

BACTERIOLOGY¹

Chairmen: H. A. DETWILER, Eli Lilly Company, and P. B. BEAMER,
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ABSTRACTS

Stimulation by Molybdenum of Bacteria Grown in the Presence of Various Sources of Nitrogen. E. A. COOK, Indiana University.—Molybdenum is essential for microbial symbiotic and non-symbiotic nitrogen fixation, and for reduction of nitrate-nitrogen by various plants and fungi. Also, several higher plants and some microorganisms are stimulated by its presence when NH_4^+ is the sole source of nitrogen. In the present study, possible effects of this element on the growth of *Pseudomonas* and *Bacillus* with various single sources of nitrogen were observed. The nitrogen compounds included NH_4NO_3 , NaNO_3 , KNO_3 , $\text{Mg}(\text{NO}_3)_2$, $\text{Ca}(\text{NO}_3)_2$, NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$, NaNO_2 , KNO_2 , aspartic acid, glutamic acid, methionine, histidine, ornithine, asparagine, glutamine, glutathione, casein hydrolysate, urea, and thiourea.

The organisms were grown in shaken flasks in a nitrogen-free basal broth which contained less than 1 $\mu\text{g}/\text{ml}$ molybdenum as determined by the thiocyanate-stannous chloride colorimetric method. Various single nitrogen sources and varying amounts of molybdenum as $(\text{Na}_2\text{MoO}_4)$ were added aseptically to the broth. After 24 hours incubation, viable cell counts were made by plating diluted aliquots of the flasks on nutrient agar.

With *Ps. fluorescens*, 250-500 $\mu\text{g}/\text{ml}$ molybdenum slightly stimulated growth when the source of nitrogen was NH_4Cl , NaNO_3 , aspartic acid, ornithine, or urea. No effect was observed with the other nitrogen compounds. In contrast, much more pronounced results were obtained with *B. subtilis*, particularly when urea was the source of nitrogen: growth occurred only in the presence of either 1-5 $\mu\text{g}/\text{ml}$ or 100-150 $\mu\text{g}/\text{ml}$ molybdenum. Stimulation of growth was noted when NaNO_3 , KNO_3 , $\text{Ca}(\text{NO}_3)_2$, or glutamic acid were used (at 1-5 $\mu\text{g}/\text{ml}$ molybdenum), when NH_4Cl or $(\text{NH}_4)_2\text{SO}_4$ were used (at 100-150 $\mu\text{g}/\text{ml}$ molybdenum), or when NH_4NO_3 was used (at 250 $\mu\text{g}/\text{ml}$ molybdenum).

A Microbiological Assay Method for Determining Submicrogram Quantities of Mn^{++} . IRWIN L. DICKSTEIN, Indiana University.—Manganese (II). Since early in the 19th century, analytical chemists have been seeking an accurate and practical method for determining small amounts of manganese (II) in various materials. Chemical, spectrochemical, and microbiological methods have been devised, but none can accurately detect quantities of the ion less than 0.10 p.p.m. Sporulation of *Bacillus subtilis*

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has been observed to be directly proportional to the concentration of manganese (II) in the environment. The assay method to be described is based on this phenomenon.

The materials to be assayed for Mn^{++} were added to 250 ml Erlenmeyer flasks containing 12 ml of double strength nutrient broth and diluted with distilled water to give a final volume of 25 ml. The flasks were autoclaved and then inoculated with 10^4 viable cells obtained from an 8 hour shaken culture of the Marburg strain of *B. subtilis*. After 18 hours at $37^\circ C$, cells from the shaken flasks were stained and the percent of sporulation observed microscopically. Standard flasks containing known amounts of Mn^{++} were always included. Standard curves were drawn in which the percent sporulation was plotted against the p.p.m. of added manganese (II). The concentrations of the ion in the unknown samples were then calculated from the standard curves. Concentrations between 0.010 and 0.090 p.p.m. were detected accurately with this method. The results compared favorably with those obtained by older methods. Materials that were assayed with good results included samples of soil, water, sewage; tissues and fluids from plants and animals; and bacterial vegetative cells and spores.

Factors Affecting the In Vitro Propagation of Rabies Virus. R. J. HOSLEY, Eli Lilly & Company.—The Alabama strain of rabies virus was propagated in a simple balanced salt solution containing glucose and minced brain from 1- to 3-day old Swiss albino mice. The medium was modified by the inclusion of various compounds under study. Virus titers were obtained for the culture flask contents at zero hours and again at 72 hours following incubation. Final titers of experimental and control cultures were compared to ascertain the magnitude and direction of the effect of various compounds or experimental conditions.

An explanation for the failure to obtain virus propagation in vitro in adult mouse brain tissue, which had been reported in the literature and confirmed in the present investigation, was sought in a study of the relationship of virus propagation to quantitative differences in free amino acid content of infant and adult mouse brain tissue. Among the free amino acids known to be present in infant mouse brain tissue but not detected in adult tissue, the compound L-(-)-tyrosine was observed to produce a significant enhancement of virus propagation when added to the culture fluid in a concentration as low as 5×10^{-4} molar. The compound did not appear to alter the rate of tissue metabolism or the pH stability of the system. The compound beta-phenylserine, chosen for study because of its structural relationship to tyrosine, was found to produce a significant inhibition of virus propagation without evidence of an effect on tissue metabolism.

These observations suggest a possible metabolic pathway of importance in the synthesis of rabies virus by host cells. It is conceivable that such a pathway, if operative in the intact host, may prove vulnerable to blocking mechanisms.

A Semi-synthetic Medium for the Cultivation of Poliomyelitis Virus. T. W. O'NEIL, Eli Lilly & Company.—A hydrolysate of lactalbumin was

selected from a group of ten hydrolyzed milk protein products for its low toxicity to monkey kidney epithelial cells. This product was then evaluated for its nutrient qualities for the purpose of developing a simpler and more economical non-proteinaceous medium than Medium 199. This medium was to be used for the tissue cultivation of poliomyelitis virus. A simplified medium containing this hydrolysate, modified Hanks' solution, penicillin and dihydrostreptomycin was evolved which gave good poliomyelitis virus titers in both monolayer and mince type tissue cultures of monkey kidney.

The chief advantages of this medium are economy, simplicity of preparation, lack of protein material, and its ability to produce good poliomyelitis virus yields in both monolayer cells and minced tissues. The ingredients are readily available and can be prepared in a short period of time.