

# Effects of Diethyl Ether and Halothane on Firefly Luciferin Bioluminescence

Issaku Ueda, M.D.

The intensity of bioluminescence from firefly lantern extracts elicited by the addition of ATP has been reported to be proportional to the amount of added ATP. The intensity of bioluminescence elicited by the addition of  $2.0 \times 10^{-7}$  mole ATP to a solubilized fraction of firefly lantern homogenate was decreased by administration of diethyl ether or halothane. Relative intensity of bioluminescence decreased linearly when plotted against the logarithmic scale of the concentrations of the anesthetics. With 6 per cent diethyl ether the dose-response curve for ATP concentration and light intensity was shifted to the right parallel to the control.

The inhibition of bioluminescence by anesthetics was reversed to control values by elimination of the anesthetic from the medium.

BIOLUMINESCENCE liberated from firefly lanterns has been studied extensively by McElroy and his coworkers.<sup>1</sup> They have isolated an enzyme, luciferase, which leads to the liberation of light from luminescent substrate, luciferin, in the presence of adenosine triphosphate (ATP) and magnesium under aerobic condition.<sup>2</sup> The intensity of the light emitted from luciferin was reported to bear a linear relation to the amount of available ATP when excess luciferin and luciferase were present.<sup>3</sup>

The present paper reports the quantitative suppression of bioluminescence emitted from the soluble fraction of firefly lantern homogenates by the administration of therapeutic concentrations of diethyl ether and halothane.

## Method

Luciferin and luciferase were prepared from the firefly (*Luciola cruciata*) obtained locally

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and dried *in vacuo* according to the method of Strehler and Totter<sup>4</sup> with modifications. Dried firefly lanterns were homogenized with cold arsenate buffer at pH 7.4 in a Waring blender at 20,000 r.p.m., for three minutes. The homogenate was centrifuged in a Hitachi 40-P ultra centrifuge<sup>\*</sup> at  $30,000 \times G$  for one hour, in the cold. The residues were discarded, and  $MgSO_4$  was added to the supernatant. This resulted in a clear amber-colored preparation which was kept cold. The final dilution was the equivalent of 10 mg. tissue in 1 ml. of 0.04 M  $MgSO_4$  and 0.1 M arsenate buffer.

The reaction for the liberation of bioluminescence was started with the addition of 0.1 ml. ATP to 1.0 ml. firefly lantern extract. Luminescence was measured with a Farrand spectrofluorometer fitted with 1P21 photomultiplier and recorded with a Brown Electronik 15 recorder.<sup>†</sup>

Diethylether and halothane were vaporized with oxygen in a Copper Kettle and diluted to the appropriate concentrations with oxygen. The concentrations of the anesthetics were calculated at Copper Kettle temperatures and oxygen flow, and were checked by analysis of the effluent gas by gas chromatography. Concentrations were expressed as volumes per cent in oxygen in the bubbled gas, not in percentage concentrations dissolved in the test medium.

Anesthetic mixtures were administered to 1.0 ml. of firefly extract in a 3-ml. quartz cuvette, by bubbling for three minutes. Inhibition of bioluminescence reached a maximum with the administration of anesthetics in 90 seconds. Controls were taken by bubbling pure oxygen through the extract. (The ATP used was obtained from the Sigma Chemical Company.)

<sup>\*</sup> Hitachi Ltd., Tokyo, Japan.

<sup>†</sup> Farrand Optical Co., Inc., New York, N. Y.

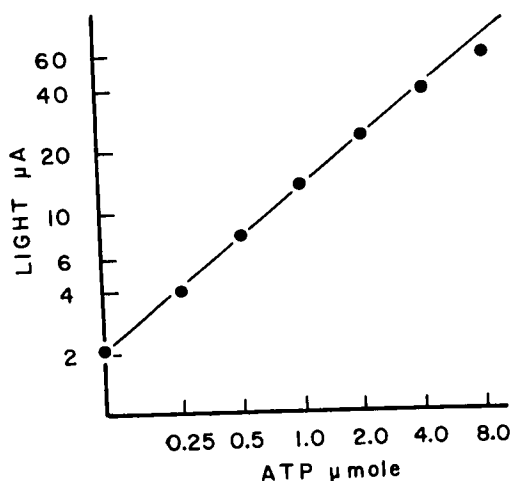


FIG. 1. Relation between intensity of bioluminescence and ATP concentration.

### Results

A linear relation was obtained between the logarithm of intensity of bioluminescence and the logarithm of ATP concentration between  $1.0 \times 10^{-7}$  and  $4.0 \times 10^{-6}$  moles as shown in figure 1. The light output from the same batch of lantern homogenate gave identical values at each ATP concentration, and the deviations were negligible. Although the values differed between batches, dose-response curves were parallel.

The administration of diethylether at concentrations of 2, 4, 6 and 8 volumes per cent decreased the relative intensity of bioluminescence elicited by the addition of  $2.0 \times 10^{-7}$  mole ATP, to 80.4, 55.8, 46.6 and 36.6 per cent, respectively, when compared to the control values. When the relative intensity of bioluminescence was plotted against the logarithm of diethylether concentration, the inhibition increased linearly with increase of anesthetic concentration (fig. 2). Similarly, administration of halothane at concentrations of 1, 2, 3 and 4 volumes per cent decreased the relative intensity of bioluminescence to 72.7, 56.7, 43.4 and 35.5 per cent, respectively. Relative intensity of bioluminescence again decreased linearly when plotted against the logarithm of halothane concentrations (fig. 3).

The intensity of bioluminescence in the presence of 6 per cent diethyl ether increased with increase of ATP concentration as shown

in figure 4. The dose-response curve was shifted to the right, parallel to the control.

Elimination of anesthetics from the reaction mixture by bubbling of oxygen for three minutes reversed the intensity of luminescence to control values.

### DISCUSSION

Biochemical aspects of the mechanisms of general anesthesia have been extensively reviewed.<sup>4,5</sup> In tissue respiration, the available evidence indicates that anesthetics inhibit electron transfer between diphosphopyridine nucleotide and coenzyme Q, to which the liberation of ATP from adenosine diphosphate is tightly coupled.<sup>5</sup> The action of general anesthetics is, therefore, assumed to be the result of a decreased liberation of high energy phosphate compounds in the brain, thereby suppressing nervous activity. The uncoupling theory postulated by Brody<sup>6</sup> also suggests that the synthesis of ATP is decreased by anesthetics.

However, these hypotheses are difficult to reconcile with the fact that the concentration of high energy phosphate compounds in the mammalian brain is not decreased during anesthesia.<sup>5</sup> It has been postulated<sup>5</sup> that the utilization of ATP may be the primary site of

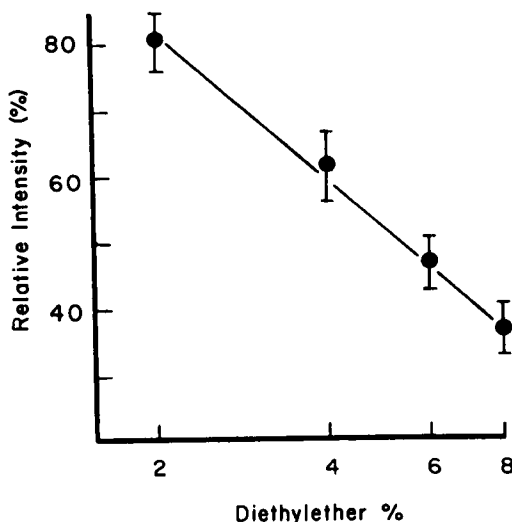
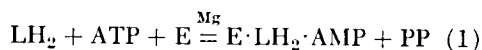


FIG. 2. Inhibition of bioluminescence by diethyl ether. Values are the means of the results from 6 batches of lantern extracts. Standard deviations are shown.

action of anesthetics rather than its synthesis. Because of the tight coupling of ATP synthesis to electron transfer, the decrease in phosphate acceptor could inhibit electron transfer, by the feedback mechanism postulated by Krebs.<sup>7</sup> From this viewpoint, the inhibition of electron transfer by anesthetics demonstrated by the previously cited investigators is a result of anesthetic activity rather than its cause. Direct evidence of suppression by anesthetics of ATP utilization, however, has not yet been reported.

The elucidation of the mechanism of luciferin bioluminescence by McElroy and his co-workers revealed that luciferin-luciferase interaction leading to light emission is a multi-staged process.<sup>8</sup> Light emission requires a substrate luciferin, an enzyme luciferase, ATP and molecular oxygen.

The initial reaction leading to light emission is the luciferase (E) catalyzed adenylation of luciferin (LH<sub>2</sub>) from ATP to luciferyl-adenylate (LH<sub>2</sub>·AMP), with the elimination of inorganic pyrophosphate (PP), requiring the divalent cation Mg.



Bioluminescence is produced when luciferyl-adenylate (LH<sub>2</sub>·AMP) is oxidized to oxy-

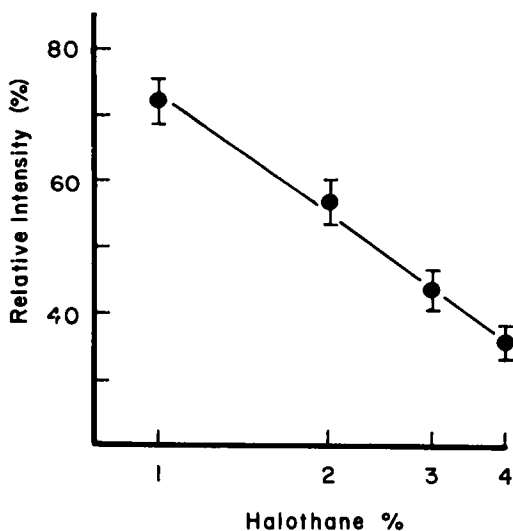


FIG. 3. Inhibition of bioluminescence by halothane. Values are the means of the results from 6 batches of lantern extracts. Standard deviations are shown.

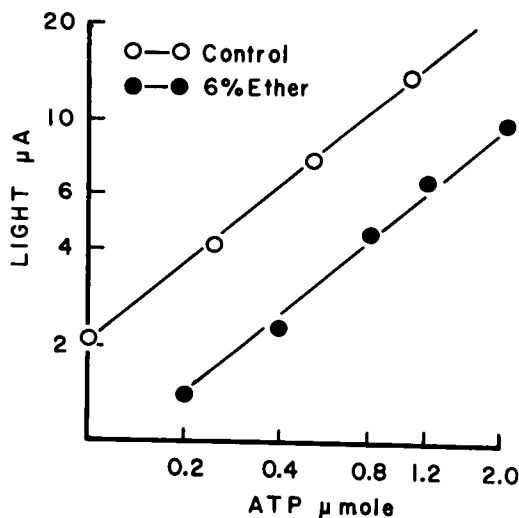
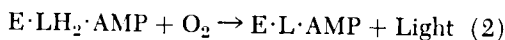


FIG. 4. Relation between the intensity of bioluminescence and ATP concentration with 6 per cent diethyl ether. Values are the results from the same batch of lantern extract. Three observations gave identical values without noticeable deviation.

luciferyl-adenylate (L·AMP) with molecular oxygen presumably the ultimate electron acceptor.



The present demonstration of a reversible suppression of bioluminescence by anesthetics leaves one of two possible sites for their action. The enzyme condensation expressed in equation 1 is ATP-dependent, and suppression at this level would provide clear-cut evidence that ATP utilization is blocked by anesthetics. A second possibility is that the energy expenditure in the oxidation of luciferyl-adenylate in equation 2 is suppressed by the anesthetics.

Although the exact site remains to be elucidated, the present study reveals that the anesthetics in therapeutic concentrations markedly inhibit firefly bioluminescence not by inhibition of ATP synthesis at the electron transfer chain.

It is difficult to extrapolate the present results to mammalian nervous activity because of the complete heterogeneity of firefly bioluminescence. It would be interesting to investigate the effect of anesthetics on ATP splitting enzymes derived from mammalian brain.

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**DIAPHRAGMATIC HERNIA** Cardiovascular collapse and death following "crash" induction is described for patients with traumatic diaphragmatic hernia. In animals a simulated lesion of this type predisposed to hypotension when airway pressure was increased. Decreased cardiac output may be due to compression or torsion of veins and right atrium, or by the large foreign body in the chest. Hypovolemia, toxicity from infarcted bowel, and electrolyte imbalance also contribute to circulatory instability. Vigorous positive pressure respiration may be the precipitating factor. Procedures recommended are: restore blood volume and electrolytes to normal, drain the stomach, avoid depressant drug premedication, intubate awake, maintain spontaneous respiration and assist respiration as little as possible. Be prepared for vasopressor administration and emergency thoracotomy at the time of induction. (Lochning, R. W., Takaori, M., and Safar, P.: *Circulatory Collapse From Anesthesia for Diaphragmatic Hernia*, *Arch. Surg.* **90**: 109 (Jan.) 1965.)

**BRACHIAL BLOCK** Experience with almost 3,300 brachial blocks was analyzed. For children most were by the axillary approach (755). Complications included two convulsions in children following lidocaine. No minor reactions were noted in children because heavy premedication was used. With adult patients who had light premedication, there were 24 minor systemic toxic reactions (1 per cent) but no convulsions. Inadequate anesthesia occurred in 2.6 per cent of supraclavicular, and 2.9 per cent of axillary blocks. Minor neuritis occurred in 1 per cent of adults. Stellate ganglion block occurred in 82 per cent of patients for whom the supraclavicular approach was used; none occurred after axillary blocks. Loss of radial artery pulsation occurred in nine children (1 per cent) following axillary block. Nine patients developed pneumothorax following supraclavicular block. There were no deaths. Choice of technique: axillary is better for children (no cooperation needed; heavy premedication), and for pulmonary disease patients where a pneumothorax would be very serious. Supraclavicular is preferred if patient cannot abduct his arm, if a high block is needed and if the patient can help by describing paresthesias. (Moore, D. C., Bridenbaugh, L. D., and Eather, K. F.: *Block of the Upper Extremity*, *Arch. Surg.* **90**: 68 (Jan.) 1965.)