solids were removed and washed with ether. The remaining ether was removed and distillation yielded 12.4 g. (59%) of colorless liquid, b.p. 76–79° at 37 mm. Further rectification afforded pure vinylene carbonate, b.p. 73–74° at 32 mm., 162° at 735 mm.; m.p. 22°; $n^{25}D$ 1.4190; d^{25}_4 1.3541. MR_D calcd. for $C_3H_2O_3$: 16.7. Found: 16.1. *Anal.* Calcd. for $C_3H_2O_3$: C, 41.9; H, 2.3. Found: C, 42.1; H, 2.4. Infrared analysis showed carbon–hydrogen absorption at 3.12 μ and strained ring carbonyl absorption at 5.48 μ.

Catalytic hydrogenation of vinylene carbonate yielded ethylene carbonate. Identity was proven by infrared absorption analysis and mixed m.p. determination.

Chlorine adds to ethylene carbonate to produce 1,2-dichloroethylene carbonate.

Diels–Alder Reaction.—Vinylene carbonate and 2,3-dimethylbutadiene in dry toluene were sealed under nitrogen in a tube and heated at 170–180° for 10 hr. A distilled (b.p. 145–147° at 4 mm.) sample of *cis*-4,5-dihydroxy-1,2-dimethylcyclohexene was crystallized to yield a colorless solid, m.p. 57.1–57.7°. *Anal.* Calcd. for $C_9H_{12}O_3$: C, 64.3; H, 7.2. Found: C, 64.6; H, 7.4.

McPHERSON CHEMICAL LABORATORY OF THE
OHIO STATE UNIVERSITY MELVIN S. NEWMAN
COLUMBUS 10, OHIO ROGER W. ADDOR

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LUMINESCENCE IN CELL-FREE EXTRACTS OF LUMINOUS BACTERIA AND ITS ACTIVATION BY DPN[1]

Sir:

The enzyme-catalyzed emission of light by extracts of luminous organisms, when a hot water extract of the same organism is added to a cold water extract which has ceased to glow (the classical "luciferin–luciferase" reaction[2]), although demonstrable in extracts of fireflies,[3,4] *Cypridina hilgendorfii*[5] and other species, has never been conclusively demonstrated in extracts of luminous bacteria. Numerous workers have indeed reported negative results.[6–10]

Some time ago Shoup and Strehler,[11] using a quantum counter[12,13] of nearly ultimate sensitivity as a light-detecting apparatus, found that acetone-dried powders of the luminous bacterium, *Achromobacter fischeri*, will give appreciable light for some minutes after mixing with water. Using the same detector, conditions for more optimal rates of luminescence have been investigated and it has now been found possible to obtain luminescence visible to the naked eye from cell-free water extracts of acetone-dried *A. fischeri*. After the luminescence has disappeared, its reappearance may be effected by adding boiled extracts of acetone-dried bacteria. Moreover, it has been found that diphosphopyridinenucleotide (DPN) is a potent substitute for the boiled bacterial extract, raising the counting rate in a typical experiment from *ca.* 30 cts./15 seconds to *ca.* 100,000 cts./15 seconds almost at once. Reduced DPN ($DPNH^+$) is an even more potent substrate for this luminescence, giving a maximal response immediately. DPN presumably requires some time to be reduced by dehydrogenase systems in the extract.

It thus appears either that DPN is closely linked to the light-emitting system as an electron transport agent and becomes rate limiting in the crude active extracts or, possibly, that DPN is bacterial luciferin.

The high sensitivity of this system to added DPN and $DPNH^+$ (*ca.* 0.01–0.1 μg./ml. gives a measurable response) suggests its possible application as an assay tool analogous to the firefly enzyme in ATP measurement.[14] A study of factors influencing the extract luminescence and its application to bioassay is in progress.

BIOLOGY DIVISION
OAK RIDGE NATIONAL LABORATORY B. L. STREHLER
OAK RIDGE, TENNESSEE

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