It has also been possible to demonstrate that the crude toxin (even from dilute solutions) may be quantitatively recovered in a smaller volume in the M.M.E.D. apparatus, operated at a pH removed from the iso-electric point of the toxin (*e.g.* pH 7.0). Using a single separation cell (capacity500 ml) as a batch process, 95% or more of the toxin has been concentrated in 50 ml at the bottom of the cell within 4 hours with a potential gradient of approximately 4 volts/cm. The final concentration in the top of the cell was one-hundredth of that present originally (see Table II).

TABLE II

CONCENTRATION OF TOXIN

Substance	*Toxicity M.L.D./ml*
Original	$2 \cdot 10^4$
Bottom fraction	$2 \cdot 10^5$
Top fraction	$2 \cdot 10^2$

Further work using the M.M.E.D. to purify tetanus toxin from both the G.S. 761 and the "Harvard" strains of *Cl. tetani* (the latter producing significantly more toxin under the conditions of these experiments) is in progress. The effect of the purification on such properties as flocculation, antigenicity, etc. will also be investigated.

The author wishes to thank Dr. POLSON for his assistance and Professor VAN DEN ENDE for his interest in this work.

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Received December 7th, 1954

Conversion of D-xylose to D-xylulose phosphate by extracts of *Pseudomonas hydrophila**

Phosphorylation studies, in which D-xylose was the pentose supplied, have been reported with cell-free extracts of *Pseudomonas hydrophila*[1], *Lactobacillus pentosus*[2] and *Aerobacter cloacae*[3]. The product of phosphorylation was first described only as an acid-stable pentose phosphate[1]. Later, using *L. pentosus*, LAMPEN[4] identified D-ribose-5-phosphate as the chief product of this system, stating, however, that D-xylose or D-xylulose phosphates could not be detected. Recent work has shown that the cell-free extracts used in phosphorylation studies of *P. hydrophila* and *L. pentosus* contained not only a kinase but also a specific xylose isomerase[5,6,7]. It, therefore, became uncertain whether D-xylose or D-xylulose was the actual substrate in the phosphorylation.

Attempts to identify the product of the phosphorylation have now revealed the presence of at least four acid-stable phosphate esters. This communication describes the identification of the first ester formed in the sequence of reactions now known to take place.

The enzyme preparation used was a fresh sonic extract of *P. hydrophila*[7]. Nucleoprotein was removed with $MnCl_2$[8] and nucleic acids and other extraneous protein with protamine sulphate[9,10]. This procedure was used to prevent the possible formation of pentose phosphate by enzymic hydrolysis of nucleic acids or of nucleoprotein. After centrifugation (14,000 r.p.m.), the supernatant solution was dialyzed against weak phosphate and then against weak bicarbonate buffers (pH 7.4–7.5) for a total of 48 h. The resulting product was used for all experimental work.

Phosphorylation experiments were carried out in an atmosphere of N_2–CO_2 in 15 Warburg vessels (27° C), each containing 0.02 *M* $NaHCO_3$, 0.01 *M* $MgCl_2$, 0.026 *M* ATP, 1.8 ml enzyme preparation and 0.018 *M* D-xylose. Conditions chosen were such that no free sugars remained at the termination of the experiments, as judged by paper chromatography of the products. Phosphorylation rates were measured by the method of COLOWICK AND KALCKAR[11]. After 90 minutes, the contents of all vessels were combined (48 ml), 0.6 ml glacial acetic acid was added and the solution was allowed to pass slowly (1–1.5 ml/min) through a large column of IR120 (H^+) ion exchange resin followed by several washings with deionized water. Protein flocculated out at the top of the column. The effluent (pH about 2.8) was collected at 0–4° C concentrated *in vacuo* at 30° C[12] to a volume of 50 ml, and treated with barium hydroxide. After removal of the adenosine phosphates at pH 8.2, the pH was quickly brought to 6.8. Four volumes of ethanol were then added to precipitate the barium salts of the sugar phosphates. The precipitate was thoroughly washed and dried.

* Issued as N.R.C. No. 3483.

The various sugar phosphates in the precipitate could not be separated by preparative paper chromatography or by paper electrophoresis. The mixture was, therefore, dephosphorylated by incubation in 0.05 *M* acetate buffer at pH 5.2 for 1 h at 30° C with a partially purified acid phosphatase preparation. Protein was precipitated with trichloroacetic acid and the resultant mixture was put through a mixed bed ion exchange resin column (IR120 (H^+) and IRA400). The free sugars were then separated by paper chromatography in phenol-water (4:1)[13] on long papers (36 inches). The ketopentose areas were made visible by applying a new combination spray. The TCA-orcinol spray of KLEVSTRAND AND NORDAL[14] was applied first (with subsequent heating) followed by aniline phthalate[15] overspray (without heating). This procedure produced a purple color with D-xylulose and a pink color with D-ribulose but gave no color with aldopentoses.

D-Xylulose was identified as one of the major components of the mixture of sugars by the specific color, by its position on paper and by conversion to its *p*-bromophenylhydrazone. The eluted material was, however, contaminated with traces of D-ribose which occupied the same position on the papers but gave the typical aldopentose reaction when sprayed with aniline phthalate alone. The D-ribose in the eluate was converted by bromine oxidation to ribonic acid which was removed from solution by passage through a mixed bed ion exchange resin. The ketopentose remaining in solution was characterized by preparation of its *p*-bromophenylhydrazone (mp: 128–9° C) by a modification of the original method of SCHMIDT AND TREIBER[16]. A mixture of this derivative and an authentic sample of D-xylulose-*p*-bromophenylhydrazone (m.p. 128.5–129.5° C) melted at 128–129.5° C. The presence of D-xylulose phosphate in the sugar phosphate fraction was thus established. Although the position of the phosphate group in the molecule could not be determined, the acid stability of all the phosphorylation products[1] suggests that the compound in question is D-xylulose-5-phosphate.

No D-xylose was detected after dephosphorylation of the sugar phosphates. Moreover, D-xylose-5-phosphate** was not further metabolized by the enzyme system. This result is in agreement with earlier findings[17] and with the suggestion that isomerization precedes phosphorylation[6].

The experimental data given in this communication establish the initial sequence in the utilization of D-xylose by extracts of *P. hydrophila* as:

$$\text{D-xylose} \rightleftharpoons \text{D-xylulose} \longrightarrow \text{D-xylulose-(5)-phosphate.}$$

The technical assistance of Mrs. ALMA HARVEY is gratefully acknowledged.

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Received December 7th, 1954

** Kindly supplied by Dr. A. E. MIRSKY, Rockefeller Institute for Medical Research, New York, from the Levene Collection.

Some properties of the casein of mouse milk (RIII)*

A protein, considered to be casein on the basis of its phosphorus content, has been prepared from the milk of mice of the RIII strain (carriers of the mammary tumor agent). Its concentration is approximately 2.8%. Some of its properties are described below.

The manner of collecting the milk and the precautions taken to avoid changes during treatment

* Supported by a grant from the American Cancer Society.