the most sensitive ever attained.

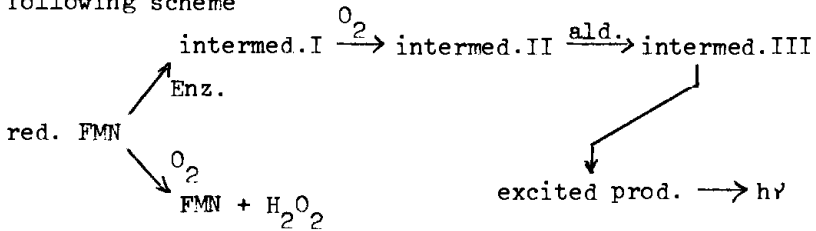
BACTERIAL LUMINESCENCE

A. Reaction mechanism

Luminous bacteria (*Photobacterium fischeri* or *Achromobacter*) were the first bioluminescent organisms to be used in biochemical analysis (Harvey, 14) and served to measure the oxygen production by plants under anaerobic conditions. The reaction requires a riboflavin compound (flavin mononucleotide or FMN) and an additional factor identified as a long-chain aldehyde which increases the light-yield by a factor ranging from 100 to 1000. The role of the latter as a substrate in the reaction has been pro-

posed as early as 1955 by McFlroy and Green (15) but not proved with some certainty until very recently. The role of reduced pyridine nucleotides was clarified around the same period when it was shown that they are merely used to reduce FMN and therefore useless in the presence of pre-reduced FMN.

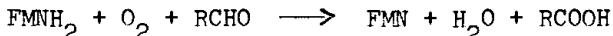
The several steps involved in the mechanism of bacterial luminescence were described ten years ago by Hastings and Gibson (16). On the basis of former experimental evidence and of their own results they proposed the following scheme



It is important to note that reduced FMN oxidizes autocatalytically in the presence of oxygen with a half-life of about 0.1 sec as shown by Gibson and Hastings (17). Taking into account that the half-life of the luminescence is of the order of 10 seconds and that the luminescence maximum occurs after about 2 seconds it must be concluded that the reduced FMN molecules are trapped very rapidly and efficiently by the enzyme and at least one long-lived intermediate may be postulated. The half-life of the luminescence is therefore independent of the enzyme concentrations over many orders of magnitude and the luminescence curve is determined solely by the reaction mechanism whereby the enzymatic complex gives rise to the excited species. The nature of the aldehyde, more especially its chain-length, is important in this respect as it will affect the decay-rate and quantum-yield of the luminescence reaction, whereas the brightness of the reaction is proportional to the FMN concentration. The absence of turn-over of the enzyme is only apparent since the addition of extra quantities of reduced FMN to an exhausted mixture gives rise again to light emission. On the other hand, the addition of fresh enzyme to a mixture after 3 seconds gives no additional light. The turn-over of bacterial luciferase was further studied by Erlanger, Isambert and Michelson (18) by fixing the enzyme to polyacrylic hydra-

zide. The stabilized enzyme showed all the main features of the native enzyme and could be used over and over without apparent limit. When kept at 4° C it remained stable in contrast with the native enzyme which tends to lose its activity rather rapidly.

One of the still debated questions is the ratio in which the several substrates react with each other. The consumption of aldehyde in particular has always been difficult to ascertain and the overall kinetic picture was often confused by the dominance of side-pathways. More recently mass spectrometry was used by Shimomura (19) to identify the end-products of aldehyde oxidation. Tetradecanoic and dodecanoic acid were obtained in roughly quantitative yields when using resp. tetradecanal and dodecanal for the light reaction. The observed quantum yield with respect to the aldehyde was found to be 0.17 ± 0.1 for chain lengths ranging from C₈ to C₁₄. No significant amount of carbon dioxide was produced. According to Hastings, Eberhard, Baldwin, Nicoli, Cline and Nealson (20) bacterial luciferase bears a single binding site for FMNH₂ and they propose therefore the following stoichiometry :

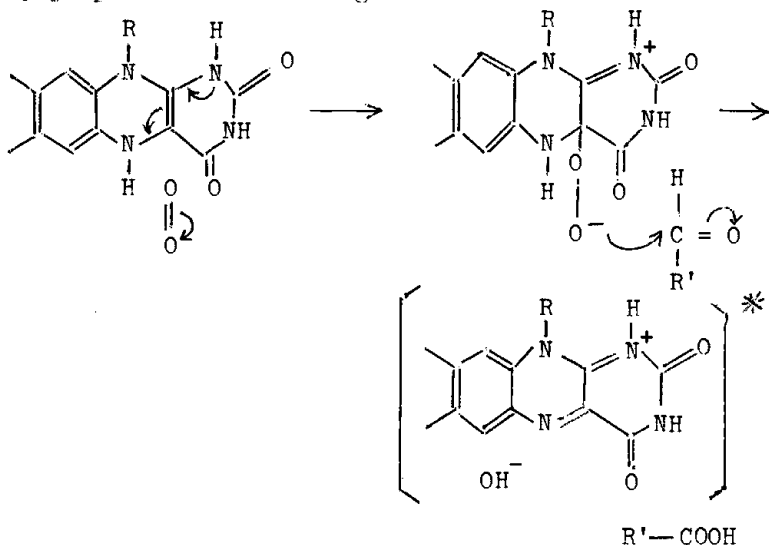


Another approach was used by Lee (21) who deduced stoichiometric ratios from the oxygen consumption and from the relative substrate concentrations leading to maximum quantum yield. According to his scheme two reduced FMN molecules would react in a coordinated way.

B. Luminescent intermediate

Study and identification of luminescent intermediates has been based mostly on the comparison of the chemiluminescence spectrum with the fluorescence spectra of known substances and, of course, on the chemical identification of the end-product of the reaction. Spectral considerations may however be impaired by the fact that the spectrum of the emitting molecule is likely to be modified by its binding to the enzyme. Energetic considerations are also fundamental and should remind us of the fact that in the case of bacterial luminescence the oxidation of one FMNH₂ molecule by oxygen creates only 27 Kcal of energy which is insufficient to produce photons with a wavelength of 490 nm. Eley, Lee, Lhoste, Lee, Cormier and Hemmerich (22) observed that the luminescence spectrum matches more

closely with that of flavin protonated at the N-1 position. By combining this observation with the need for an energy supply from the aldehyde Eberhard and Hastings (23) have recently proposed the following mechanism :



C. Structure of the enzyme

The high quantum yields observed in bioluminescence may be ascribed to the specific role of the enzyme which protects the excited molecular species from quenching. The microenvironment surrounding the substrate in the active site appears therefore to be of paramount importance and gives additional interest to the study of the enzyme structure. Bacterial luciferase was shown to consist of two sub-units (24,25) whereas kinetic experiments by Meighen and Hastings (26) showed the existence of a single flavin binding site per dimer.

Numerous mutants of bacterial luciferase have been isolated from which it could be deduced that both sub-units are not functionally equivalent (27). One of these mutants has been the subject of particular attention and was designated as MAV by Hastings, Weber, Friedland, Eberhard, Mitchell and Gunsalus (28) who isolated it some years ago. It differs slightly from *Photobacterium fischeri* with respect to the molecular weights and the composition of the subunits (25). It is further characterized by the longer life-time of the intermediate products, which

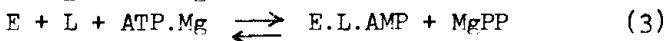
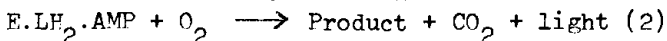
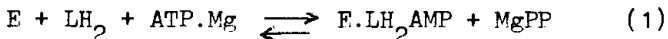
makes it a "slow" luciferase. Optimum quantum yield is obtained with decanal as compared with tetradecanal for the *Photobacterium fischeri* strain.

FIREFLY LUMINESCENCE

A. Reaction mechanism

Firefly luminescence is characterized by the existence of a specific substrate, luciferin, which has become commercially available since a few years, and by the need for ATP. The system was developed into an assay method for this substance by Strehler and Totter (29).

The general reaction scheme of firefly bioluminescence may be summarized as follows (LH₂ = luciferin; L = dehydroluciferin)



Step (1) is reversible and shows a striking similarity with the activation of aminoacids by synthetases. Both the L and D isomers of LH₂ react with ATP although only the D-isomer is susceptible to give off a photon. ATP reacts as a complex with Mg⁺⁺ which associates stoichiometrically with its β and γ phosphate groups, leaving the α phosphate group free to bind with the enzyme.

Oxidation of the activated substrate occurs in step (2). During many years it has been considered that L.AMP was the end-product of the reaction. It is indeed tightly bound to the enzyme ($K_{diss} = 5.10^{-10}$) and was thought therefore to cause end-product inhibition. Pyrophosphate, which inhibits step (1), would on the contrary reverse the inhibition caused by end-product accumulation (step 3) with the simultaneous release of ATP (8). Such cyclic mechanism was supposed to account for the long-lived tailing of the luminescence curve but in the light of more recent knowledge this mechanism has to be reconsidered.

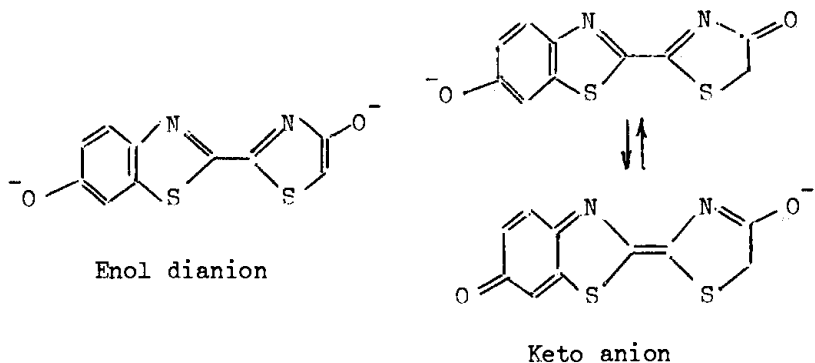
The elimination of carbon dioxide was demonstrated to be roughly quantitative in the presence of luciferase (30) using C¹⁴-labeled luciferin. AMP is probably ejected before the emission of light can occur (see also 31 and 32).

The activation of luciferin is a two substrate reaction and kinetic experiments performed by Denburg, Lee

and McElroy (33) show the binding of LH_2 and $\text{Mg}\cdot\text{ATP}$ to occur in a random order. Luciferin enhances the binding of ATP slightly and vice-versa. The binding of ATP is influenced to a greater extent by the ionic strength of the medium (34): The K_m for $\text{ATP}\cdot\text{Mg}$ increases with increasing ionic strength according to the Hofmeister series ($\text{SCN}^- > \text{I}^- \sim \text{NO}_2^- > \text{Br}^- > \text{Cl}^-$) but is not influenced by the nature of the cation (Na^+ , K^+ or NH_4^+). The anion inhibition is instantaneously reversible and non competitive with respect to $\text{ATP}\cdot\text{Mg}$; it may therefore be ascribed to a general ionic strength effect expressed as a decrease in attraction. The K_m of luciferin is not affected in this particular instance.

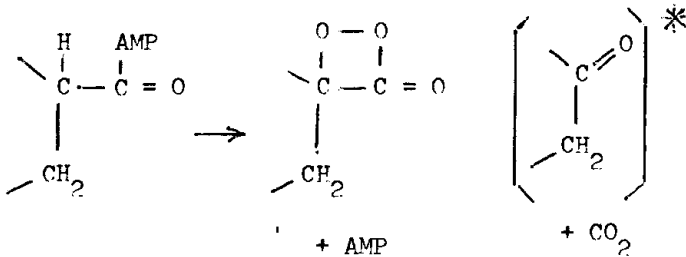
B. Luminescent intermediate

As stated above it has become evident that dehydro-luciferin is not responsible for the observed luminescence in fireflies. Spectral studies have shown that at neutral or basic pH the quantum yield is 1 and the emitted light yellow-green (562 nm). In acid medium the quantum yield is depressed and the spectrum shifts to the red (maximum at 614 nm). This shift can be explained by the predominance, at low pH, of another distinct emitting species. The red emission observed in vitro as well as in vivo may be ascribed to the keto anion of decarboxylated luciferin (9 and 31); the yellow-green light observed in vivo would be emitted by the enol dianion (32).



Similar and other considerations have led to the conclusion that the most probable intermediate is a dioxetane ring, known to give highly efficient electronically excited fragments (35, 36, 37 and 38).

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Some controversy still exists about the origin of the oxygen incorporated into the dioxetane ring, either molecular oxygen or water (39, 40).

C. Structure of the enzyme

Firefly luciferase has a molecular weight of 100,000 and consists of two subunits of molecular weight 50,000. At low concentration it is always dissociated. Using equilibrium dialysis and fluorimetric techniques it was possible to identify two sites for ATP (or AMP), two for LH_2 , one for $\text{Mg} \cdot \text{ATP}$ and one for $\text{L} \cdot \text{AMP}$ per molecular weight of 100,000 (33, 34 and 41) whether the enzyme is dissociated or not. The subunits are similar but not identical. The $\text{ATP} \cdot \text{Mg}$ site is identical with one of the sites for ATP which acts as a competitive inhibitor with respect to $\text{ATP} \cdot \text{Mg}$. The role of the second ATP site is not clear as yet and could possibly have a regulatory function. The enzyme binds only one AMP in the presence of $\text{ATP} \cdot \text{Mg}$ and none in the presence of an excess of ATP.

ANALYTICAL APPLICATIONS OF BIOLUMINESCENCE

A. Instrumentation and counting technique

One should first of all be aware of the fact that the analytical applications of bioluminescence are based on the measurement of reactions rates. On mixing of the reagents luminescence curves usually show a sharp rise followed by a more or less rapid decay. The decay-rate will depend on the specific reaction being measured and also on the experimental conditions. Many measuring instruments used up to now have been aimed at the measurement of the peak intensity of the luminescence curve, which used to be considered as the most representative of the substrate concentration. Analog instruments used for this purpose con-

sist essentially of a photomultiplier with its associated electronics, connected to a fast recorder or oscilloscope. In a commercial version (Biometer, Dupont Instruments) the maximum is automatically recorded and displayed on a digital register.

Scintillation counters do not allow to record luminescence peaks of short duration (a few seconds or less). However, experiments performed with such counters have shown that one is not necessarily bound to the measurement of this peak. In the case of firefly luciferase and under the proper conditions, the luminescence decreases exponentially over a rather long period of time (half an hour or more), while remaining proportional to the ATP concentration (42, 43, 44, 45). This enables one to use the integral mode of counting which is, in practice, the only compatible with the use of scintillation counters, and at the same time makes flow-monitoring possible. A lin-log plot of the luminescence versus time gives straight lines which run perfectly parallel over a very wide concentration range and whose slopes are proportional to the enzyme concentration.

In the case of bacterial luminescence, when the reduced FMN is produced in situ by the oxidation of NADH_2 , the concentration of the dehydrogenase associated with the luciferase can be adjusted in such a way that a flattened luminescence peak is obtained allowing for the necessary time to perform the countings (46, 47, 48). With some systems a steady state may be achieved whereby the reduced substrate is produced continuously in the presence of the luminescent system.

For the assay of ATP integration of the luminescence is currently started after a fixed time interval following mixing of the reagents. This principle has been automated in the "ATP Photometer" developed quite recently by JRB Inc., San Diego Calif. In our hands a time interval of a few minutes proved adequate, giving ample time to introduce the sample into the counter.

Although scintillation counters possess some features that are useless for bioluminescence measurements one can take advantage of their high performance as automated quantum counters

- they can be used for discrete as well as for flow measurements (when used in the "repeat" mode)
- they have a very high sensitivity
- the integral technique allows to obtain better statis-

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- tics; speed of mixing is also less critical
- they have a wide dynamic range
 - discriminator, gain and high voltage settings permit an accurate choice of the best counting conditions
 - advantage can be taken of the sample changer for automation purposes (49)
 - digital results are obtained which can easily be used by data processing equipment (50).

Bioluminescence countings may often be impaired by the phosphorescence of the vials introduced into the counter. A fixed cell, remaining in the counter, was therefore developed together with a semi-automatic micro-transferator for the introduction of small-sized samples. Transfer occurs by suction by means of a motor-driven microsyringe (51).

The choice of the high voltage and discriminator settings is a problem to which sufficient attention has not always been paid, when trying to achieve maximum sensitivity. The settings used for the scintillation counting of tritium are not adequate, contrary to what has sometimes been advocated. Overlapping of the photomultiplier noise with the luminescence spectra is an important factor since one operates with the coincidence disabled and at room temperature where the noise is evidently higher. Higher voltages are not necessarily better as discussed in one of our previous papers (51) and may give rise to memory effects at high counting frequencies. High counting rates may further be at the origin of erratic results due to double photon pulses (43).

Bioluminescence measurements are preferably performed around room temperature. This temperature may be stabilized in a very simple way by using small sample tubes inserted into regular scintillation vials filled with water (43, 48).

Partial automation of bioluminescence assays was achieved by means of a conventional auto analyzer equipment (52) or a Hamilton precision liquid dispenser (49), in both cases in conjunction with a liquid scintillation spectrometer.

B. General methodology

1. Firefly luminescence : The assay of ATP has been dealt with by many authors since this method was introduced by Strehler and Totter in 1952 (29). Whether one measures the initial luminescence flash, or the light intensity after a fixed delay, in both cases results could be obtained which were shown to be proportional to the concentration of ATP which acts as the limiting component in the system. It is of course essential that this concentration does not exceed half-saturation of the enzyme, but this is not likely to be the case. Indeed, the half-saturation concentration of firefly luciferase is 35 $\mu\text{g/ml}$ (10^{-7} mole/ml), i.e. several orders of magnitude more than the quantities actually assayed.

The sensitivity of the method is determined by that of the measuring equipment but is also affected by the residual luminescence of commercial enzyme preparations. Unfortunately purification of the enzyme by crystallization (54) is neither practical nor economical on a small scale. In common practice the blank value is usually lowered by ageing the enzymatic preparation before use. Methods have also been suggested, based on gel filtration (55, 56, 57). Luciferin, dehydroluciferin and ATP are easily separated from luciferase on low porosity gels, and sensitivities of 10^{-14} - 10^{-15} mole ATP can be attained with the purified preparations. Higher porosity gels may also be used to free the luciferase from other interfering enzymes which affect the specificity of the reaction. Nielsen and Rasmussen (55) were able to lower the adenylate kinase (myokinase) content 500 times but the NDP kinase (trans-phosphorylase) is removed less efficiently. These enzymes are responsible for the production of ATP in the presence of resp. ADP and other nucleoside triphosphates. Since the ATP is formed but gradually after the addition of the luciferase, interference by these enzymes is indicated by a modification of the shape of the luminescence curve. However, in most routine cases the results will not be impaired.

Several methods have been tested for the extraction of ATP from biological material : n-butanol (57), trichloroacetic acid (58), perchloric acid (59), ethanol (58, 60, 61), tris-buffer, etc. Perchloric and trichloroacetic acid interfere with the luminescence and should therefore be avoided and tris-buffer is usually to be preferred.

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Interfering authocyanins and phenolic compounds extracted with the ATP from plants could be eliminated by means of polyvinylpyrrolidone (58).

When assaying ATP in living material it is important to ascertain that its content has not been modified during sampling, storage or extraction (59, 61, 62). The best results are obtained by injecting the sample rapidly into a greater volume of boiling water or buffer taking care to keep the temperature as close as possible to 100° C. Thanks to the extremely high sensitivity of the ATP assay the dilution effect will in general not be harmful.

2. Bacterial luminescence

Bacterial luciferase is specific for FMN but its interest lies also in the fact that it occurs associated with a dehydrogenase that makes the system suitable for the measurement of reduced NAD in the presence of an excess of FMN (44, 46, 47, 48, 63, 64, 65). The same system reacts also with NADP but to a lower extent.

Reduced FMN being rapidly oxidized in the presence of oxygen its assay must be performed after reduction "in situ" by means of NaBH_4 (63) or in the presence of an excess of NADH_2 (FMN being in this case the limiting component).

The sensitivity of the present method reaches far beyond that of fluorimetric techniques (10^{-13} - 10^{-14} mole, 47, 64). Moreover, many substances likely to interfere in fluorimetric assays will not affect the bioluminescence. The proportionality between substrate concentration and luminescence holds over many orders of magnitude as for ATP luminescence.

A method has been proposed recently by Brodin et al. (65) for the assay of pyridine nucleotides in mixtures. Selective measurements of either NADH or NADPH is performed after oxidation of the unwanted nucleotide by means of a highly specific enzymatic system. An alternative method is to reconstitute the reduced nucleotide to be measured, resulting in a continuous light emission.

Both bacterial and firefly luciferase are but poorly stable and their efficiency for light production should therefore be tested with each batch of samples. The effect of interfering substances will also preferably be taken into account by the addition of an internal standard.

C. Analytical applications

Many of the actual but also potential applications of firefly bioluminescence have already been reviewed by Strehler and Totter as early as 1954 (53) and more recently by Strehler (8). They can be subdivided according to the substance being assayed:

1. assay of the luminescence substrate (ATP)
2. assay of related substrates which can be converted directly into ATP (ADP, AMP) or may yield ATP in the presence of ADP (phosphoenolpyruvate, creatinephosphate, nucleosidetriphosphate, etc.). The method has been extended recently to c-AMP (66). In some cases the substrate concentration may be deduced from the consumption of ATP (e.g. glucose)
3. assay of enzymes : e.g. hexokinase, pyruvate kinase, creatine kinase, etc.

Recent applications are concerned with the measurement of phosphodiesterase (67) and ATP-sulphurylase (68, 69).

Although bacterial bioluminescence was introduced more recently as an analytical method its potential field of application is at least as broad as that of firefly luciferase. Many compounds which are either convertible in dehydrogenase reactions or can lead to a dehydrogenase step can indeed be subjected to analysis.

NADH is currently produced in separate reactions preceding the light yielding reaction. The following substances were for instance assayed by Brodin et al. (64).

- glucose : NADPH was formed in a coupled hexokinasedehydrogenase reaction. Sensitivity : 0.5-6.0 pmole in aliquot of 1.1 μ l.
- malate : the unfavourable equilibrium for the NADH formation was overcome by using an NAD analog and hydrazine as a trapping agent.
- NAD : reduced with ethanol and alcoholdehydrogenase in the presence of semicarbazide as a trapper.

In our laboratory (Gerlo, unpublished results) the oxidation of glucose-6-P was performed in presence of the luminescent system, giving rise to a rather steady light yield.

During recent years bioluminescence has been involved in the determination of biomass and its application to soil and water ecology. With this in mind systematic studies were undertaken on the ATP level and its variations

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in bacteria (57, 70) and other microorganisms, chiefly marine algae (62). Biomass determination on the basis of ATP content is a simple and rapid technique compared with slower and more tedious classical methods as plate-counting and microscopic examination. It is based on the assumption that 1°/ no ATP is associated with non-living particulate material, 2°/ that the ratio of ATP to other parameters is fairly constant. As far as this last assumption is concerned it was shown that in marine bacteria the ATP content ranges from 0.3 to 1.1 % of the cell carbon, the average being 0.4 % (70). Similar studies on marine algae (62, 71, 72) showed a considerable uniformity in ATP concentration in diverse algae ranging in size from less than 1 $\mu\text{g C/cell}$ to 215,000 $\mu\text{g C/cell}$. The average value of 0.35 % relative to C is very close to that reported for bacteria. Biomass profiles of sea-water could in this way be obtained on 1-2 liter samples down to depths of 3500 m. (73, 74). The samples were filtered on 47 mm membranes of 0.45 μ pore-size and immediately immersed in boiling tris-buffer to extract ATP and inactivate the enzymes (61).

ATP determinations in lake sediments were performed by Lee et al. (75, 76).

CONCLUSIONS

Bioluminescence has been used up to now for the assay of various substances and many more are now awaiting to be assayed by the same method. The conditions for each specific substance need of course to be standardized as for any other enzymatic assay, but it may be anticipated that the array of applications will be extending steadily, together with the further development of suitable instrumentation.

It should be observed that ATP, FMN and NAD(P) are involved more or less directly in all biochemical reactions and that a great number of substrates and enzymes are therefore likely to be assayed in some way by the techniques of bioluminescence. Because of their high sensitivity only minute samples are needed and they might therefore appear very suitable in the future in the field of clinical chemistry.

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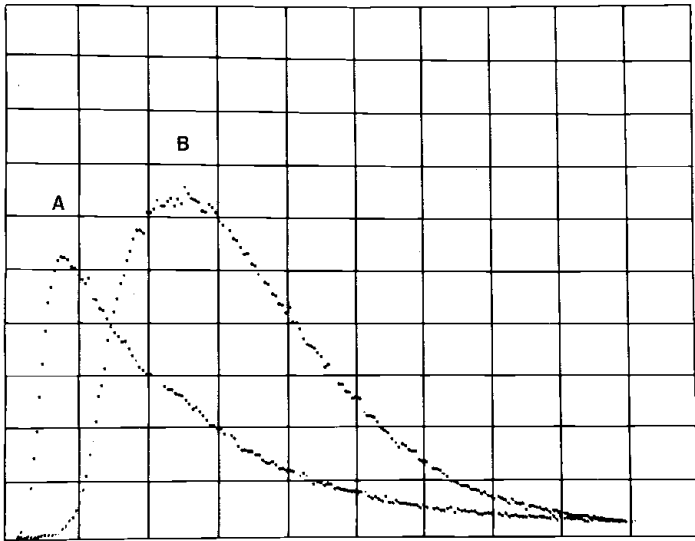
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** Editorial Note. This volume has now been published. *Chemiluminescence and Bioluminescence* (M.J. Cormier, D.M. Hercules & J. Lee, eds.) Plenum Press, New York (1973).



Comparison of photomultiplier background (A) and bioluminescence (B) spectra. Curves obtained with a SA41 multi-channel analyzer connected to a SL20 scintillation counter (Intertechnique)