Preliminary Notes

Phlorizin as a competitive inhibitor of the active transport of sugars by hamster small intestine, *in vitro*

As reviewed by LOTSPEICH[1] and CRANE[2], evidence has accumulated in recent years to indicate that phlorizin inhibition of intestinal absorption and tubular reabsorption of sugars is closely related to effects of this glycoside on cell permeability. For example, phlorizin prevents the entry of sugars into ascites tumor cells[3], the entry and accumulation of galactose in rabbit kidney-cortex slices[4] and the expansion by insulin of the galactose space in the eviscerate rat[5].

Until recently, however, it has not been possible to obtain direct evidence that phlorizin does, in fact, inhibit entry of sugars into intestinal epithelial cells through their mucosal or brush-border surfaces. Reports from this laboratory[6,7] and other experiments to be published show that the overall process of intestinal active transport of sugars by hamster small intestine, *in vitro*, has two components; namely, (1) an entry component which is dependent upon the presence of Na^+ but independent of energy supplies (*i.e.*, it occurs in the absence of oxygen and in the presence of 4,6-dinitro-*o*-cresol; conditions which prevent active sugar transport) and, (2) an accumulation component which is dependent upon both Na^+ and energy supplies. Phlorizin at low concentrations has been shown to inhibit the entry component and

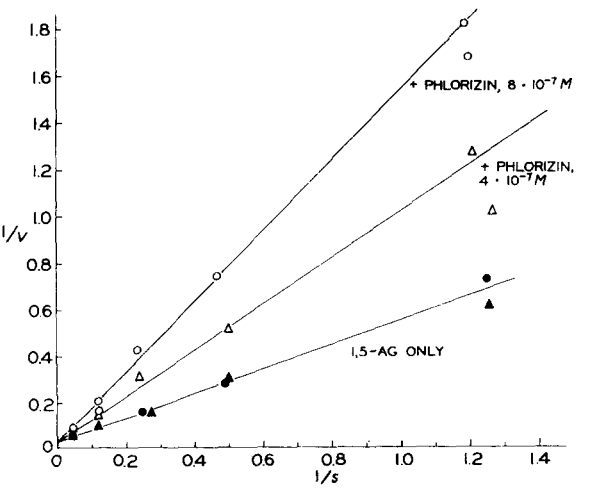

Fig. 1. Competitive inhibition of the active transport of 1,5-anhydro-D-glucitol by phlorizin. The experiments were performed as previously described[9]. Small strips or rings of everted hamster intestine were incubated for 10 min at 37° in Krebs–Henseleit bicarbonate buffer containing various concentrations of tritium-labeled 1,5-anhydro-D-glucitol (1,5-AG) with or without the addition of phlorizin at the concentrations stated in the figure. The amount of tritium label entering the tissue was measured by liquid scintillation spectrometry. The data are taken from two different experiments indicated by triangles and circles.

consequently the overall process of active transport[6,7]. Experiments consistent with this interpretation of the action of phlorizin have been reported previously for the accumulation of galactose in rabbit kidney-cortex slices[4] and subsequently for the metabolism and active transport of glucose by rat small intestine[8].

The nature of this inhibition of sugar entry now appears to be explained by the experiments illustrated in Figs. 1 and 2 which were carried out by the intact-strip method of CRANE AND MANDELSTAM[9]. The inhibition observed is clearly competitive with the actively transported compounds[10] 1,5-anhydro-D-glucitol and 6-deoxy-D-glucose, yielding an apparent K_i for phlorizin of $4.5 \cdot 10^{-7}$ M in the first case

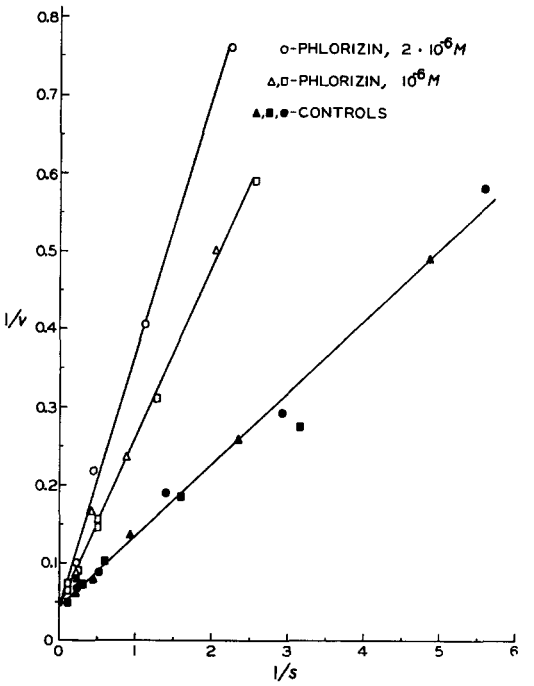

Fig. 2. Competitive inhibition of the active transport of 6-deoxy-D-glucose by phlorizin. The experiments were carried as described in the legend to Fig. 1 except that 6-deoxy-D-glucose was used and assayed by the DISCHE AND SHETTLES method[14]. The data are taken from three different experiments indicated by triangles, squares and circles. The two experiments at the 10^{-6} M level of phlorizin were carried out 3 months apart.

and $8 \cdot 10^{-7}$ M in the other. Inasmuch as all actively transported sugars appear to share the same pathway[11,12], it seems reasonable to interpret these experiments as indicating a competition between phlorizin and the sugars for a specific site on a "carrier" in the brush-border membrane.

Since phlorizin, in the concentration range employed, cannot be detected within the cells by the available analytical methods, it cannot be decided whether phlorizin enters the cells at an undetectably slow rate or whether its attachment completely immobilizes the "carrier". Owing to the strictly competitive nature of the inhibition shown in Figs. 1 and 2, a secondary attachment of the aglycone moiety such as that postulated for phloretin inhibition of sugar transport in the human red cell[13] would

appear not to be an essential part of the inhibition observed here. Phloretin is less than 1% as active against intestinal sugar transport as is the full glycoside.

This work was supported by grant No. 11216 from the National Science Foundation.

Department of Biological Chemistry, Washington University Medical School, St. Louis, Mo. (U.S.A.)

FRANCISCO ALVARADO*
ROBERT K. CRANE

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Received October 27th, 1961

* On leave from the Department of Enzymology, Instituto Marañon, Centro de Investigaciones Biologicas, C.S.I.C., Madrid (Spain).

The synthesis of a rhamnolipid by enzyme preparations from *Pseudomonas aeruginosa*

Following the log phase of growth, *Pseudomonas aeruginosa* is known to produce and excrete a glycolipid into the media[1,2]. JARVIS AND JOHNSON[1] suggested the following structure for this glycolipid:

L-rhamnosyl-(1→3?)-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoic acid

Recently a possible glycosyl donor for the synthesis of rhamnose glycosides, deoxyribosylthymine diphosphate rhamnose, has been isolated from several different types of bacteria[3,4]. The enzymic preparation of TDP-rhamnose has been described[5]. We wish to report the enzymic synthesis of the above rhamnolipid by enzymes occurring in *P. aeruginosa* (ATCC 7700) from TDP-rhamnose, β-hydroxydecanoyl-CoA, and either endogenous or exogenous acceptors.

Cell-free extracts of *P. aeruginosa* were prepared as previously described[5]. TDP-L-[^{14}C]rhamnose (23000 counts/min/μmole) was prepared enzymically[5]. Rhamnolipid was isolated and crystallized from cultures of *P. aeruginosa*. Acid hydrolysis of the rhamnolipid permitted the isolation of β-hydroxydecanoic acid (m.p., 44.5–45.0°,

Abbreviation: TDP, deoxyribosylthymine 5′-pyrophosphate.