p-**Ethylaminobenzamide.**—A mixture of 6.8 g. (0.05 mole) of *p*-aminobenzamide, 7.8 g. (0.05 mole) of ethyl iodide and 5 g. of sodium bicarbonate in 75 cc. of 35% aqueous alcohol was refluxed on the steam-bath for eight hours. Most of the solvent was distilled off, water added, and the solid filtered off. The product was dissolved in 70 cc. of dilute hydrochloric acid, cooled in ice water and to it, with stirring, was added a solution of 3 g. of sodium nitrite in 15 cc. of water. The nitroso derivative was filtered off, washed with water and dried; crude yield, 5.5 g. It was dissolved in 180 cc. of hot absolute alcohol and filtered with charcoal. Dry hydrogen chloride was bubbled into the cooled and well stirred filtrate at a rapid rate until all the solid went into solution. The clear solution was stirred at room temperature for four and one-half hours, filtered with charcoal, and evaporated almost to dryness. Water was added to dissolve the hydrochloride which separated and the solution was made alkaline with ammonium hydroxide. On standing, the product separated out. It was recrystallized from hot water.

p-**Propylaminobenzamide.**—A mixture of 10.8 g. of *p*-aminobenzamide, 60 g. of zinc dust, 100 cc. of glacial acetic acid, and 200 cc. of absolute alcohol was refluxed on a steam-bath with stirring. Five grams of freshly distilled propionaldehyde was added over a period of one hour and refluxing continued for one hour longer. The solid was filtered off and the filtrate was steam distilled to remove the alcohol and acetic acid. The residue (about 750 cc.) was cooled and the solid which separated was filtered off and recrystallized from dilute alcohol. Compound IX was prepared by a similar procedure.

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COMMUNICATIONS TO THE EDITOR

THE NATURE OF CYPRIDINA LUCIFERIN

Sir:

Johnson and Eyring have recently stated[1] that "'luciferin' apparently contains both coenzyme (I or II) and a flavin prosthetic group." Ball and Ramsdell[2] are led to "suspect that flavin-adenine dinucleotide may play some role in the luminescent mechanisms of the firefly." Since *Cypridina* luciferin is oxidizable[3] reversibly[4] this latter suggestion is a plausible possibility. The more definite conclusion of Johnson and Eyring[1] is *a priori* a very attractive one because of the fundamental importance of the coenzymes and flavins in cellular oxidations and the occurrence of flavins in a number of oxidases. Unfortunately, a reëxamination of the experimental material on luciferin does not entirely confirm their conclusion.

In regard to the absorption spectrum of *Cypridina* luciferin, comparison should be made with the flavins rather than the flavoproteins. Oxidized riboflavin has maxima at about 3600 and 4500 Å.[5] while reduced riboflavin is colorless. Reduced luciferin concentrates show a maximum at about 4300 Å.[6] and hence, since this is probably due to luciferin, it is a colored compound. This band disappears after brief aeration and a new band appears at about 4700 Å. The 4700 Å. band disappears after prolonged exposure of the solution to air while a band at about 3600 Å. appears.

Johnson and Eyring report[1] that luminescence occurs after treatment of "luciferase" solution with $Na_2S_2O_4$, reduced coenzymes or riboflavin. Such an experiment is difficult to interpret if the observed luminescence was faint. It is easily possible for the partially dark adapted eye to see one millionth part of the light emitted by the luciferin from a small amount of *Cypridina*.[7] In any case if reduced riboflavin is to be regarded as a substrate in the luminescent reaction identical with or analogous to luciferin, the amount of light emitted should be large and should be related to the amount of substrate oxidized.

Indirect studies on the oxidation–reduction potential of luciferin[4] place it near toluhydroquinone and hydroquinone.[8] The oxidation reduction potential of riboflavin is 0.4 or 0.5 v. lower than this at *p*H 7.0, while that of coenzyme is still lower. It has been reported that luciferin concentrates contain no nitrogen,[9] although the sensitivity of the method in relation to the amount of partially purified luciferin was not stated. In unpublished experiments of Dr. M. Kunitz, attempts to measure coenzyme I in luciferin preparations were unsuccessful. Here the sensitivity was such that the luciferin sample could hardly have contained as much as 2% of coenzyme.

The available data, therefore, although they may not exclude the conclusion of Johnson and Eyring, certainly give it little support.

(1) Johnson and Eyring, THIS JOURNAL, **66**, 848 (1944).
(2) Ball and Ramsdell, *ibid.*, **66**, 1419 (1944).
(3) Harvey, *J. Gen. P.*, **1**, 133 (1918).
(4) Anderson, *J. Cell. & Comp. Physiol.*, **8**, 261 (1936).
(5) Warburg and Christian, *Biochem. Z.*, **298**, 150 (1938).
(6) Chase, *J. Biol. Chem.*, **150**, 433 (1943).
(7) Harvey, *Science*, **57**, 501 (1923).
(8) This suggests the desirability of investigating luciferin as a possible link between flavins and oxygen.
(9) Chakravorty and Ballentine, THIS JOURNAL, **63**, 2030 (1941).

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COLORIMETRIC TESTS FOR DDT AND RELATED COMPOUNDS

Sir:

The revolutionary development of the insecticide DDT,[1] the major portion of the technical product being 2,2-bis(*p*-chlorophenyl)-1,1,1-tri-

(1) Annand, *J. Econ. Entomol.*, **37**, 125 (1944); Froelicher, *Soap and Sanit. Chem.*, **20** (7), 115 (1944).