irrespectively of whether the ascending or the descending technique is used. The use of an ordinary alarm clock makes the apparatus inexpensive but if the equilibration time must exceed eleven hours another type of timer is required.

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A Note on the Action of Firefly-Extracts on ATP

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Use of the enzyme system from *Photinus pyralis* for the determination of minor amounts of ATP has been suggested and further a method implying it has been described by Strehler and Totter [1]. In order to study the extent of the postulated linear dependence of light intensity on ATP concentration experiments were carried out with different amounts of firefly-extracts and of ATP.

The experiments were carried out in small test tubes closed with a rubber stopper. ATP solution, enzyme extract and either arsenate buffer or water was mixed by rapid shaking. Light intensity was then determined by a photometer. If we suppose that light intensity is a direct measure of the reaction velocity and accept that the phototube (type RCA 1-P-21) is linear in the spectral area in question [2], then the photometer readings directly express reaction velocity in arbitrary units. It is known that oxygen is required for the reaction but this is supposed to be present in a concentration that is sufficient for the reaction, for two reasons: First it

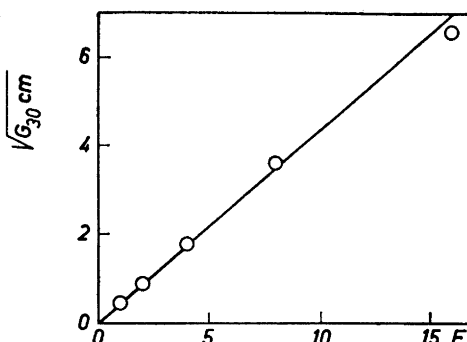

Fig. 1. Abscissa: Concentration of firefly-extract in arbitrary units. Ordinate: Square root of galvanometer deflection measured 30 sec. after start of experiment. Each experiment was made with a mixture of 500 μl water, 100 μl ATP solution (34 μg/100 μl) and 200 μl firefly-extract. t = 25.1°C.

is demonstrated by Hastings *et al.*[3] that only decreasing oxygen pressure to a very minute fraction of the normal has any influence on the reaction and secondly, we have demonstrated that bubbling of atmospheric air through the test tubes did not restore activity when the light intensity had fallen to approximately zero. Yet activity could be restored by the addition of either ATP or firefly extract de-

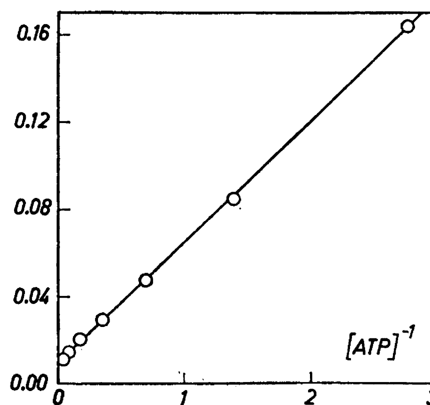

Fig. 2. Abscissa: Reciprocal of ATP concentration in ml · μg^{-1}. Ordinate: Reciprocal of galvanometer deflection. Experiments performed with 500 μl water, 250 μl enzyme solution and 250 μl ATP solution. t = 22.5°C.

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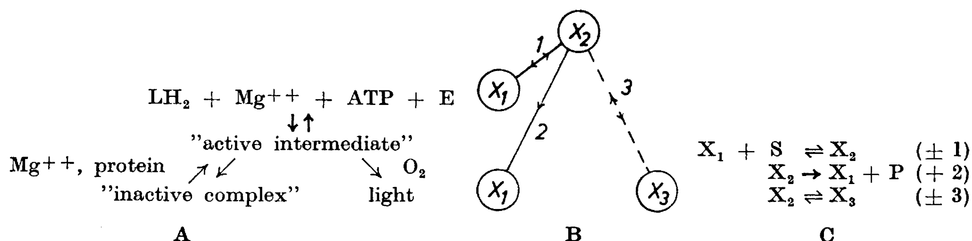

Fig. 3. A. Reaction scheme suggested by McElroy *et al.*[5] for the luciferase reaction. (E = enzyme;LH_2 = reduced luciferin). *B.* Reaction scheme suggested by Christiansen [6] to be the general scheme for a reaction following the Henri-Michaelis chronometric integral. (X_1, X_2 and X_3 are different forms of the enzyme or combinations of enzyme and substrate.) *C.* The same as *B*, written as ordinary equations. (S = substrate and P = products.)

pending on the initial ratio between these two components.

The effect of varying the amount of firefly extract is shown in Fig. 1. It is seen that light intensity measured 30 sec. after mixing the reagents is proportional to the square of the concentration of firefly extract. This is in good agreement with the commonly accepted fact that the extract contains two components that are important for the reaction, the enzyme luciferase and another component, luciferin [3,4].

The dependence of reaction velocity on ATP concentration was studied too. It is seen from Fig. 2 that the reaction kinetics is in accordance with the Henri-Michaelis scheme, when initial velocity is measured 30 sec. after mixing. Frequent readings during initial few minutes do, however, not show reaction kinetics in accordance with a normal Henri-Michaelis reaction, as the extremely rapid initial fall in light intensity is not caused by a decrease in the concentrations of the reactants [3]. To explain this it has been suggested by McElroy *et al.*[5] that part of the luciferase in one way or another is reversibly inactivated (Fig. 3). The scheme suggested for this particular reaction is then identical with the one proposed by Christiansen to be the general scheme for the simplest enzymatic reaction. It is reasonable to suggest that the luciferase reaction differs from the simple enzymatic reactions by approaching steady state with respect to reaction ± 3 (Fig. 3) after a few minutes instead of immediately.

A similar reaction mechanism is described by Andersen [7] in the case with arylsulphatase A, only in this case steady state with respect to the inactivating reaction was reached about 60 min. after the initiation.

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