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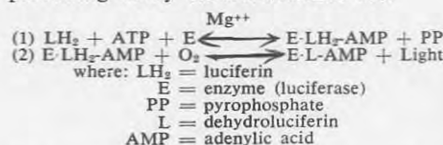
A rapid method for detection of microorganisms by ATP assay; its possible application in virus and cancer studies

The rapid detection of living material and the measurement of changes in metabolism are important problems common to such diverse activities as biological warfare defense, medical research, and the search for extraterrestrial life (Levin, 1963). The ubiquity of adenosinetriphosphate (ATP) in all forms of terrestrial life is generally accepted (Huennekens and Whitely, 1960) thus making it an indicator of life at all levels of cellular organization. Since the energy requirements for all biological reactions are directly or indirectly supplied through ATP, any change in cellular integrity should be reflected in the kinetics of ATP metabolism. Thus, pathological changes might be monitored.

In 1960, this laboratory proposed a bacteria detection technique based on the firefly bioluminescent assay for ATP (McElroy, 1947, 1957; Billen et al., 1953; Strehler & Totter, 1954). This investigation[1] is now in an advanced state, and feasibility has been demonstrated (Resources Research, Inc., 1963 a, b, c) with a wide variety of microorganisms.

The reaction steps (Seliger et al., 1961) producing firefly bioluminescence are:



$$(1)\ LH_2 + ATP + E \overset{Mg^{++}}{\longleftrightarrow} E{\cdot}LH_2{\text{-}}AMP + PP$$
$$(2)\ E{\cdot}LH_2{\text{-}}AMP + O_2 \longrightarrow E{\cdot}L{\text{-}}AMP + \text{Light}$$

where: LH_2 = luciferin
E = enzyme (luciferase)
PP = pyrophosphate
L = dehydroluciferin
AMP = adenylic acid

With all of its other components present in excess, the reaction becomes quantitatively specific for ATP.

Two types of light detection apparatus are currently being used. One consists of a light chamber which permits a cuvette containing a solution of firefly lantern extract to be rotated into the chamber to face a photomultiplier tube. A rubber plug above the cuvette is then pierced with a hypodermic needle through which the material suspected of containing ATP is introduced. The photomultiplier tube monitors the light produced by the reaction. The resulting emf is then amplified and displayed on an oscilloscope screen. A Polaroid camera may be used to record the sweep generated. In the second system, the test material can be introduced into a reaction chamber by hypodermic syringe, capillary rise, or aerosol spray. The light is detected by a Photovolt Model 520-Special photometer, and its intensity is recorded on a Photovolt Model 44 linear recorder.

While responses have been obtained with whole cells, disruption of the cells is required for quantitative assay of ATP. An ultrasonic oscillator is used for this purpose.

[1] Supported by the Bureau of Naval Weapons, U.S. Navy.

In a typical test, 1.0 ml of a 0.5% solution of commercially available lyophilized firefly lantern extract[2] is placed into a cuvette which is then positioned in the light detection chamber. The buffered extract contains luciferase, luciferin, and magnesium. Sufficient dissolved oxygen for the reaction is present in the solution. A suspension of the material to be assayed is subjected to ultrasonic vibration for one to several minutes. One-tenth ml of the suspension is then drawn into a hypodermic syringe and immediately injected through the light-proof seal into the cuvette. The reaction reaches maximum light intensity in less than 0.5 seconds and then decreases exponentially for several minutes. The area under the curve generated by either type of recording device is proportional to the quantity of ATP present. Since, under uniform conditions, the maximum intensity is directly proportional to the total ATP content and also to the total area under the curve, it can be used as a direct, quantitative measure of ATP. The entire procedure can be executed and recorded in less than 2 minutes. Figs. 1 and 2 compare responses obtained with standard ATP and a suspension of yeast cells, both with the oscilloscope unit. As seen, 750 cells of *Saccharomyces cerevisiae* were readily detected.

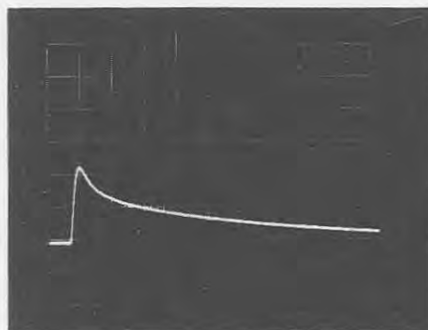


FIG. 1. Light response obtained from 10^{-3} gamma ATP by firefly bioluminescent reaction. Maximum deflection 42 mv, horizontal sweep 5 sec/cm.

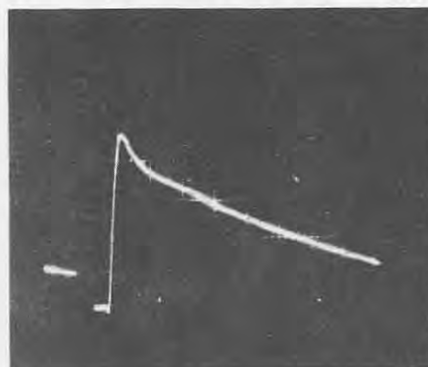


FIG. 2. Light response obtained from 750 cells of *S. cerevisiae* by firefly bioluminescent reaction. Maximum deflection 48 mv, horizontal sweep 5 sec/cm.

[2] Worthington Chemical Corp., Freehold, N. J.

TABLE 1. Cells from which positive ATP responses were obtained by firefly bioluminescent Assay

Arthrobacter simplex
Azotobacter agilis
Azotobacter indicus
Bacillus cereus
Bacillus subtilis
Bacillus subtilis spore suspension
Chlorella pyrenoidosa
Escherichia coli
Micrococcus cinnabareus
Penicillium spores
Pseudomonas delphinii
Pseudomonas fluorescens
Saccharomyces cerevisiae
Serratia marcescens
Staphylococcus epidermidis
Streptomyces bobiliae
Xanthomonas beticola
Xanthomonas campestris
Erythrocytes (mouse)
Leucocytes (mouse)
Axillary region (mouse)
Liver, kidney (mouse)
Kidney (monkey)

To date, the cells listed in Table 1 have been tested, and all have produced positive responses. The currently developed sensitivity of the method utilizing the more sensitive Photovolt unit permits the detection of 10^{-7} gamma of ATP. It is believed that this sensitivity can be still further increased.

During preliminary experiments to extend the usefulness of this technique, attempts were made to detect possible differences in ATP content of normal and virus-infected tissue. A suspension of monkey kidney tissue was divided into aliquots, several of which were inoculated with similar cells infected with an adenovirus[3]. Both sets of cultures were incubated for 3 days and ATP determined as described above. On an equal volume basis, the infected culture contained approximately 10 times as much ATP as the uninfected. The fact that the ATP in small amounts of tissue culture can be rapidly assayed indicates that the method can detect viral biological warfare agents in addition to bacterial ones if the former are delivered in tissue culture.

The mouse and monkey tissues listed in Table 1 were obtained[4] in both cancerous and noncancerous form. The noncancerous material was taken from the same animals and organs as that containing the malignancies. In each of five tissues cited, the

[3] Cultures were supplied by John Nemes, Dept. of Microbiology, School of Medicine, Georgetown University.

[4] Tissues were supplied by James Larkin, Toxicology Dept., Hazleton Laboratories, Inc.

ATP content of the cancerous material was significantly lower, ranging from 10 to 30% of that present in equal aliquots of the noncancerous.

The virus and cancer experiments, although in a very early stage, indicate a possible method for studying the kinetics of virus infection, cancer development, and other disease mechanisms. This suggests that the bioluminescent ATP assay may also become useful as a diagnostic tool.

Whether or not extraterrestrial life might be detected by this technique would depend on the similarity of the biochemistry of the life found to that of life on Earth. The detection of extraterrestrial life through ATP assay, therefore, would be an important step in establishing a possible relationship between the two forms of life.

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