ities, as well as by the relative number of abnormal organisms present. Thus, organisms growing in methylcholanthrene exhibited abnormalities within 30 to 32 days regardless of the different concentrations used. Here only a few organisms were affected. Survival following this treatment averaged 5 to 8 days. Scharlach red yielded almost identical results. However, 3:4 benzpyrene proved to be more effective. Paramecia growing in this hydrocarbon exhibited abnormalities within 12 to 14 days in all of the concentrations employed. These abnormal organisms rarely survived for more than 5 days. Furthermore, the number of organisms affected was considerably greater than either of the other agents employed. Cytological studies of abnormal organisms that were fixed and stained, gave no indication that either the macronucleus or micronucleus were affected or involved.

Conclusion. These results indicate that exposure of *Paramecium caudatum* to the carcinogens employed in these experiments, causes the production of certain abnormalities regardless of the different concentrations of the carcinogenic agents used. The order of potency for these carcinogens, as demonstrated by the time of appearance of abnormalities, is as follows: 3:4 benzpyrene; methylcholanthrene, and Scharlach red.

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Cell-Free Enzymes of *Azotobacter vinelandii.**

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Bach and coworkers[1] have reported nitrogen fixation by a cell-free extract of *Azotobacter* cells. Roberg[2] was unable to confirm their results. The possession of a cell-free nitrogen-fixing enzyme system would greatly facilitate studies on the mechanism of nitrogen fixa-

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1 Bach, A., Jermoljeva, Z., and Stepanjan, M., *Compt. rend. acad. sci.* (U.S.S.R.), 1934, **1**, 22.

2 Roberg, M., *Jahrb. f. wiss. Bot.*, 1936, **83**, 567.

tion. With this in view, studies reported here describe preliminary investigations on various cell-free enzymes obtainable from *Azotobacter vinelandii*.

Cultures of *A. vinelandii* were grown in Burk's[3] nitrogen-free medium with vigorous aeration for 24 to 30 hours at 30°. Cells were removed from the liquid medium with a Sharples supercentrifuge, washed by resuspending in half-strength Burk's[3] salt solution and again recovered by centrifuging. The washed cell paste was ground† with pH 7.0 phosphate buffer according to the method of Wiggert, Silverman, Utter and Werkman.[4] The glass and larger cell fragments in the ground material were removed by 30 minutes centrifugation at 4500 rpm in an angle head centrifuge. The supernatant was further clarified by 30 minutes' centrifugation at 24,000 rpm in the Beams air driven concentration centrifuge (rotor radius approximately 3 inches). These extracts were diluted with an equal volume of pH 7.0, M/15 phosphate buffer and passed through a Berkefeld N or Mandler 15 filter. Such filtrates showed no growth in a nitrogen-free medium.

Cell-free juices of *Azotobacter* exhibit a powerful hydrogenase. Methylene blue reduction under an atmosphere of hydrogen proceeded at a rate approximately ten times the endogenous reduction rate under helium or vacuum. Methylene blue reduction also revealed hydrogenase activity in toluene preserved cells and in acetone dried powders prepared by the method of Bovarnick.[5]

Gas uptake of cell-free *Azotobacter* juice as measured in the Warburg respirometer in a 95% hydrogen 5% oxygen atmosphere was 540 cmm per hour per mg nitrogen content of the juice, whereas gas uptake under 95% He 5% oxygen was but 15 cmm. This hydrogen-oxygen uptake was inhibited 90% by M/1000 potassium cyanide and 75% by M/100 sodium azide.

The presence of succinic, malic and lactic acid dehydrogenases in cell-free filtered preparations of *Azotobacter* was demonstrated by the methylene blue reduction technic. No activity was evident with glucose, glutamic acid or formic acid as substrate. Sodium succinate oxidation in the Warburg respirometer supported an oxygen

[3] Burk, D., and Lineweaver, H., *J. Bact.*, 1930, **19**, 389.

† Professor Werkman and his colleagues kindly demonstrated the method and certain modifications to the authors. Details of the modified method will be published from Professor Werkman's laboratory.

[4] Wiggert, W. P., Silverman, M., Utter, M. F., and Werkman, C. H., *Iowa State Coll. J. Sci.*, 1940, **14**, 179.

[5] Bovarnick, M., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 191.

uptake over 6 times the endogenous rate of uptake. Nilsson[6] observed no succinic dehydrogenase activity in vacuum dried or air dried *A. chroococcum* cells, though he obtained methylene blue reduction on hexosediphosphate, ethyl alcohol and glucose with such preparations. The wash water from Nilsson's[6] dry cells contained hexosediphosphate dehydrogenase activity in a cell-free state.

Krampitz and Werkman[7] have recently reported that an oxalacetic acid decarboxylase can be obtained in cell-free juices of *Micrococcus lysodeikticus*. A similar enzyme is present in both cell-free juices and acetone dried cells of *A. vinelandii*. A cell-free preparation released CO_2 from a solution of oxalacetic acid at a rate of 384 cmm per hour per mg nitrogen content of the preparation. This rate was several fold as great as the spontaneous decomposition of oxalacetic acid in the presence of a boiled enzyme preparation. Alpha-ketoglutaric acid was also decarboxylated but at a much slower rate than oxalacetic acid. No activity was evident with pyruvic acid as substrate.

Using the oxidation of ascorbic acid, which spontaneously reduces cytochrome *c*, as a measure of cytochrome oxidase activity, no increase in oxygen uptake was detected when purified heart muscle cytochrome *c* was added to *Azotobacter* juice. This indicates either that the cytochrome oxidase system is not active in the juice studied, or more likely, since cyanide-sensitive oxygen uptake on a succinate substrate could be demonstrated, that heart muscle cytochrome *c* can not act in the cytochrome system of *Azotobacter*. Keilin and Harpley[8] have described a similar lack of activity of heart muscle cytochrome *c* on the cytochrome system of *Escherichia coli*.

Summary. Succinic, lactic and malic dehydrogenases and hydrogenase were demonstrated in cell-free juices of *Azotobacter vinelandii* by the use of the methylene blue reduction technic. Oxygen uptake with sodium succinate as substrate and hydrogen-oxygen uptake with hydrogen as substrate were observed in the Warburg respirometer. Cell-free juices contained oxalacetic acid and alpha-ketoglutaric acid decarboxylases, but not pyruvic acid decarboxylase. Though succinate oxidation was cyanide-sensitive, addition of heart muscle cytochrome *c* did not stimulate the oxidation of ascorbic acid, suggesting that the cytochrome system differs from the animal cytochrome system.

6 Nilsson, R., *Arch. Mikrobiol.*, 1936, **7**, 598.

7 Krampitz, L. O., and Werkman, C. H., *Biochem. J.*, 1941, **35**, 595.

8 Keilin, D., and Harpley, C. H., *Biochem. J.*, 1941, **35**, 688.