of this investigation, however, the manufacturer of heptachlor has succeeded in synthesizing an isomer of heptachlor epoxide (mp 159–160°) which has been found by infrared spectrophotometric analysis to be identical with the epoxide isolated from animal tissues.

The concentrations of heptachlor epoxide found in the milk before, during, and after the administration of heptachlor are presented in Fig. 1. From these

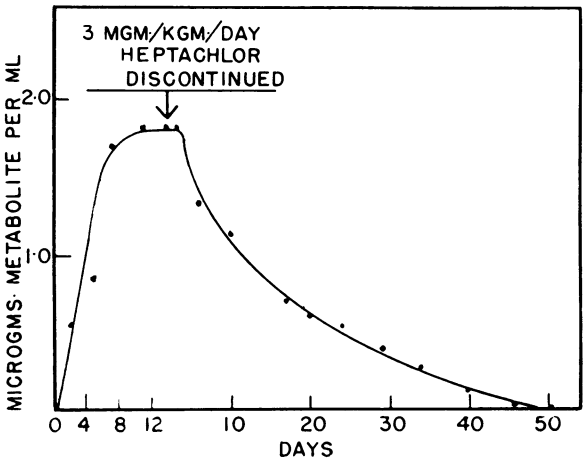

FIG. 1. Excretion of heptachlor epoxide in milk of a dairy cow fed heptachlor.

data it is evident that within 9 days after starting on the heptachlor regimen, the concentration of heptachlor epoxide in the milk rises rapidly to a maximum and constant level of 1.8 ppm. Since the metabolite is concentrated in the butterfat of the milk and the milk averaged 4.1% butterfat, the concentration of metabolite in the butterfat would be equivalent to 44 ppm. After discontinuing the administration of heptachlor to the cow, the concentration of the epoxide in the milk gradually decreases. However, it is not until 51 days after cessation of the treatment that the milk appears to be free of traces of heptachlor epoxide. Although the presence of added heptachlor in milk can be determined in levels as low as 0.02 ppm, negative results were obtained for heptachlor under the described dosage schedule.

From these findings it is apparent that the metabolism of heptachlor in the cow is similar to that of the dog and rat with respect to the storage of the epoxide metabolite. The prolonged secretion of the heptachlor epoxide in the milk after the administration of heptachlor had ceased is probably due to the slow mobilization of the epoxide stored in the body fat.

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Manuscript received June 30, 1953.

A Method for the *in vitro* Culturing of Potato Tubers

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Magrou (*1*), in 1938 and in a series of succeeding papers, discussed tuberization in potatoes, basing his work in part on material cultured on Knop's solution. More recently Stewart (*2*) has described a method for the aseptic culturing of callus masses derived from potato tubers. The present report outlines a simple method whereby tubers can be obtained *in vitro* in sterile culture.

The culture medium used is that of White (*3*), modified by the addition of 2.5 mg/l of pantothenic acid. Etiolated shoots from the eyes of potato tubers that have been maintained in the dark serve as source material for the tubers. The shoots are removed and placed for 20 min in a 12% solution of Clorox to which a small quantity of detergent has been added. Following this treatment, the shoots are placed under a flap of sterile paper towelling where the nodes are excised, using flamed instruments, and placed in the culture bottles.

At the present time the cultures are maintained at 26° C in the dark. Within 3 days of the establishment of the cultures, roots and stolons may be observed growing out from the primordia at the nodes. Most frequently the stolons continue to elongate while forming several branches. After several weeks tubers appear occasionally, mostly on the secondary branches (Fig. 1) and usually above the surface of the agar. Some have been noted, however, below the surface of the culture medium (Fig. 2). Tuberization, in addition, has been observed in cultures stored in a lighted room and at room temperature.

In other preliminary experiments it has been possi-

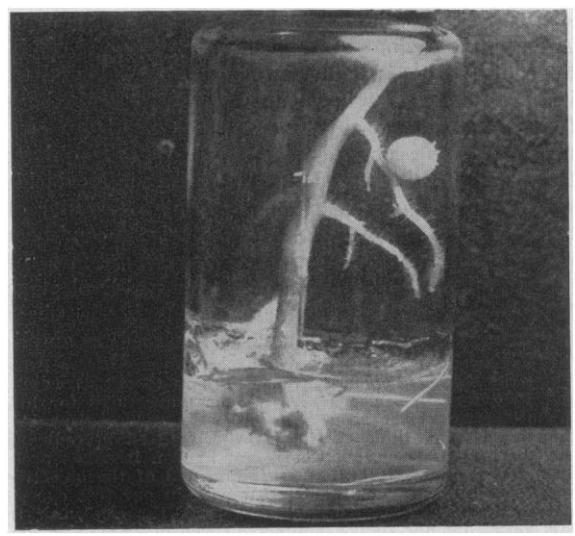

FIG. 1. Typical tuber formed after 3 wk of culture. × 1.5.

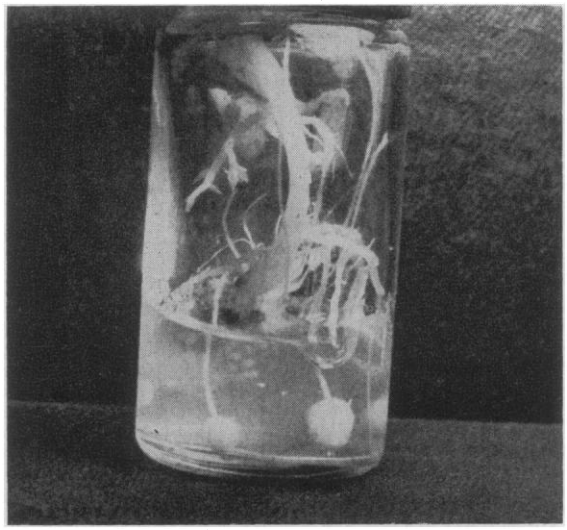

FIG. 2. Tubers formed from stolons that have grown below the surface of the medium. × 1.5.

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

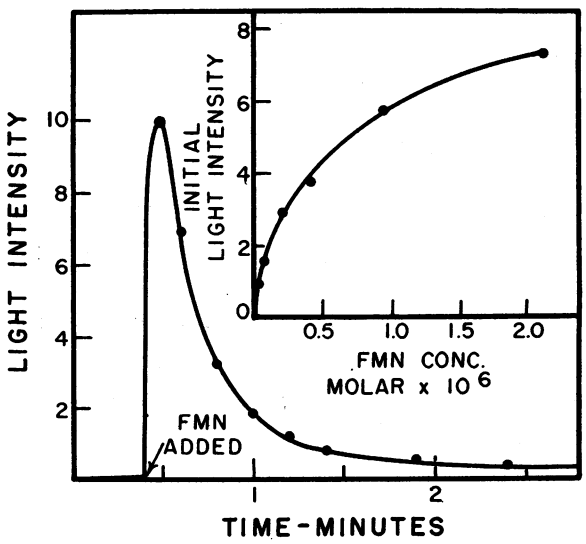

FIG. 1. The effect of riboflavin phosphate on light production. The reaction mixture contained partially purified enzyme in a phosphate buffer at pH 6.8. The DPNH was added at zero time and FMN was added at 25 sec. The course of light production is indicated at the left. The effect of FMN concentration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

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