# FORMATION OF TREHALOSE FROM GLUCOSE IN THE RENAL CORTEX

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Summary. Trehalose-$^{14}$C was found when rabbit renal cortical slices were incubated with glucose-U-$^{14}$C. This finding is consistent with our working hypothesis that trehalose is an intermediate in the reabsorption of glucose by the kidney.

It has been suggested that kidney trehalase, which specifically hydrolyzes trehalose (1-$\alpha$-D-glucopyranosyl-$\alpha$-Dglucopyranoside) to two glucose moieties, functions in the active transport of glucose from the glomerular filtrate (Sacktor, 1968). Trehalase was localized exclusively in the renal tubules, although blood trehalose was never detected (Grossman and Sacktor, 1968). In several mammalian species the enzyme had sufficient activity to account for the rate of glucose transport, *in vivo*, and in the rabbit the enzymes were found which, in sequence, could effect the biosynthesis of trehalose from glucose (Sacktor, 1968). On the other hand, Van Handel (1969) injected glucose-$^{14}$C into a rabbit and failed to find any trehalose in the kidney 10 or 20 min later and questioned the participation of trehalose in glucose resorption. It is possible, however, that the design of Van Handel's experiments may not have been favorable for showing the conversion of glucose to trehalose. The high activity of trehalase in the renal cortex may severely limit accumulation of the disaccharide. Furthermore, the transit time for the reabsorption of glucose across the tubule cells is only about 10 sec (Chinard *et al.*, 1959). We have been conducting parallel studies and have found that labeled trehalose is demonstrable when renal cortical slices are incubated briefly (1 min) with glucose-U-$^{14}$C. These preliminary findings, which are consistent with our working hypothesis on the mechanism of active transport of glucose, are reported in this communication.

## Methods and Materials

Rabbit kidney cortical slices, about 100 mg per slice, were prepared by the method of Kleinzeller et al. (1967). Pieces of cortex and slices were kept in incubation medium at 0°C, under a steady flow of 100% $O_2$, until used. The phosphate-saline medium of Krebs (1950), containing 10 mM sodium acetate, and adjusted to pH 7.4, was used. Prior to the addition of the slices, 0.2 mM carrier glucose containing 2 x $10^6$ dpm glucose-U-$^{14}C$ was added to the incubation flasks, the flasks were flushed with $O_2$ and equilibrated at 30°C in a metabolic shaker. Two to three slices were incubated at a time and at the end of 1 min they were rapidly rinsed in glucose-free phosphate-saline buffer and immediately frozen in liquid $N_2$. The slices, about 3.5 g, were homogenized in cold 12% perchloric acid and centrifuged at 12,000 x g. The cold supernatant was neutralized with 5N KOH and centrifuged. The clear extract was placed on a mixed bed ion exchange column. The anion exchange resin was Dowex AG 1X8 (100-200 mesh), $HCO_3^-$ form, and the cation exchange resin was Dowex AG 50WX8 (100-200 mesh), $H^+$ form. The neutral sugars were eluted with water and lyophilized.

Glucose and trehalose were separated by two dimensional thin layer (silica gel-G) chromatography essentially as described by De Stefanis and Ponte (1968). The solvent system in each direction was chloroform: acetic acid: water (3.0: 3.5: 0.5 v/v). Sugars were detected by the naphthoresorcinol reagent of Lato et al., 1968). Corresponding areas for glucose and trehalose were scraped and eluted with water. Radioactivity was measured in a Tri-Carb liquid scintillation counter using a scintillation mixture of toluene: Triton X-100 (4:1), containing 5.5 g 2,5-diphenyloxazole and 125 mg 1,4-bis[2-(5-phenyloxazolyl)] benezene per 1. Recoveries of glucose and trehalose were identical, about 80%. In one experiment, Biogel-P2 (200-400 mesh) was used to separate glucose from trehalose. Chromatography was performed in the cold with a column 120 cm x 2.5 cm in dimension, eluting with 0.01 M phosphate buffer, pH 7.0. Carrier trehalose was added to the neutral extract to locate the peak or spot.

Glucose and trehalose were measured enzymatically, as described previously (Sacktor and Wormser-Shavit, 1966). Purified trehalase was prepared from blowfly (Phormia regina) abdomens by a modification (Sacktor, 1968) of the procedure of Friedman (1960). The enzyme had a specific activity of 24 μmoles trehalose hydrolyzed x $min^{-1}$ x $mg^{-1}$ protein.

Glucose-U-$^{14}C$ was obtained from Schwarz BioResearch, Inc. Prior to use it was purified by deionization with a mixed bed ion exchange column, and by passage through charcoal (Whistler and Durso, 1950). Essentially all the counts appeared in a single spot with the same $R_f$ as glucose.

## Results and Discussion

Data showing the conversion of labeled glucose to trehalose by kidney cortical slices are given in Table I. Experiment A describes one of three studies in which the sugars were separated by thin layer chromatography. In this experiment, the neutral sugars, obtained after passage through ion exchange

Table I. Formation of Trehalose by Renal Cortical Slices

| | Expt. A | | Expt. B | |
|---|---|---|---|---|
| | Trehalose | Glucose | Trehalose | Glucose |
| Apparent Trehalose, initial separation | 3890 | - | 9280 | - |
| Rechromatographed, after elution and incubation without trehalase (control) | 2600 | 1290 | 9280 | 0 |
| Rechromatographed, after elution and incubation with trehalase | 410 | 3520 | 2910 | 3250 |
| Trehalose Glucose (trehalase) less Glucose (control) | 2230 | - | 3250 | - |

Data are reported in dpm. In experiments designated A and B, the sugars were separated by thin layer chromatography and Biogel column chromatography, respectively. Values are calculated to account for the total dpm in the apparent trehalose area.

columns, had approximately 3 x $10^5$ dpm. Almost 4 x $10^3$ dpm, or 1.3%, cochromatographed in a 2-dimensional system with carrier trehalose. The trehalose area, which was clearly distinct from the glucose spot, was scraped from the plate and the sugar eluted. The eluate was lyophilized and the apparent trehalose was dissolved in a minimum of water. The sugar solution was divided in half: one part was incubated for 2 hrs with purified trehalase; the other was incubated in an identical manner but without trehalase. In the control incubation, 67% of the radioactivity rechromatographed as trehalose. In contrast, rechromatography of the apparent trehalose, after treatment with trehalase, showed only 11% of the dpm remaining in the trehalose area, whereas 90% of the radioactivity migrated as glucose. Thus, the actual trehalose found, calculated from the glucose formed from apparent trehalose incubated with trehalase less that found in the mixture incubated without the specific disaccharidase, had over 2.2 x $10^3$ dpm, representing 57% of the apparent trehalose and 0.7% of the total neutral sugar. In two other experiments, a similar pattern was observed. The deionized extracts contained approximately 10 x $10^4$ and 9 x $10^4$ dpm, of which 1.8 and 2.2%, respectively, of the neutral sugars was apparent trehalose. The true trehalose, calculated as described above, had 0.3 and 0.4%, respectively, of the radioactivity of the total neutral sugar extract.

An experiment in which the deionized sugars were separated on a Biogel P-2 column is also summarized in Table I. Rechromatography of the apparent trehalose peak indicated the complete absence of glucose. The rechromatographed sugar was then lyophilized and incubated with trehalase. Now, over 3.2 x $10^3$ dpm, or 53% of the sugar chromatographed as glucose. This represents 0.4% of the radioactivity in the total neutral sugar eluate. The efficacy of the Biogel system in separating glucose from trehalose is illustrated in Fig. 1.

The demonstration with two chromatographic systems that renal cortical slices form trehalose from glucose is clearly in opposition to the observations of Van Handel (1969). The present results are therefore consistent with our

working hypothesis that trehalose and trehalase participate in the active transport of glucose. Data on the turnover of trehalose in kidney relative to the rate of glucose transport have yet to be obtained.

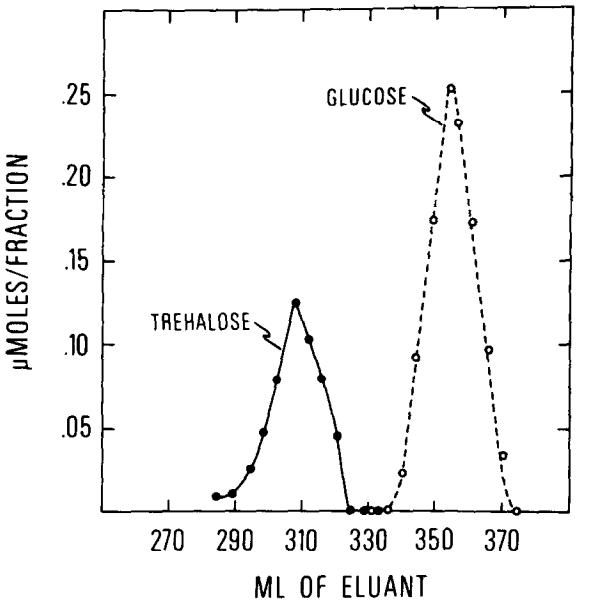


Fig. 1. Separation of trehalose and glucose on Biogel-P2 columns. Solid circles and line illustrate a rechromatograph of the apparent trehalose peak before treatment with trehalase. Open circles and dashed line illustrate the stoichiometric formation of glucose stemming from the trehalose, shown by the solid line, after incubation with trehalase.

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