# METABOLISM OF PENTOSES BY CLOSTRIDIA

## I. Enzymes of Ribose Dissimilation in Extracts of *Clostridium perfringens*[1]

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Evidence has accumulated indicating that the clostridia ferment glucose by means of a system similar to the Embden-Meyerhof-Parnas (EMP) pathway (Elsden, 1952). In the case of *Clostridium perfringens*, for instance, most of the enzymes of the EMP pathway have been demonstrated in cell free extracts (Bard and Gunsalus, 1950; Shankar and Bard, 1955). Further evidence for the operation of the EMP pathway in *C. perfringens* has been provided in the form of studies involving tracer distribution in the products of the fermentation of $C^{14}$-labeled glucose (Paege *et al.*, 1956). The utilization of pentoses by members of the genus *Clostridium* has received less attention. Reports in the literature concerning this phase of carbohydrate metabolism in the clostridia have been restricted generally to a listing of the pentoses metabolized and to the products of pentose metabolism. For example, Prévot and Taffanel (1942) reported that *Clostridium novyi*, *Clostridium saprotoxicum*, and *Clostridium hemolyticum* metabolized arabinose and xylose, producing ketones, lactic acid, ethanol, and fatty acids of low molecular weight. Bolcato *et al.* (1952) studied the fermentation of xylose and arabinose by *Clostridium acetobutylicum*. They isolated and identified as fermentation products triose phosphates and pyruvic acid, and concluded on the basis of this evidence that pentose was fermented by means of a $C_2$-$C_3$ cleavage.

It was felt that the metabolism of pentoses by members of the genus *Clostridium* was worthy of further study. Experiments conducted with *C. perfringens* revealed that this organism could be induced to ferment D-ribose, but not D-xylose or L-arabinose. The products included carbon dioxide, hydrogen, ethanol, and volatile acids (Cynkin, 1957). In the present report, evidence is presented indicating that extracts prepared from *C. perfringens* grown on D-ribose possess ribokinase and phosphopentoisomerase activity, and are able to convert ribose-5-phosphate to hexose monophosphate.

### MATERIALS AND METHODS

*C. perfringens* strain BP6K from the Cornell University collection was maintained by transfer in heart infusion broth containing D-ribose. Mass cultures of cells were grown in a medium containing 1 per cent yeast extract (Difco), 1 per cent tryptone (Difco), and 0.5 per cent $K_2HPO_4$. A 20 per cent solution of D-ribose was sterilized by filtration and added to the medium to yield a final sugar concentration of 1 per cent. Cultures were incubated at 37 C for 5 hr, harvested by centrifugation, and washed once with distilled water before being used in the preparation of cell free extracts.

Two general methods were used for the preparation of extracts. In the first method, the culture was suspended in distilled water and subjected to sonic oscillation. In the second method, extracts were prepared by grinding with alumina.

D-Ribose was obtained from various commercial sources. Ribose-5-phosphate was obtained from Schwarz Laboratories as the barium salt. Triphosphopyridine nucleotide (TPN) and the potassium salt of adenosine triphosphate (ATP) were obtained from the Sigma Chemical Company.

The method of Horecker *et al.* (1954) was employed for the demonstration of "ribokinase" activity, in which the disappearance of free pentose was followed after the phosphate esters were removed as ethanol-insoluble barium salts.

[1] Part of the material presented here was taken from a thesis submitted by the senior author to the Graduate School of Cornell University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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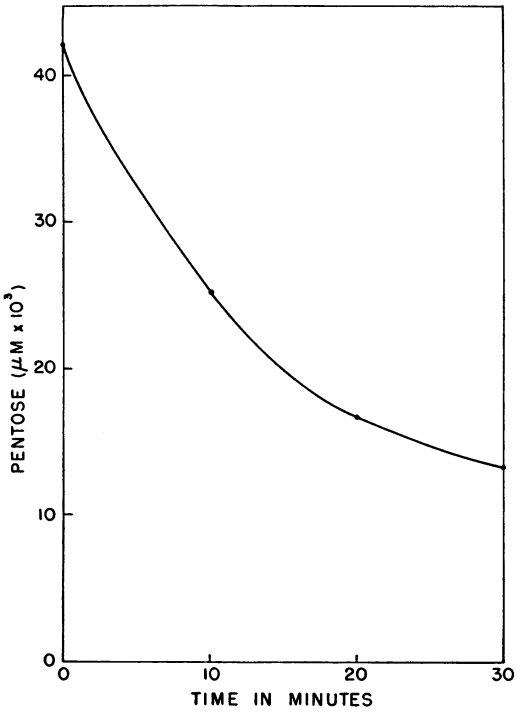


*Figure 1.* Disappearance of free pentose in the presence of ATP. In these experiments, a cell-free extract was prepared by treating a 20 ml suspension containing about 20 mg (dry weight) of cells per ml in a Raytheon 50 Watt, 9 kc Oscillator for 60 min. Reactions were conducted at room temperature. The reaction mixture contained the following ingredients (in a total volume of 0.6 ml): tris(hydroxymethyl)aminomethane buffer (pH 7.3), 15 μmoles; $MgCl_2$, 10 μmoles; cysteine (pH 7.4), 5.2 μmoles; ATP, 4 μmoles; extract, 0.1 ml; D-ribose, 2.0 μmoles. The ribose was added last. At the intervals shown above, a 0.1 ml sample was removed, added to 0.5 ml absolute ethanol, and treated with 0.01 ml of a saturated barium acetate solution. The resulting precipitate was removed by centrifugation, and a 0.1 ml aliquot of the supernatant was analyzed for pentose.

"Phosphopentoisomerase" activity was demonstrated using the method of Axelrod (1955), in which a modification of the carbazole procedure of Dische and Borenfreund (1951) was employed for the detection of ketoses. Pentose was estimated by the method of Mejbaum (1939). Hexose monophosphate was estimated by following TPN reduction spectrophotometrically in the presence of glucose-6-phosphate dehydrogenase.

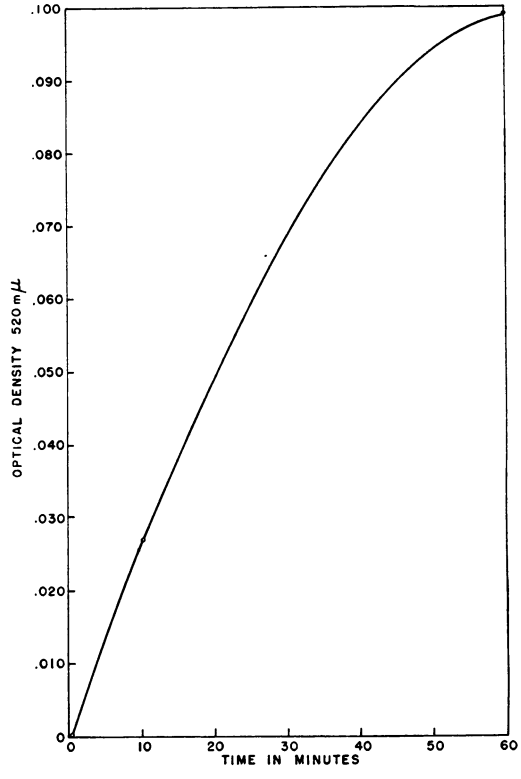


*Figure 2.* Formation of ketose from ribose-5-phosphate. In these experiments, cell-free extracts were prepared as follows: a paste containing 21 g of cells (wet weight) was ground manually with 50 g of "levigated" alumina. Twenty-five ml of a 0.9 per cent KCl solution were stirred in with the mixture, and the preparation was stored overnight. A clear supernatant solution was obtained after two centrifugations at 20,000 × G for 45 min. All operations were performed at 4 C. The substrate, ribose-5-phosphate (barium salt), was dissolved (1 mg/ml) in 0.1 M tris(hydroxymethyl)aminomethane buffer, pH 7.3, immediately before use in order to minimize spontaneous isomerization. One-half ml of this solution was placed in each of three colorimeter tubes at 37 C. One-tenth ml of enzyme solution was then added to each tube. At each time interval indicated, one tube was treated with 6.0 ml of 23 N $H_2SO_4$, 0.2 ml of a 0.12 per cent carbazole solution in absolute ethanol, and 0.2 ml of a 1.5 per cent aqueous solution of L-cysteine hydrochloride. Each tube was then incubated at 37 C for 30 min. Optical density at 520 mμ was determined. The tube treated at 0 min was used as a colorimetric blank.

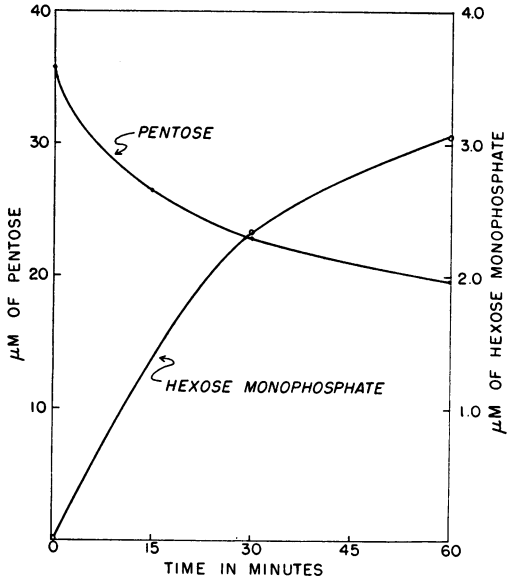


*Figure 3.* Formation of hexose monophosphate from ribose-5-phosphate. The cell free extract was prepared by treating a 20 ml suspension containing about 100 mg (dry weight) of cells per ml in a Raytheon 200 watt, 10 kc oscillator for 20 min. Debris was removed by centrifugation at 20,000 × G for 45 min. The reaction was begun by adding 0.2 ml of 0.05 M ribose-5-phosphate to 1.0 ml of extract. The incubation temperature was 30 C. At the intervals shown above, a 0.2 ml sample was removed, added to 0.8 ml distilled water, boiled for 2 min, chilled, and centrifuged. The supernatant was analyzed for pentose and for hexose monophosphate.

The assumption was made here that phosphohexoisomerase was present in the extracts (Shankar and Bard, 1955).

## RESULTS

*Ribokinase activity.* When D-ribose was incubated with a cell free extract in the presence of ATP, the disappearance of pentose was observed (figure 1). No attempt was made to identify phosphorylated products. However, in the experiments described below, ribose-5-phosphate was assumed to be the product of the phosphorylation of ribose (Cohen *et al.*, 1951; Sable, 1952; Horecker *et al.*, 1954).

*Phosphopentoisomerase activity.* In the quantitative determination of this enzyme, the color density produced in the cysteine-carbazole reaction would be standardized by comparison with the color produced by an equilibrated reaction mixture (Axelrod, 1955). In the present experiment, however, an increase in color density was to be regarded as qualitative evidence for isomerase activity, i. e., ketose production from ribose-5-phosphate. The observed increase in optical density, shown in figure 2, presumably is the result of ketose production, and this has been considered as evidence for the isomerization of ribose-5-phosphate.

*Conversion of pentose phosphate to hexose monophosphate.* When ribose-5-phosphate was incubated with extracts, pentose disappearance could be demonstrated. This disappearance was accompanied by the formation of hexose monophosphate (figure 3). Attempts to demonstrate intermediates such as triose phosphate, assayed by measuring diphosphopyridine nucleotide reduction spectrophotometrically, or heptulose, using a modification of the orcinol reaction (Horecker *et al.*, 1953), were unsuccessful. Assuming the presence of phosphohexoisomerase, the concentration of hexose monophosphate estimated by the method described in figure 3 is the sum of the concentrations of glucose-6-phosphate and fructose-6-phosphate. It should be pointed out that the extracts used in these experiments do not metabolize glucose-6-phosphate as estimated by following, spectrophotometrically, the reduction of di- or triphosphopyridine nucleotide.

## DISCUSSION

The transketolase-transaldolase sequence of reactions, in which the conversion, pentose phosphate → heptulose phosphate → hexose phosphate, occurs, has been associated with an "Oxidative Cycle", in which, theoretically, hexose can be completely oxidized to $CO_2$ and $H_2O$ without the involvement of the tricarboxylic acid cycle (see Gunsalus *et al.*, 1955, for references). In addition, the fermentation of D-ribose by cell-free extracts of bakers' yeast (Gibbs *et al.*, 1955), and the fermentation of D-ribose and D-xylose by intact resting cells of *Aerobacter aerogenes* (Altermatt *et al.*, 1955) have been shown to proceed through the transketolase-transaldolase reaction sequence. However, since bakers' yeast and *A. aerogenes* are facultative aerobes, it might be argued that the fermentation of pentoses by means of this

mechanism is a perversion of the normal function of the sequence in the oxidative cycle.

In the present study, alternate functions cannot be assigned so readily to the transketolase-transaldolase sequence. In the first place, *Clostridium perfringens* is an obligate anaerobe, so that the sequence cannot be placed in an oxidative role. In the second place, the absence of glucose-6-phosphate dehydrogenase makes it difficult to envision the involvement of the sequence in a cyclic mechanism. It would seem, then, that in *C. perfringens*, the primary, if not sole, catabolic function of the enzymes of the transketolase-transaldolase sequence is the dissimilation of pentose.

SUMMARY

Cell free extracts of *Clostridium perfringens* have been shown to possess ribokinase and phosphopentoisomerase activity. The conversion of pentose phosphate to hexose monophosphate in these extracts was also demonstrated.

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