ATP: ATP disodium $5H_2O$ crystalline, Sigma grade 99 to 100%. 5 mg of ATP were diluted to 5 ml with pH 7.8 0.01 *M* glycylglycine buffer; further dilutions were carried out as needed.

Instrumentation: The light produced by the addition of ATP to the reaction mixture was measured in an Aminco Chem-Glow photometer using a Heathkit-Servo recorder. The reaction is initiated by the addition of 0.1 ml of ATP into a test tube containing 20 µl of a mixture of luciferin, luciferase, and magnesium. Following the addition of the ATP, the contents are vigorously mixed on a Vortex mixer for 5 sec and then placed into the chamber. The flash height is read 7 sec after the addition of ATP. It has been ascertained that this method is much more reproducible than the injection procedure used by other investigators.

Optimum conditions for assay: It was first necessary to show that in the presence of all components (ATP, luciferin, Mg^{++}, and O_2) the rate of the reaction was directly proportional to the luciferase concentration. These data, in which the concentration of luciferin, ATP, MG^{++}, and O_2 were kept constant but the amount of luciferase was varied, are shown in Figure 1. These data are plotted as log of arbitrary light units versus log of 10 times the enzyme concentration expressed in ml. It can be seen that the response is directly proportional to the enzyme concentration. Activity is defined as the maximum flash height in mV (or any arbitrary unit) achieved when ATP is added to the mixture.

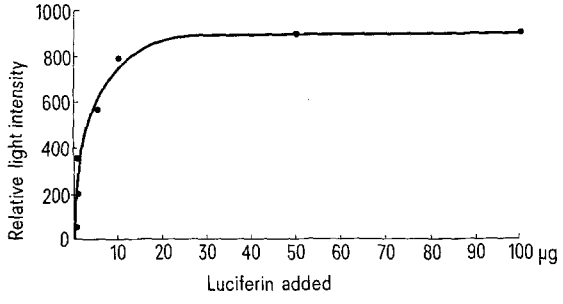

Fig. 2. Effect of luciferin concentration on light emission.

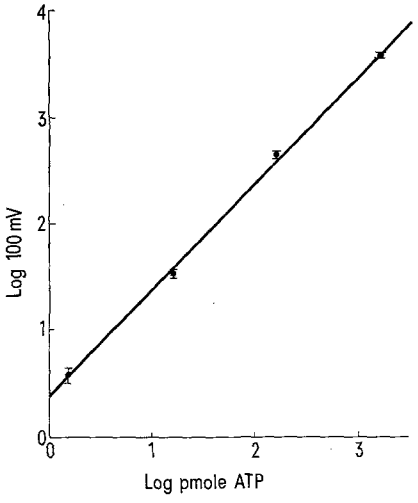

Fig. 3. The effect of ATP concentration on light production.

Effect of luciferin concentration: Figure 2 shows the effect on the light intensity of varying luciferin concentrations in the presence of a fixed amount of enzyme and ATP.

Problem of inherent light: Many methods involving firefly luciferase suffer from the problem of inherent light. We have found that by preparing a premix of pure luciferin and partially purified luciferase and permitting the mixture to age for 24 to 36 h in the cold, that the inherent light decreases to a constant low level with no loss of sensitivity with respect to ATP.

Standardization of the method: The data discussed above have led to the establishment of a standard method for the assay of ultramicro amounts of ATP. These data indicate that the preparation of a premix leads to conditions offering the maximum sensitivity with respect to ATP at the lowest inherent light level. The procedure has been standardized in the following manner: 1 mg of pure luciferin that has been maintained under nitrogen in the deep freeze is dissolved in 10 ml of glycylglycine buffer, 0.01 *M*, pH 7.8, containing 1×10^{-3} *M* EDTA (this can be used immediately or gassed with nitrogen and frozen); the 40–50 fraction of luciferase, as shown in the Table, is prepared and used immediately or frozen; and 0.01 *M* $MgSO_4$ is prepared in demineralized water. The premix is then prepared by adding equal volumes of luciferin, luciferase, and $MgSO_4$. This mixture is then allowed to stand in the cold for a minimum of 24 h before using; it is never frozen. This premix can be used up to a week.

Figure 3 shows the sensitivity of the assay covering a range of 1.56 pmole to 1560 pmoles of ATP.

Zusammenfassung. Eine schnelle Methode zur Bestimmung von ATP in picogramm-Mengen wird beschrieben. Die Grundlage für die Methode ist eine Reaktion zwischen ATP und einem Inkubationsansatz, der Luciferin, Mg^{2+} und partiell gereinigte Luciferase enthält, und ist zur Bestimmung von ATP in biologischem Material von unterschiedlicher Beschaffenheit (Bakterien, Pflanzen- und Tiergewebe) geeignet.

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