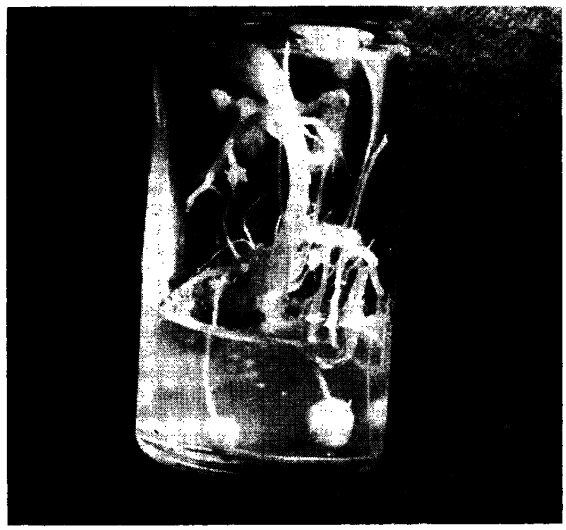

FIG. 2. Tubers formed from stolons that have grown below the surface of the medium. × 1.5.

ble to produce tubers in one-third of the initiating cultures within 3 wk. It is felt that the application of this technique will prove invaluable both in the understanding of tuberization and the furthering of potato scab research.

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Manuscript received June 25, 1953.

The Requirement of Riboflavin Phosphate for Bacterial Luminescence[1]

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Recent reports on the factors essential for luminescence in extracts from the crustacean, *Cypridina hilgendorfii*, fireflies, and luminous bacteria indicate that the dark reactions leading to the excitation of the corresponding luciferins are fundamentally different in these three forms. The essential components for *Cypridina* luminescence appear to be a labile luciferin (a chromopolypeptide), luciferase, and oxygen (*1, 2*). On the other hand, in addition to luciferin, luciferase, and oxygen, light production in firefly extracts requires adenosinetriphosphate (ATP) and an inorganic ion (*3*). Recently Strehler (*4*) has reported that reduced diphosphopyridine nucleotide (DPNH) is essential for light production in aqueous extracts of acetone powders of luminous bacteria. He suggests that DPN is either closely linked to the light-emitting system or is bacterial luciferin. We would like to report that a flavin is essential for DPNH to function in initiating light emission in bacterial extracts. Riboflavin phosphate (FMN) has been found to be the only flavin derivative that will function in this capacity. Doudoroff has previously shown that certain dim strains of luminous bacteria would luminesce brightly when grown in the presence of riboflavin (*5*). In addition to FMN, DPNH, and the enzymes, there appears to be at least one other factor (presumably bacterial luciferin) that is necessary for light production.

Aqueous extracts of the salt water bacterium, *Achromobacter fischeri*, were obtained by suspending freshly grown cells in distilled water. After lysis the debris was removed by centrifugation. An active preparation that emits light with DPNH was precipitated from the aqueous extract by adjusting the pH to 4.5 with HCl. Further purification was achieved by $(NH_4)_2SO_4$ fractionation. In the latter procedure, appreciable loss of enzymatic activity was observed. However, much of this activity could be recovered by adding the yellowish supernatant from the acid precipitation to the various $(NH_4)_2SO_4$ fractions. Several experiments suggested that the active principle in the supernatant was a flavin. Tests with riboflavin and flavin adenine dinucleotide demonstrated that both were potent inhibitors of the luminescent reaction. However, riboflavin phosphate was an adequate substitute for the supernatant factor. An enzyme preparation that failed to emit light with DPNH alone was readily obtained by irradiating one of the $(NH_4)_2SO_4$ fractions with a strong ultraviolet light (Keese lamp). The response of the irradiated enzyme to both FMN and DPNH are shown in Fig. 1. Spectrophotometric

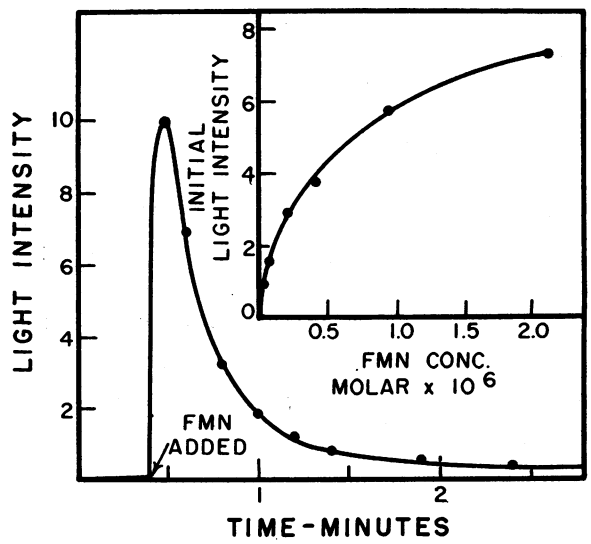

FIG. 1. The effect of riboflavin phosphate on light production. The reaction mixture contained partially purified enzyme in a phosphate buffer at pH 6.8. The DPNH was added at zero time and FMN was added at 25 sec. The course of light production is indicated at the left. The effect of FMN concentration on initial light intensity is shown in the inset curve.

[1] Contribution No. 54 of the McCollum-Pratt Institute.

measurements at 340 mμ also indicate that FMN is essential for DPNH oxidation, although most of this activity depends upon a nonluminescent reaction. In the presence of excess DPNH and FMN, the partially purified preparations rapidly lose their ability to emit a bright light. However, the supernatant from a fresh, boiled enzyme preparation rapidly restores light production, thus indicating the presence of a heat-stable factor that is essential for luminescence. This is analogous to the destruction of both firefly and *Cypridina* luciferins during luminescence, suggesting, therefore, that this substance can be legitimately classified as bacterial luciferin.

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Manuscript received July 1, 1953.

Extinction of Light by Filter Passing Matter in Chesapeake Bay Waters[1, 2]

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Over one hundred water samples were tested for the presence of dissolved coloring matter (*1*) in order to determine whether coloring matter in solution is of importance in the extinction of light in Chesapeake Bay waters. These samples were taken during the months of April and August, 1951, at stations located in Chesapeake Bay and adjacent sounds and rivers. Most of the samples were obtained from the surface, a few from a 10-ft depth, and, at four stations, from the surface to the bottom at 10-ft intervals. To avoid changes due to biological activity during storage (*2*), the samples were processed and analyzed immediately after being pumped aboard. As soon as filtering with a fine Berkefeld filter was completed, the absorbency (A_s) (*3*) of the sample was determined at 20- to 25-mμ intervals of wavelength with a Beckman model DU spectrophotometer having a 50-cm liquid absorption cell.

Examination of the typical absorbency curves for filtered samples in Figs. 1 and 2 shows a lack of significant color absorption peaks. The regular increase in absorbency with decreasing wavelength becomes of measurable magnitude at approximately 550 mμ and can be partially accounted for by the presence of fine filter passing suspensoids which, when combined with whatever dissolved coloring matter is present, act as a yellow substance (*4*) by absorbing and scattering light from the shorter wavelength end of the visible

[1] Contribution No. 13 from the Chesapeake Bay Institute.
[2] Results of work carried out for the Office of Naval Research of the Navy Department, the State of Maryland (Dept. of Research and Education), and the Commonwealth of Virginia (Virginia Fisheries Laboratory).

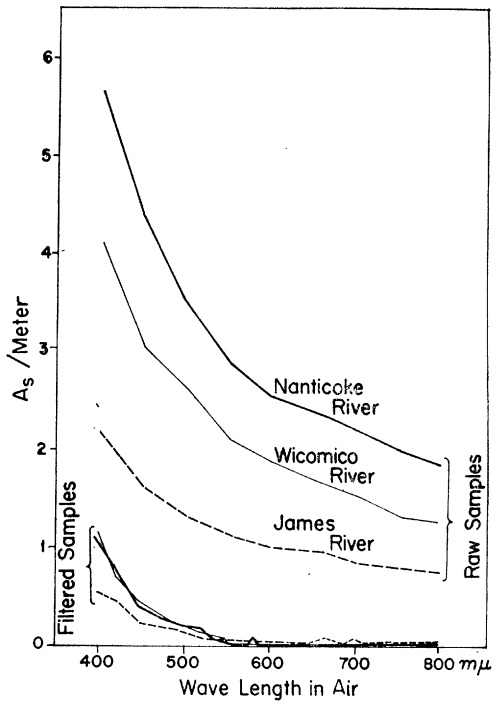

FIG. 1.

spectrum. The filter passing materials caused the greatest extinction in the violet, less in the blue, and tend to become unimportant in the green.

It should be noted that the color curves for samples

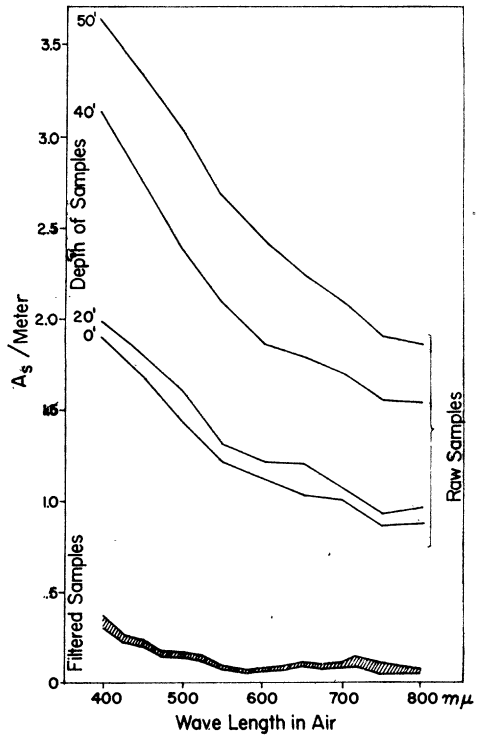

FIG. 2.