Short Communications

The Mechanism of Active Intestinal Transport of Sugars

By J. E. G. BARNETT, A. RALPH and K. A. MUNDAY

Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 5NH, U.K.

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Recent studies of structural requirements for active intestinal transport (Barnett, Jarvis & Munday, 1968; Barnett, Ralph & Munday, 1969, and unpublished work) strongly support the proposal (Crane, 1960) that the hydroxyl function at C-2 of the sugar is essential for active intestinal transport in the hamster intestine. In these studies no sugar, including D-mannose, without a hydroxyl group in the *gluco* configuration at C-2 was transported by an Na⁺-dependent carrier. Each other hydroxyl group of D-glucose can be replaced by hydrogen with only diminution rather than loss of active transport, and replacement of the hydroxyl groups at C-3 and C-6 of the sugar by fluorine or at C-3 of the sugar by chlorine gave high rates of Na⁺-dependent transport, since these atoms appear to replace oxygen in hydrogen-bonding of the sugar to the carrier.

By contrast, we now find that all replacements so far tested of the hydroxyl group in the *gluco* configuration at C-2, including replacement by hydrogen, fluorine and chlorine, lead to complete loss of Na⁺-dependent active transport and the ability to competitively inhibit Na⁺-dependent transport. Sugars (initial concentration 1 mM) were incubated at 37°C for 30 min in the presence of 145 mequiv. of Na⁺/l and the final tissue/medium ratios determined as previously described (Barnett *et al.* 1968, 1969). The ratios were: 3-deoxy-3-fluoro-D-glucose, 26.6; 3-chloro-3-deoxy-D-glucose, 24.2; D-galactose, 7.5; D-mannose, 0.21; 2-deoxy-D-glucose, 0.17; 2-chloro-2-deoxy-D-glucose, 0.19. In the absence of Na⁺ the corresponding values were: D-galactose, 0.30; D-mannose, 0.18; 2-deoxy-D-glucose, 0.17; 2-chloro-2-deoxy-D-glucose, 0.18. Difficulties with the assay of 2-deoxy-2-fluoro-D-glucose prevented accurate determination of a tissue/medium ratio, but it was less than 0.5. There was no detectable inhibition of D-galactose accumulation in the presence of 20 mM-2-deoxy-2-fluoro-D-glucose. 2-Deoxy-D-glucose is well recognized as a non-transport sugar (Caspary, Stevenson & Crane, 1969), as is D-mannose, despite a recent report of slight stimulation by Na⁺ of tissue accumulation (Bihler, 1969), which we did not observe in our preparation. These results suggest that the carrier-

sugar bond at C-2 is of a different nature from the hydrogen bonds previously proposed for the interactions at the other sugar positions (Barnett *et al.* 1968, 1969) and that a covalent bond may exist between C-2 of the sugar and the carrier protein, which would also explain the wide variations in the maximum rate of transport, *V*, found in our previous investigations.

Crane (1965) has proposed that the driving force for active intestinal transport is the Na⁺ concentration gradient across the brush-border membrane, maintained by the sodium pump. The sugar binds strongly to the carrier in the presence of Na⁺ but weakly in its absence, and so the concentration of sugar in equilibrium with the carrier is inversely related to the Na⁺ concentration. A low intracellular Na⁺ concentration relative to the extracellular concentration allows a high sugar concentration inside the cell relative to that outside. In a recent modification of this theory Caspary *et al.* (1969) have suggested a multi-stage process that involves conformational change without a covalent bond between sugar and carrier. Since the carrier system is visualized as being capable only of equilibrating with the sugar on each side, any covalent carrier-sugar complex that will fit with the important, well-established, features of this mechanism must be formed and broken without net utilization of energy. A possibility for such a system, which is consistent with the observation that ¹⁸O is retained at C-2 of the sugar during transport (Crane & Krane, 1959), is shown in Scheme 1. The sugar is absorbed on to the carrier in the presence of Na⁺, the hydroxyl group at C-2 then cleaves an intramolecular ester on the membrane to form an ester bond between sugar and membrane, and finally the intramolecular ester is regenerated with release of the sugar to the inside of the cell in a low-Na⁺ environment.

It is difficult to explain the considerable differences in the maximum rates of transport of the sterically similar 3-fluoro-3-deoxy-D-glucose (71 mM/10 min) and 3-deoxy-D-glucose (41 mM/10 min) in a process in which sugars are simply absorbed on to a mobile carrier. The proposed covalent bond is, however, adjacent to C-3 and so the overall rate of

translocation might be expected to be affected by the differences in electronic effects of the hydrogen and fluorine during the formation and breaking of the covalent bond.

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