(Carboethoxymethyl)-(β-carboethoxyethyl) sulfide, obtained by the reaction of thioglycolic ester with acrylic ester in the presence of a small amount of piperidine, was cyclized by means of sodium metal in benzene suspension. The product, thiophan-3-on-4-carboxylic ester,[2] $C_7H_{10}O_3S$, boiled at 96° at 4 mm. and gave a phenylhydrazone, $C_{13}H_{16}N_2O_2S$, m. p. 100–101°[3] from aqueous ethanol (Calcd.: C, 59.1; H, 6.1; N, 10.6. Found: C, 59.1; H, 6.0; N, 10.7), and a semicarbazone, $C_8H_{13}N_3O_3S$, m. p. 176° (Calcd.: N, 18.2. Found: N, 18.1). The ester, on acid hydrolysis, gave thiophanone-3, $C_4H_6OS$, b. p. 83–85° at 29 mm., darkens on standing, semicarbazone, $C_5H_9N_3OS$, m. p. 196° dec., dinitrophenylhydrazone, $C_{10}H_{10}N_4O_4S$, m. p. 179° dec. (Calcd.: N, 19.8. Found: N, 19.7).

2-Methylthiophan-3-on-4-carboxylic ester, $C_8H_{12}O_3S$, b. p. 93–95° at 4.5 mm., was obtained by cyclization of (α-carboethoxyethyl)(β-carboethoxyethyl) sulfide. On hydrolysis it gave 2-methylthiophanone-3, $C_5H_8OS$, b. p. 82° at 28 mm., unstable in air, semicarbazone, $C_6H_{11}N_3OS$, m. p. 185–186°, dinitrophenylhydrazone, $C_{11}H_{12}N_4O_4S$, m. p. 161–162° (Calcd.: C, 44.7; H, 4.1. Found: C, 44.6; H, 4.2).

Circumstances made necessary the termination of our studies on synthetic thiophanes in August, 1942. It is intended to resume these unfinished investigations after the war.

(2) The assigned formula is based on analogy; see Prill and McElvain, THIS JOURNAL, **55**, 1235 (1933).

(3) Karrer and Schmid (ref. 1, p. 127) obtained two phenylhydrazones, m. p. 141.5–142.5° and m. p. 167°; possible explanations to account for this discrepancy cannot be investigated at this time.

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RECEIVED APRIL 21, 1944

## THE NATURE OF THE LUCIFERIN-LUCIFERASE SYSTEM

*Sir:*

In the luminescent system extracted from *Cypridina*, the substrate, luciferin, is dialyzable, while the enzyme, luciferase, is not.[1] Reduced luciferin undergoes reversible dark oxidation by oxygen or ferricyanide, and apparently irreversible luminescent oxidation by luciferase plus oxygen. Like dihydrocoenzyme, with an absorption maximum at 3400 Å., considerably purified, reduced luciferin solutions have an absorption peak near 3200 Å. On adding oxygen this absorption diminishes, while a new peak at 4300 Å. appears, shifting quickly to 4700 Å., then disappearing.[2] In presence of oxygen, aqueous luciferin is unstable. The absorption at 4700 Å. resembles that of certain flavoproteins, and practically coincides with the luminescence maximum at 4750 Å. The energy of this luminescent transition corresponds to 59,430 calories as compared to 57,340 calories available by direct oxidation of two hydrogen atoms on glucose, the substrate known to increase tremendously the luminescence of washed luminous bacteria.

We have now observed that a concentrated solution of luciferase, after prolonged dialysis with several hundred volumes of distilled water, and storage for some eighteen months in a refrigerator, will luminesce with oxygen, following reduction by (1) $Na_2S_2O_4$, (2) reduced Coenzyme I solution (partially reduced by pure hydrogen and platinized platinum, then added anaerobically to luciferase), (3) reduced riboflavin (similarly treated), (4) washed cells of *E. coli* plus glucose, and (5) growing culture of *E. coli*.[3] A portion of the luciferase solution gave luminescence repeatedly on successive additions of an excess of hydrosulfite then oxygen, but finally ceased. Luminescence continuous for some hours resulted when hydrogen, plus slight oxygen impurity, was passed through some luciferase solution containing platinized asbestos. Oxidized coenzyme, oxidized riboflavin, ferrocyanide, or glucose without bacteria, as expected, did not reduce the luciferase.

A consideration of the above facts indicates that the luminescent system consists of a pyridine nucleotide plus flavoprotein. According to the absorption spectrum, "luciferin" apparently contains both Coenzyme (I or II) and a flavine prosthetic group, the former component providing a reductant, and the latter, after loose combination with its specific protein, comprising molecules excitable by oxidation. Some of the excited molecules radiate and are not destroyed, but others, failing to radiate are destroyed by their absorbed energy. This phenomenon causes the "irreversible reaction" of luminescence. Such destruction of non-radiating excited molecules is responsible for the familiar degradation of riboflavin in solution in the light, and also phthalhydrazides during luminescent oxidation.

Thus, in luminous bacteria, light emission presumably occurs when flavoprotein, reduced by hydrogen from suitable substrates (*e. g.*, glucose) via the dehydrogenase–coenzyme system, is oxidized directly by oxygen. The effects of cyanide on oxygen consumption and luminescence, respectively, indicate that most of the hydrogen proceeds step-wise, by electron transfer, through the cytochrome–heme system to oxygen. With chlorophyll substituted for the related heme molecule, the same system of catalysts operating in the reverse direction would lead to photosynthesis. In luminescence two hydrogens are oxidized for the quantum emitted, while in photosynthesis single hydrogens are made available.

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(1) Harvey, "Living Light," Princeton University Press, Princeton, N. J., 1941.

(2) Chase, *J. Biol. Chem.*, **150**, 433 (1943).

(3) Doctors Chase, Schlenk and Kunitz kindly supplied experimental materials.