and K^+ for the K^+ site[4]. NAGAI, IZUMI AND YOSHIDA[15] have suggested that Na^+ may induce an alteration in the affinity of substrate binding to the enzyme.

The physiochemical properties of Tl^+ which may be important in allowing Tl^+ to substitute for K^+ have been discussed by BRITTEN AND BLANK[12]. The cause of the apparently unique ability of Tl^+ to activate the ATPase and phosphatases with an affinity 10 times greater than K^+ remains unknown.

The results suggest that Tl^+ and K^+ act at a common site in the activation of acetylphosphatase or *p*-nitrophenylphosphatase. These studies also provide further evidence for a close association between the properties of the K^+-activation site of the microsomal $(Na^+ + K^+)$-ATPase and K^+-phosphatases.

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Evidence against the involvement of the carbonyl group in the glucose transport mechanism of human erythrocytes

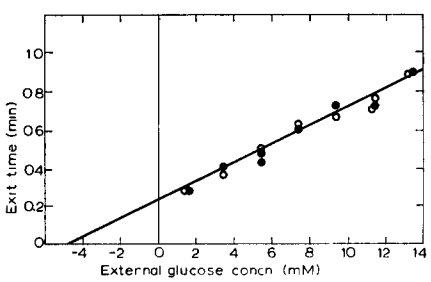
The nature of the interaction between sugars and the carrier which has been postulated to facilitate their transport across the erythrocyte membrane is obscure. LANGDON AND SLOAN[1] studied the incorporation of [^{14}C]glucose which occurred upon borohydride reduction and proposed an imine as an intermediate. ROSE *et al.*[2] have more recently suggested that a carbinolamine may be involved. However, reduction under the conditions described by LANGDON AND SLOAN[1] fails to inhibit glucose

transport[3]. The more extensive glucose incorporation reported here also leaves transport unaltered. LEFEVRE has found that 1,5-anhydro-D-glucitol, a close structural analog of glucose is transported[4] and the inhibition of this transport by glucose reported here may imply that the hydroxyl on C-1 is unessential.

Previously[3] the transport kinetics were investigated in reduced cells in which a moderately large amount of glucose had been incorporated into the membrane. Similar studies in which much more extensive incorporation was obtained are more conclusive. The following reduction procedure was devised. A 25 % suspension of human erythrocytes (5 ml) was incubated for 20 min in 100 mM [^{14}C]glucose (6.8 μC/mmole) at 37°. Aliquots (0.01 ml) of 0.22 M $NaBH_4$ (in 0.02 M NaOH) were added at 2-min intervals over a period of 90 min. The pH was maintained at 7.4 by the addition of 2 M acetic acid. During the reduction the extent of hemolysis was about 10 and 44 % of the glucose (as determined by a glucose oxidase method) was reduced. At the end of the reduction, the cells from a 3.0-ml aliquot were centrifuged, washed twice and quantitatively transferred to 10 ml of 80 mM glucose and incubated at 37° for 1 h. A 0.25-ml portion of the packed erythrocytes was suspended in 0.75 ml of 80 mM glucose in isotonic buffer. The exit of glucose from these preloaded cells was studied at 37° by the method of SEN AND WIDDAS[5]. This indicated (Fig. 1) that the transport of glucose in the reduced cells was unaffected (K_m = 5 mM and t_0 = 0.2 sec). The remainder of the reduced suspension was centrifuged, the cells were washed and the protein was extracted and counted. Glucose incorporation was 21 nmoles/mg stromal protein. This is 4 times the maximum amount of incorporation obtained by LANGDON AND SLOAN[1]. The essential point of these experiments is that even when relatively massive amounts of glucose are incorporated into stromal protein, there is no inhibition of transport as would be expected if a molecule of glucose had been irreversibly bound to the transport site. These studies are inconsistent with the model proposed by LANGDON AND SLOAN[1] and the interpretation suggested by ROSE *et al.*[2]. LANGDON AND SLOAN'S[1] evidence rests primarily on an interaction of the kinetics of glucose incorporation during borohydride reduction. LEFEVRE[4] has questioned this approach. These kinetic studies can also be criticized on an entirely different basis. The expression derived by LANGDON AND SLOAN[1] demands that the concentrations of borohydride and glucose remain constant during the course of reduction. It was noticed, however, that attempts to increase the extent of incorporation by increasing the reduction time gave only about one third as much labeling as these equations predicted. This suggested that the glucose and borohydride concentrations might not remain constant as these authors reported. In the buffer used borohydride was found to be fairly stable but it disappeared rapidly from the erythrocyte suspension. This is due to the low capacity of the buffer which is rapidly exhausted by borohydride hydrolysis, the pH rising to 9.5 where borohydride is fairly stable. When erythrocytes are present the pH rose to 8.1 where borohydride is much more rapidly hydrolyzed. These studies using the iodometric method of LYTTLE *et al.*[6] gave no information about the fate of the borohydride. For this vacuum distilled [^{3}H]borohydride was used. The borohydride remaining in a mixture of erythrocytes and glucose is reacted with an excess of formaldehyde in alkali to form methanol. After acidification and precipitation of the protein with trichloroacetic acid, the counts in solution are from methanol and from tritium incorporation into soluble materials. The counts in the latter are determined by hydrolyzing the borohydride in an aliquot of the original reaction

mixture before precipitating the protein. Under the conditions used the concentration of borohydride drops to one half in 45 sec and is essentially zero after 3.5 min. There was extensive incorporation of tritium into the soluble fraction which is probably attributable to the reduction of glucose. These results are consistent, although not directly comparable with those of PECSOK[7] and DAVIS AND SWAIN[8], who studied borohydride hydrolysis in buffered aqueous solutions. The expression derived by LANGDON AND SLOAN[1], particularly at longer reduction times, is strongly influenced by borohydride concentration. The serious instability of the reducing agents therefore suggests that the kinetic arguments advanced by these authors may not be justified.

LEFEVRE[4] has reported that 1,5-anhydro-D-glucitol, in which hydrogen is substituted for the hydroxyl group on C-1 of glucose and which thus lacks the potential aldehyde functional group, is transported across the erythrocyte membrane. If it can be demonstrated that glucose and its deoxy analog are transported by the same system, it would be reasonable to conclude that this aldehyde is not essential for transport. A series of tubes containing 0.5 ml of packed human erythrocytes preloaded to 25 mM with 1,5-anhydro-D-glucitol were equilibrated at 25° and 1 ml of isotonic buffer was added at zero time. The efflux of sugar was stopped at various time intervals by the addition of 10 ml of an ice-cold solution of 2 mM $HgCl_2$, 1.25 mM KI and 2 % NaCl (ref. 9). The cells were isolated, lysed and the protein was precipitated. An aliquot of the supernatant was analyzed by periodate oxidation using a modification (oxidation was carried out at 90–100° for 30 min and standards were run simultaneously) of the method of SALO[10]. In a similar experiment the exit of the anhydro sugar from cells preloaded to 25 mM 1,5-anhydro-D-glucitol and 75 mM glucose (in isotonic buffer) was investigated. The rate of exit of the anhydro sugar is much slower in the presence of glucose (Fig. 2). While there is no net flux of glucose across the membrane, the glucose carrier sites are saturated and there is a rapid exchange between the internal and external compartment. It is possible that the anhydro sugar is transported by an independent system which nevertheless has a high affinity for glucose. It is more likely that the decrease in the rate of exit of 1,5-anhydro-D-glucitol in the presence of glucose is the result of competition between these two sugars for a limited number of sites on the internal surface of the membrane and that they share the normal glucose transport system. Subject to the above reservation, these



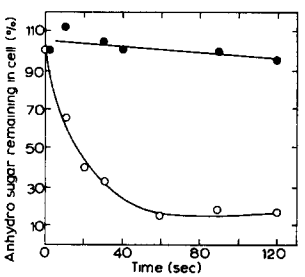


Fig. 1. Transport kinetics of cells reduced with multiple additions of borohydride. Control cells to which borohydride was not added (○—○) and cells reduced in the presence of 100 mM glucose by the addition of small aliquots of borohydride over 90 min (●—●) transport glucose equally well.

Fig. 2. Transport of 1,5-anhydro-D-glucitol from cells in the presence (●—●) and absence (○—○) of 75 mM glucose are compared. Glucose strongly inhibits the exit of 1,5-anhydro-D-glucitol.

studies argue against the involvement of glycosidic, hemiacetal and acetal linkages and their thiol analogs as intermediates in the transport of monosaccharides.

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2-Deoxyglucose transfer in rabbit intestine

KLEINZELLER *et al.*[1] have recently reported that the sugars, 2-deoxyglucose and 2-deoxygalactose, are accumulated by rabbit kidney slices to concentrations in excess of those in the bathing medium. The accumulation of these sugars is not dependent on the presence of Na^+ in the medium and is unaffected by ouabain. However, the ability of the slices to accumulate other sugars such as glucose, galactose and 3-*O*-methylglucose was found to be entirely Na^+ dependent. These observations clearly suggest that sugar transport in kidney is not necessarily a function of the Na^+ concentration in the medium and raises questions concerning the concept of a sugar transport system driven by differences in Na^+ concentration between extra- and intracellular fluid. Since the existence of a Na^+-independent sugar transport system in the intestine would have important implications regarding the "Na^+ gradient hypothesis"[2], we felt it worthwhile to examine the transport of 2-deoxyglucose in rabbit intestine. It has been reported[3] that neither 2-deoxyglucose nor 2-deoxygalactose is actively transported by hamster intestine, but there is the possibility of a species difference.

Transmural fluxes of 2-deoxyglucose were determined with the apparatus described by SCHULTZ AND ZALUSKY[4]. Distal ileum from New Zealand White Rabbits (sacrificed by intravenous injection of pentobarbital) was mounted as a flat sheet between two chambers with identical bathing solutions containing 5 mM 2-deoxy-