## Formation of D-ribulose from D-gluconate in guinea-pig liver

Recent studies[1] in this laboratory on the substrate specificity of DPN–L-gulonic acid dehydrogenase from guinea-pig liver revealed that D-gluconic acid was also dehydrogenated, as shown by the increase of absorbancy at 340 mμ. D-Gluconic acid might easily be produced *in vivo* from glucose by D-glucose dehydrogenase in liver or by other enzyme systems. SMILEY AND ASHWELL[2] also reported that a similar enzyme dehydrogenates the aldonic acids with L-hydroxy group at the third carbon atom. The present paper deals with the reaction products from D-gluconic acid by this dehydrogenation using uniformly labeled [$^{14}$C]gluconic acid.

The enzyme was purified by the method reported by ISHIKAWA[3] in this laboratory. [$^{14}$C]Gluconic acid uniformly labeled was obtained by electrolytic oxidation with NaBr from uniformly labeled [$^{14}$C]glucose (0.1 μC/μmole) and purified with Dowex-1. The reaction was performed in double-side-arm Warburg flasks as described in the legend to Fig. 1. After a 2-h incubation at 37°, $CO_2$ was absorbed in KOH after addition of trichloroacetic acid, and precipitated as $BaCO_3$. Decarboxylation, determined with a Q-gas counter, amounted to 894.1 ± 5.6 counts/min (infinite thickness) in the first experiment, 1004.6 ± 5.9 counts/min in the next under the same conditions, while nonenzymically 66.8 ± 2.0 counts/min were recorded. The remaining carbon chain was identified as ribulose as follows. The 10 mg of ribulose syrup obtained from epimerization of D-arabinose[4], containing D-ribose and D-arabinose in smaller amounts, was added as carrier to the deproteinized mixture. After the mixture was desalted and concentrated, it was dissolved in 0.005 *M* $Na_2B_4O_7$

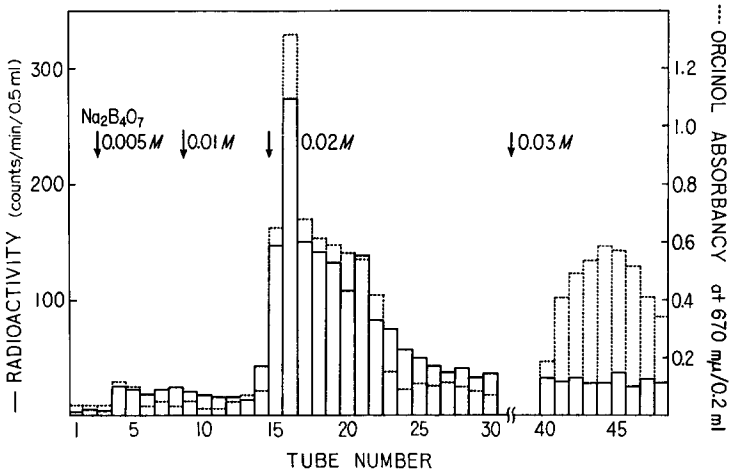


Fig. 1. Elution curve of the reaction product. The reaction mixture contained: 15 μmoles (1.5 μC) sodium gluconate in side-arm I; 30 μmoles DPN, 100 μmoles nicotinamide, 0.2 μmole $MnCl_2$, 0.8 ml 0.1 *M* glycine buffer, pH 10.5, and 1.0 ml of the enzyme solution in main chamber; 50% trichloroacetic acid in side-arm II; and 0.2 ml conc. KOH in central well; total volume, 3.2 ml. A Dowex-1-borate 0.9 × 12.5 cm column was used for the fractionation and the volume of each fraction was 10 ml. Radioactivity is expressed in solid lines as counts/min/0.5 ml. Absorbancy at 670 mμ is expressed in dotted lines as estimated after 0.2 ml of the fraction was reacted with orcinol and diluted to 4.0 ml.

Abbreviation: DPN, diphosphopyridine nucleotide.

and analysed on a Dowex-1-borate column as shown in Fig. 1. Radioactivity was concentrated in the first orcinol-positive peak. Experiments with non-labeled gluconate (100 $\mu$moles in an otherwise identical experiment) gave rise to an orcinol-positive peak in the same position. These peaks were concentrated after being passed through a Dowex-50-hydrogen column, and boric acid was removed as the methanol complex. The resulting syrup showed a violet color by spot test with anthrone, and its orcinol reaction showed a ratio of absorbancy at 670 m$\mu$ to that at 540 m$\mu$[5], which suggested the presence of keto-pentose. The autoradiograph of the syrup thus obtained showed the same $R_F$ value as that of ribulose. The product was also subjected to reaction with *o*-nitrophenylhydrazine according to GLATTHAAR's procedure[4]. The resulting hydrazone was fractionated on an alumina column and its radioactivity was counted. The first yellow band eluted with ethyl acetate was *o*-nitrophenylhydrazine. Following that, elution by acetone, *n*-propanol, ethanol, and methanol produced peaks but no radioactivity was detected in them. Radioactivity was eluted with methanol–water (1:1), and this orange peak appeared in the same position as authentic ribulose *o*-nitrophenylhydrazone (m.p. 168°). 10 mg of ribulose *o*-nitrophenylhydrazone was added to the eluate and the specific activity of the crystal was determined during 5 recrystallizations. The weight of the hydrazone was estimated from the molar extinction coefficient ($6.0 \cdot 10^3$) at 432 m$\mu$, and the samples were counted in the state of infinite thinness. The specific radioactivity was found to be almost constant during the recrystallization as follows. 1, 170.1 $\pm$ 17.4; 2, 138.5 $\pm$ 9.1; 3, 166.0 $\pm$ 11.9; 4, 158.3 $\pm$ 10.2; 5, 180.9 $\pm$ 13.4 counts/min/mg. This enzyme also dehydrogenated 6-phosphgluconate[5] slowly, but slight alkaline phosphatase activity was also detected in the preparations used. Further purification of this enzyme is now going on.

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## Selective formation of α-amylase by non-growing cells of *Pseudomonas saccharophila*

Several years ago, RICKENBERG AND LESTER[1] reported that a mutant of *Escherichia coli* produced 5 % as much β-galactosidase when induced under certain non-growing conditions as was produced during logarithmic growth. They suggested that the