37 – 6 A LIFE DETECTION INSTRUMENT BASED ON THE FIREFLY BIOLUMINESCENT REACTION

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The rapid detection of small populations of microorganisms is frequently important in such varied activities as clinical medicine, public health, sterility testing, bacteriological warfare detection, and the forthcoming search for extraterrestrial life. Currently used standard methods require one to several days for a microbiological determination. A unique approach toward a rapid method to determine total bacterial populations is described in this paper. It is based upon the ubiquity of adenosine triphosphate (ATP) in all known life, and the specificity, sensitivity, and rapidity of the firefly bioluminescent reaction as a quantitative assay for that biochemical compound.

The firefly emits light through a reaction involving its enzyme (luciferase), a substrate (luciferin), oxygen, magnesium, and ATP (1-6). The ATP-free reaction mixture may be prepared from firefly lanterns and stored for use. The injection of ATP into the reaction mixture will initiate the bioluminescent reaction. The amount of light emitted is proportional to the quantity of ATP injected when the other reactants are present in excess. Previous work reported (7) on this method and associated instrumentation by one of our groups (HLI) indicated that more advanced instrumentation might achieve extraordinary sensitivity.

The heart of the new life detection instrument is a 16-stage ITT photomultiplier tube. It is fitted with a thermo-electric cooling element to obtain a regulated photocathode operating temperature of -40° C. Using a phototube accelerating voltage which yields maximum signal to noise ratio for low illumination levels, this tube produces a stable background and consistent results when tested with a standard light source. Pulse counting, with variable pulse amplitude discrimination, is used to increase signal to noise ratios in the measurement of low light levels. Amplified phototube output pulses are counted by an electronic counter with a numerical read-out and are applied to a pulse stretcher and integrator for simultaneous presentation on a strip chart recorder.

The bioluminescent reaction takes place in a light-tight chamber which holds a movable rack containing eight cuvettes. Prior to ATP assaying, 0.3 ml. of the reaction preparation is added to each cuvette in the rack. The cuvettes may be remotely positioned one at a time in front of a lucite light pipe leading from the reaction chamber to the photocathode of the detector. A temperature controlled water bath maintains the cuvettes and contents at any desired temperature between 5° C. and 50° C., normally 25° C.

The sample to be tested is subjected to ultrasonic vibration for one minute to rupture any cells present and release the ATP. An 100 microliter syringe is then loaded with the sample. The syringe mounts inside of the reaction chamber directly over the reaction position. To inject a sample volume, 80 p.s.i. is applied to an hydraulic cylinder from an external gas supply through a fast-acting electrical solenoid valve. The speed of the injection and the amount injected are controlled by the pressure and the time interval, respectively. The pressure is adjusted by a manual bleeder valve. The time interval during which the pressure is applied is selected through an electronic timer. Typical injections may range from 1.0 µl. in 0.25 milliseconds to 100 µl. in 125 milliseconds.

The speed of mixing of the injected sample and the enzyme is a determinant of the reaction rate. High reaction rates are desirable to maximize the signal to background noise ratio. To improve mixing and viewing, the syringe needle is immersed in the reaction mixture so that the tip of the needle is centered in the optical system.

The pulse counting circuit is gated by a second adjustable electronic timer. Normally the count period is synchronized to begin with the initiation of the injection cycle, and continues for a period of from one to 30 seconds after the injection is completed. The light output from the reaction increases rapidly to a peak within several seconds after injection. An exponential decay may then last for a period of minutes depending on the mixing speed and the quantity of ATP injected. The two factors determining the optimum counting period are the signal to noise ratio and the magnitude of the observed value.

Strict attention must be given to chemical cleanliness when conducting experiments in the sub-gamma quantity range to prevent contamination with extraneous ATP. Sterilization and chemical cleaning of appropriate parts have been conducted by autoclaving, boiling in hydrochloric acid and extensive rinsing. Airborne matter is prevented from settling in cuvettes by clean, sterile covers which the syringe needle penetrates.

Figure No. 1 shows a simplified block diagram of the ATP Detection Instrument.

Figure No. 2 shows the ATP Detection Instrument packaged in a console. The reaction chamber, made of stainless steel, is on the right end of the console with the hydraulic cylinder mounted on the top. The upper right-hand panel contains the adjustable electronic timers to start and stop the injection cycle and to start and stop the pulse counting interval. The lower

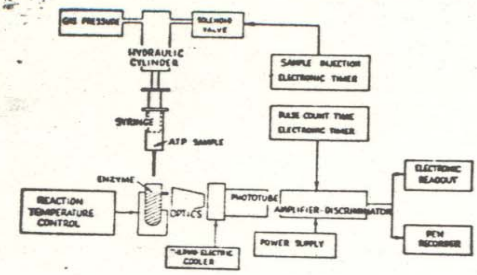

Fig.1. Block Diagram of ATP Detection Instrument.

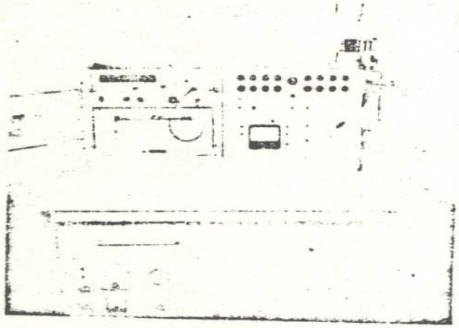

Fig.2. Console ATP Detection Instrument.

right-hand panel contains the immersion fluid temperature control and a front panel controlled shutter to protect the phototube when the reaction chamber is opened to room light. The phototube is located in a cylindrical heat shield behind this panel.

The upper right panels contain the electronic pulse counter and read-out, the pulse amplifier, discriminator, and the digital to analog converter to drive the strip chart recorder located in the lower left-hand panel position. The drawer panel contains the hydraulic pressure controls and the electrical controls and temperature read-out for the phototube thermo-electric cooler.

Figure No. 3 is a typical ATP concentration curve obtained with the instrument. The abscissa is the number of pulses counted in a five-second period following an injection, and the ordinate is the quantity of ATP contained in the injected volume. The solid line gives the average of a set of eight to 16 similar reactions; the dotted lines are at plus and minus two standard deviations. These results were obtained during early development of the instrument and do not represent the ultimate sensitivity. Since then, a better determination of the optimum counting period for very low levels of ATP has been made. In addition, the previously persistent "inherent" light present in the enzyme extract has been substantially reduced through partial purification of the luciferase. These improvements now permit the detection of quantities of ATP as small as 10^{-8} gamma, making the instrument one of the most sensitive chemical analytical tools extant.

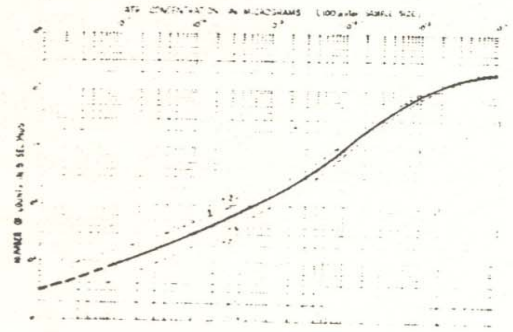

Fig.3. ATP Concentration Curve.

In tests with bacterial suspensions, the ATP released from several hundred cells of such species as Escherichia coli or Pseudomonas fluorescens has been readily detected. Only several minutes are required for sample preparation and completion of the test. It is anticipated that further progress will improve sensitivity another one or two orders of magnitude.

Plans are currently being made by the Goddard Space Flight Center for miniaturization of the instrument. A series of rocket-borne instruments will be flown for in-flight determinations of the microbiological profile of the Earth's atmosphere. Subsequently, the device might be used in planetary explorations for extraterrestrial life.

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References

1. McElroy, W. D., Proc. Nat. Acad. Sci. 33, 342, 1947.
2. McElroy, W. D., Hastings, J. W., Coulombre, J., and Sonnenfeld, V., Arch. Biochem. and Biophys. 46, 399, 1953.
3. Hastings, J. W., McElroy, W. D., and Coulombre, J., J. Cell. and Comp. Physiol. 42, 137, 1953.
4. McElroy, W. D., and Green, A., Arch. Biochem. and Biophys. 64, 257, 1956.
5. Bitler, B., and McElroy, W. D., Arch. Biochem. and Biophys. 72, 358, 1957.
6. Airth, R. L., Rhodes, W. C., and McElroy, W. D., Biochem. Biophys. Acta 27, 519, 1958.
7. Levin, G. V., Clendenning, J. R., Chappelle, E. W., Heim, A. H., and Rocek, E., BioScience 14, 37, 1964.