

was used as source of the enzyme. The conversion of deoxyribose 1-phosphate to deoxyribose 5-phosphate was followed using a method described previously³. It was found that the rate of conversion of synthetic deoxyribose 1-phosphate was nearly identical to that obtained with the enzymatically prepared substrate. The necessity for an activator for optimal reaction rate was thus not confirmed. In order to account for the apparent discrepancies in the two sets of experiments it was assumed that variations in the procedures involved in the preparation of phosphodeoxyribomutase might result in two different forms of the enzyme: one phospho-enzyme acting without activator or another dephospho-enzyme which required a sugar 1,5-diphosphate as cofactor. The above assumption was tested by studying the phosphodeoxyribomutase reaction in the presence of ³²P added as orthophosphate and using the non-activator requiring enzyme. The deoxyribose 5-phosphate formed was isolated by paper chromatography and it was shown to contain significant amounts of radioactive phosphate. This indicated that ³²P had been incorporated into the enzyme followed by transfer of the radioactive phosphorus from the phospho-enzyme with the formation of deoxyribose 5-phosphate. The phosphodeoxyribomutase reaction would then be analogous to the phosphoribomutase reaction as proposed by Klenow⁴.

When deoxyribose 1-phosphate was enzymatically prepared in the presence of ³²P, radioactivity was also detected in a substance which had the same mobility as ribose 1,5-diphosphate by electrophoresis. Furthermore treatment at room temperature with an acetate buffer pH 4.0 converted as expected the substance to a radioactive substance which had the same chromatographic mobility as deoxyribose 5-phosphate. It is therefore assumed that deoxyribose 1,5-diphosphate is formed from deoxyribose 1-phosphate by a deoxyribose 1-phosphate transphosphorylase present in the liver phosphorylase which was used for the preparation of deoxyribose 1-phosphate.

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Assay of Aldolase Activity Utilizing the 3-Methyl-2-benzothiazolone Hydrazone Test

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A new reagent, 3-methyl-2-benzothiazolone hydrazone (MBTH) has recently been introduced in quantitative photometry for the determination of aliphatic aldehydes¹, carbazoles, azodyes, stilbenes, aromatic amines² and tetrapyrrols like bilirubin and biliverdin³.

The reaction with aliphatic aldehydes includes the following three steps: (1) Condensation between MBTH and the aldehyde to form a colorless product (an azine). (2) Oxidation of excess MBTH by ferric chloride in acid media to form a reactive cation. (3) Condensation between the two products to form a highly colored cationic pigment.

The MBTH test, probably the most sensitive method yet known for detection of aliphatic aldehydes¹, gives, when applied to glyceraldehyde phosphate, a highly colored blue pigment with a broad absorption maximum at 610 m μ and a molar extinction coefficient exceeding 50 000. Based on this finding, a new method has been developed for the assay of aldolase activity.

A mixture of fructose 1,6-diphosphate, veronal buffer, MBTH and the enzyme, e.g. hemolysed red cells, is incubated at 37°, pH 8.6, for 15 min, and the liberated triosephosphates are trapped by the MBTH as they are being formed (step 1). The reaction is terminated by the addition of TCA, and after removal of the proteins, aliquots of the TCA extract are diluted and ferric chloride is added. A blue color is slowly produced (steps 2 and 3) and can be read in a spectrophotometer at 610 m μ after 30 min at room temperature. The color, which is stable for at least 2 h, is proportional to the amount of glyceraldehyde phosphate liberated in the reaction.

The advantages of the new MBTH method are simplicity and sensitivity. The simplicity makes the procedure particularly suited for routine analyses. The sensitivity, if fully exploited, may be used to determine minute amounts of aldolase or to reduce the incubation time considerably. Thus, if required, the incubation time, using hemolysed red cells as enzyme, may be reduced to 3 min. The dis-

advantage of the method is the broad reactivity of the MBTH reagent, and therefore one has to be particularly careful with the control incubations.

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The Degradation of Na-alginate by Enzymes from *Alginovibrio aquatilis*

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The depolymerization of polyuronic acids by enzymes from various sources seems to involve in most cases a special hydrolysis mechanism, called transesterification, the first products being 4,5-unsaturated uronides¹⁻³.

The degradation of Na-alginate (Diganat from Smith & Zoon, Bergen, Norway) by *Alginovibrio aquatilis* Meland⁴ has been studied, and the results indicate that at least two enzymes, or sets of enzymes, are needed for the complete hydrolysis of alginate to the compounds being oxidized by the bacterium.

An extracellular enzyme (alginate) produced during growth on Na-alginate and obtained in purified form, hydrolyzed alginate to a mixture of oligouronides, which were isolated as their Ba-salts insoluble in 50 % ethanol. The products had the spectrum typical for α , β -unsaturated acids, and by paper chromatography, this mixture could be separated into 5 different compounds or groups of compounds. Incubation of the isolated mixture of oligouronides with alginate gave no further degradation.

The rapid decrease in viscosity of the alginate solution relative to the increase in reducing power, absorption at 232 $m\mu$ and the amount of β -formyl-pyruvate forming material are evidence for alginate being an endo-acting enzyme.

Incubation of a mixture of oligouronides or oligouronides purified by paper chromatography, with crude cell-free extracts of the bacteria (grown on Na-alginate as substrate) caused a 1-3 fold increase in compounds giving β -formyl-pyruvate by periodate oxidation. The results indicate that the bacterium contains a new enzyme, which completes the hydrolysis of the oligouronides formed from alginate by the action of alginate.

Paper chromatography of the final products, isolated by Sephadex-G 25 filtration, revealed compounds which behaved like 5-keto-4-deoxyuronic or 2-keto-3-deoxy gluconic acid. The reaction mechanism of the hydrolysis of the oligouronides by the intracellular enzyme thus appears to be identical to that of the reaction catalyzed by alginate.

Some results connected to the optimal conditions for the production of alginate will be discussed. Addition of Ca^{2+} to the medium, and particularly a lowering of the growth temperature had a significant effect on the yield of alginate. Growth of *A. aquatilis* at 2-4°C increased the yield 10-50 times compared to the level of alginate assayed in cultures at 25°C.

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Relationships of Enzymology to Cancer

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Review of enzymes involved in the neoplastic processes of cancer and their role in diagnosis, prognosis, therapy, and definition