ANNUAL PROGRESS REPORT

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January 12, 1954

For Period: 1 January 1954
31 December 1954

NR: 160-160

Contract: Nonr - 994(00)

Rate: $3,080.00

Contractor: The University of Texas

Principal Investigator: Gerald R. Seaman

Title of Project: Research on Adaptive Metabolism of Protozoa.
SUMMARY OF RESULTS:

A subculture of T. pyriformis S has been established in media lacking in added riboflavin and thiamine. Microbiological assays indicate synthesis of the omitted vitamins by the ciliates. Ability to synthesize lysine, arginine, and methionine, as well as riboflavin, has been attained by a second subculture. Synthesis of the amino acids by this culture has been demonstrated by quantitative paper chromatography.

Homogenates of cells grown in the absence of added thiamine synthesize the vitamin in vitro from 2-methyl-5-hydroxymethyl-6-aminopyrimidine and 4-methyl-5-(6-hydroxymethyl)-thiazole. Although methylene blue, ATP, and high concentrates of Mg" and Ca" were included in these incubation mixtures, the absolute requirements for the reaction have not been ascertained.

In addition to being required for the oxidation of \( \alpha \)-keto acids, thioctic acid has been found to be required for the oxidation of acetate according to the equation:

\[
\text{ATP + CoA + acetate} \rightarrow \text{acetyl-CoA + pyrophosphate + AMP}
\]

These experiments have been carried out with enzymes prepared from liver of dog, beef, duck, and pigeon.

The acetate-activating system from vertebrate tissues has been separated by ammonium sulfate precipitation. One fraction contains the acetate-activating enzymes, but does not respond to the procedure for removing thioctic acid from enzymes. A second fraction, which in itself
exhibits only very slight activating activity, when added to the first fraction, enables removal of thiocytic acid. It therefore appears that the second fraction functions by splitting bound thiocytic acid from the enzyme protein; liberated thiocytic is then adsorbed and removed in the procedure. Evidence has also been obtained for the presence of enzymes necessary for "activation" of thiocytic acid in the acetate reaction.

PLANS FOR THE FUTURE:

The requirements for the in vitro synthesis of thiamine by Tetrahymena will be ascertained. In addition, enzymatic synthesis of the thiazole and pyrimidine moieties will be attempted. In vitro syntheses will be examined for other nutrients with enzymes prepared from appropriately "deficient" cells.

Further investigations will be undertaken to determine the generality of the thiocytic acid requirement in acyl transferring reactions and additional investigations of the mechanism of the "thiocytic splitting" and "thiocytic activation" enzymes should provide valuable insight as to the active coenzyme form of the vitamin.

REPORTS:

Participation of Thiocytic Acid in the Acetate-Activating Reaction. Presented at the D Vitamin Symposium, University of Texas, Austin, Texas, December 2-5, 1953.