The metabolism of L-rhamnose by *Escherichia coli*

In this note are described preliminary studies on two enzymic steps in the metabolism of L-rhamnose by *Escherichia coli*.

Washed cell suspensions of *E. coli*, strain B, when grown in the presence of rhamnose, fermented this sugar with the production of acid and small amounts of gas. When water extracts (dialyzed 3–4 hours) of acetone powders of these cells were incubated anaerobically with rhamnose and the reaction mixture analyzed, a ketose was detected by means of the cysteine-carbazole test[1]. Paper chromatographic techniques showed that this material had a greater R_F value than rhamnose in the solvent mixtures butanol-pyridine-water (3:2:1.5) and butanol-ethanol-water (5:1:4). When eluted from the papers it gave positive tests for ketose (abs. max. 545 mμ), methyl pentose[2] (abs. max. 400 mμ) and reducing sugar[3]. The orcinol test for pentoses was negative.

From the results of these tests it would appear likely that the compound formed is rhamnoketose. This hypothesis is further supported by the fact that the ketose prepared by refluxing L-rhamnose with pyridine, a common method for the preparation of ketoses from their respective aldoses, has similar properties to the ketose obtained in these experiments.

The addition of adenosine triphosphate (ATP) and rhamnose to such dialyzed acetone powder extracts of *E. coli* resulted in the formation of a phosphorylated compound as determined by the manometric method of COLOWICK AND KALCKAR[4]. In Table I are shown the results obtained with the cysteine-carbazole test on the supernatants of samples of the reaction mixtures treated with either $Ba(OH)_2$ and $ZnSO_4$, or with trichloracetic acid. The ketose which formed in the vessel containing rhamnose, ATP and extract was almost completely removed by the $Ba(OH)_2 - ZnSO_4$ treatment which precipitates phosphorylated compounds as well as proteins. The results of this experiment suggested that in the presence of ATP, rhamnoketose phosphate was probably formed.

TABLE I

KETOSE PHOSPHATE FORMATION FROM RHAMNOSE BY *Escherichia coli*

Incubation system	*Colorimeter readings at 545 mμ in the cysteine-carbazole test per 0.1 ml of sample*			
	Initial		*Final*	
	$Ba(OH)_2 + ZnSO_4$	*TCA***	*$Ba(OH)_2 + ZnSO_4$*	*TCA***
1. Rhamnose + ATP + extract	0.030	0.043	0.059	0.604
2. Rhamnose + extract	0.021	0.018	0.511	0.530
3. ATP + extract	0.016	0.025	0.014	0.024
4. Rhamnose + ATP	0.031	0.039	0.028	0.034

The complete system contained 0.002 *M* L-rhamnose, 0.0033 *M* ATP, 0.025 *M* $NaHCO_3$ and 1 ml of extract. Total volume 3 ml. Time of incubation 1.5 hours. Temperature 33°. Warburg flasks were gassed with a mixture of 5% CO_2 and 95% N_2 before tipping the ATP and rhamnose.

* Duplicate cups to which protein precipitants were added at the start of the experiment represent the initial values shown.

** Trichloracetic acid.

When the newly formed phosphate compound was separated by means of paper chromatography and eluted from the paper it gave positive tests for bound phosphorus, ketose, methyl pentose and reducing sugar.

The material was 80–90% hydrolyzed after 10 minutes at 100° in *N* HCl, which suggests that this compound may be rhamnoketose-1-phosphate.

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Received April 15th, 1955