STUDIES ON THE MECHANISM OF THE INTESTINAL ABSORPTION OF SUGARS

III. MUTUAL INHIBITION, *IN VITRO*, BETWEEN SOME ACTIVELY TRANSPORTED SUGARS*

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SUMMARY

Mutual inhibition of intestinal active transport *in vitro* between glucose and galactose, glucose and 1,5-anhydro-D-glucitol, galactose and 1,5-anhydro-D-glucitol, glucose and 6-deoxyglucose and 6-deoxyglucose and 1,5-anhydro-D-glucitol has been observed. The data provide evidence that is consistent with the assumption that intestinal active transport of sugars is mediated by a single common process.

INTRODUCTION

Recent studies on the specificity of intestinal absorption of sugars[1-4] have brought to fourteen the total number of sugars and related compounds known to be transported against an apparent concentration gradient by preparations of hamster intestine, *in vitro*. A comparison of the structures of these compounds** shows that they possess in common a pyran ring, a methyl or substituted methyl group at carbon-5 of the ring and a hydroxyl group in the glucose configuration at carbon-2. Thus, it has been concluded[2] that these three features are the minimal structural requirements for intestinal active transport. Compounds such as mannose, fructose and the pentoses which are not transported against a concentration gradient lack one or more of these structural features.

The question of next importance is whether all fourteen of the actively transported compounds are transported by the same process or whether there are several processes each of which transports only one or a few of these compounds and which provide,

* Although numbers were not assigned to them when they were published, references 1 and 3 describe experiments on the mechanism of the intestinal absorption of sugars and should be considered to be papers number one and two, respectively, of this series. A preliminary report of portions of this work was made at the Forty-third Annual Meeting of the American Society of Biological Chemists held at Atlantic City, New Jersey, April 13 to 17, 1959.

** These compounds are D-glucose, 1,5-anhydro-D-glucitol, 2-C-hydroxymethyl-D-glucose, D-glucoheptulose, 3-O-methyl-D-glucose, 3-deoxy-D-glucose, D-galactose, 4-O-methyl-D-galactose, D-allose, 6-deoxy-D-glucose, 6-deoxy-D-galactose, 6-deoxy-6-fluoro-D-glucose, 7-deoxy-D-glucoheptose and α-methyl-D-glucoside.

collectively, the observed overall specificity. An answer to this question would materially aid studies of the mechanism of intestinal absorption. If there is only one process, a number of possible endergonic reactions of sugars may be excluded from participation in the process on the basis of which functional groups of the sugar molecule are not essential for transport[1,3]. The present report provides evidence, in terms of mutual inhibition between some of the actively transported compounds, that is consistent with a conclusion that all of them are transported by a single process.

MATERIALS AND METHODS

The present experiments were carried out by the intact strip technique using the materials and methods described in the preceding paper[5]. The volume of medium, the sugar concentrations and the duration of incubation were varied as noted below. The incubation temperature was 37°.

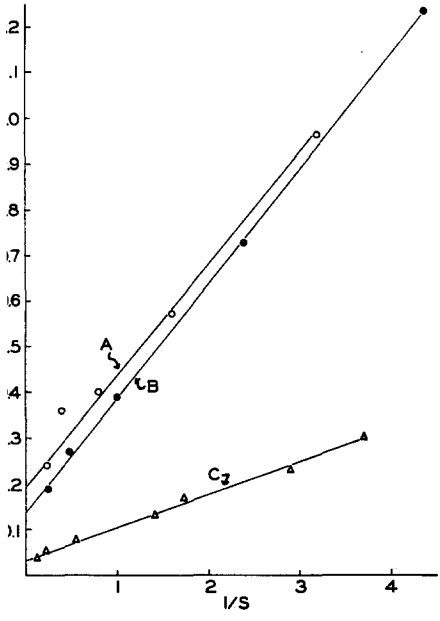
EXPERIMENTAL

Determination of apparent K_m *values*

Transport of sugar against an apparent concentration gradient by *in vitro* preparations of small intestine exhibits MICHAELIS-MENTON[6] kinetics; that is, the rate of transport increases with sugar concentration in the manner predicted by this theory. In previous studies, apparent K_m values were determined for glucose with rat[7] and guinea pig[8] intestine, for galactose with rat[9] intestine and for 1,5-anhydro-D-glucitol and 6-deoxy-D-glucose with hamster[10] intestine. In the present studies, the K_m values have been redetermined not only because of possible species variation but also because of differences in the way the transport rate is measured. In the previous studies, transport was measured by the movement of sugar through the entire intestinal wall from the mucosal to the serosal surface. In the present studies, transport is measured by the accumulation of sugar within strips of tissue. Thus, the barrier to diffusion presented by the tissues underlying the epithelial cells has been eliminated as a factor in the measured rate and in the apparent K_m (see ref. 5). The K_m values found with the present method are significantly different from those found with other *in vitro* methods.

Apparent K_m values for glucose, galactose and 1,5 anhydro-D-glucitol were determined. The data from which these were calculated are presented in a linear form[11] of the MICHAELIS-MENTEN equation[6] in Figs. 1 and 2. Two experiments were performed with glucose (curves A and B, Fig. 1) each of them designed to minimize the influence of utilization on medium and tissue sugar concentrations, but in different ways. In Expt. A, the volume of the incubation medium was adjusted to provide approximately the same total amount of glucose at each concentration level. The volumes used ranged from 640 ml at the lowest concentration to 20 ml at the highest. Since the amount of tissue in each vessel was about the same, the proportions of total glucose utilized were nearly equal. In Expt. B, advantage was taken of the fact that in a medium free of calcium and magnesium ions, the addition of 0.02 *M* NaF resulted in 85 % inhibition of glucose utilization with only 15 % inhibition of the rate of glucose accumulation. The K_m values obtained in these two experiments did not differ greatly (see legend to Fig. 1) and the average of them, $K_m = 1.5$ m*M*, was taken as the apparent K_m for glucose with intact strips.

The experiment with D-galactose, curve C in Fig. 1, was set up like Expt. A, above. The volumes of medium ranged from 50 ml at the lowest concentration of galactose to 10 ml at the highest. The experiment with 1,5-anhydro-D-glucitol (Fig. 2), on the other hand, did not require precautions of this sort inasmuch as utilization of



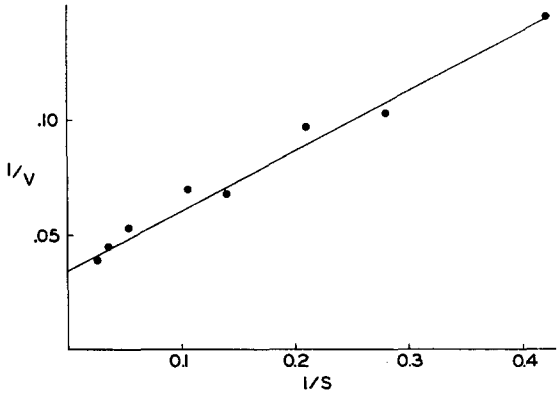


Fig. 2. Relationship of the rate of active transport of 1,5-anhydro-D-glucitol by intact strips to the external concentration. S = the initial medium concentration, V = the final tissue concentration. Incubation was for 10 min. The extrapolated value for K_m = 7.4 mM.

Fig. 1. Relationship of the rate of sugar active transport by intact strips to the external concentration of sugar. Expt. A: Glucose. Incubation was for 8 min. The extrapolated value for K_m = 1.25 mM. Expt. B: Glucose. Calcium and magnesium ions were omitted from the buffer. NaF, 0.02 M was added. Incubation was for 10 min. The extrapolated value for K_m = 1.8 mM. Expt. C: Galactose. Incubation was for 10 min. The extrapolated value for K_m = 2.2 mM. For further experimental details, see the text.

1,5-anhydro-D-glucitol was barely measureable. Utilization of this compound does not proceed beyond phosphorylation by hexokinase and the ester which accumulates inhibits the hexokinase reaction[12]. This ester was not included in the analyses of tissue concentrations inasmuch as it was removed in the deproteinization procedure employing barium hydroxide and zinc sulfate[5]. The K_m value for galactose was 2.2 mM and for 1,5-anhydro-D-glucitol, 7.4 mM.

In these experiments the velocity, v, was taken as equal to the final tissue concentration. Thus, the values do not represent initial rates, as would be desirable. It has been assumed that the same proportionality exists between these rates as would have been found between initial rates.

Mutual inhibition between actively transported sugars

Mutual inhibition of transport was studied with the following pairs of sugars: glucose and galactose, glucose and 1,5-anhydro-D-glucitol, galactose and 1,5-anhydro-D-glucitol, glucose and 6-dexoyglucose and 6-deoxyglucose and 1,5-anhydro-D-glucitol. Experiments were designed in the following way: Each sugar was used at a single concentration which was chosen on the basis of the relative K_m values. Intact

strips from the small intestine of 3 or 4 fasted animals were divided among nine flasks. One sugar of a pair was added to three of the flasks, the other sugar to another three, and both sugars to the remaining three. Periods of incubation were selected, on the basis of preliminary experiments, with the view to obtaining demonstrable tissue accumulation of both sugars without having the tissue concentration approach too closely the apparent maximal or steady state value[5]. After incubation, the tissue and medium in all nine flasks were analyzed by methods, described in the preceding paper[5], which were specific for each of the sugars used.

The results of these experiments are given in Fig. 3 and 4 and Table I. In Fig. 3 is shown the full time course of an experiment with glucose and galactose and in Fig. 4 of one with galactose and 1,5-anydro-D-glucitol. These curves demonstrate that inhibition occurs at the earliest time periods and continues throughout the experiment.

Fig. 3. Mutual inhibition of active transport between glucose and galactose. The initial concentrations were 1.1 mM for glucose and 5.5 mM for galactose.

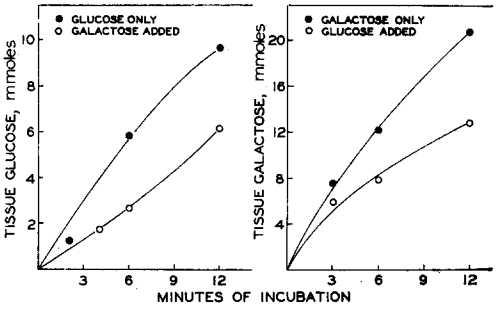
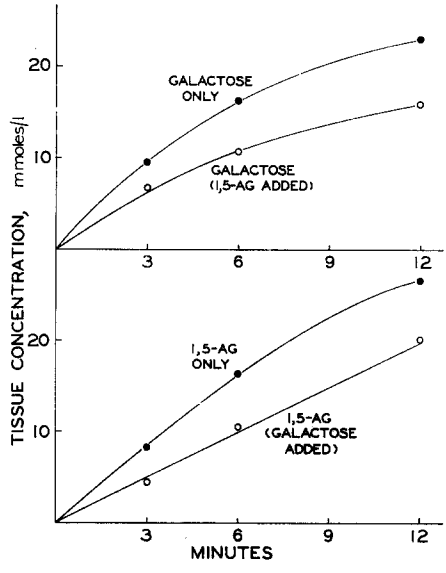


Fig. 4. Mutual inhibition of active transport between galactose and 1,5-anhydro-D-glucitol. The initial external concentrations were 3.8 mM for galactose and 34 mM for 1,5-anhydro-D-glutitol.



In many experiments, however, the degree of inhibition changed throughout the time course. For example, the inhibition of glucose by another sugar was always found to be less at the later time periods. One reason for this seems to be the fact that nearly the same maximal level of glucose was attained in the presence of the second sugar as in its absence. However, there also appears to have been an influence of accumulated tissue glucose on the accumulation of the second sugar so that the degree to which the second sugar was inhibited increased with time, as shown for galactose in Fig. 3. An explanation for this phenomenon is not available in the absence of a knowledge of the mechanism of active transport.

The data in Table I show that mutual inhibition occurred with all pairs of sugars tested. Because of the changing degree of inhibition with time, the earliest time period was chosen in each instance for the purpose of calculation. Where K_m values determined by the intact strip technique were available, the theoretical degree of inhibition expected under the conditions of the experiment was also calculated. For the pairs, glucose and galactose and glucose and 1,5-anhydro-D-glucitol the inhibition found

TABLE I

MUTUTAL INHIBITION BETWEEN VARIOUS PAIRS OF SUGARS

The initial external concentrations are given in parentheses below the name of the sugar. All data are taken from experiments designed as described in the text. Curves for the full time period (8 to 12 min) were obtained and were similar to the curves shown in Figs. 3 and 4. The methods of assay are described in the preceding paper[5].

Test compound	*Compound added*	*Time period (min)*	*Tissue concentration (mM)*	*Per cent inhibition*	
				Found	*Calculated*
lucose (1.1 m*M*)	None	3	3.0		
	Plus galactose	3	1.25	58.5	59.0
[-^{14}C]galactose (5.5 m*M*)	None	3	7.5		
	Plus glucose	3	5.9	21.4	15.8
lucose (1.0 m*M*)	None	2	2.76		
	Plus 1,5-anhydro-D-glucitol	2	1.67	44	63.5
,5-anhydro-D-[^{3}H]glucitol (25 m*M*)	None	2	2.4		
	Plus glucose	2	1.0	58	61.5
alactose (3.8 m*M*)	None	3	9.5		
	Plus 1,5-anhydro-D-glucitol	3	6.4	32.6	63
,5-anhydro-D-[^{3}H]glucitol (34 m*M*)	None	3	8.2		
	Plus galactose	3	5.1	37.8	19.5
lucose (0.86 m*M*)	None	6	4.2		
	Plus 6-deoxyglucose	6	3.1	26	—
-[^{3}H]deoxyglucose (0.86 m*M*)	None	6	2.0		
	Plus glucose	6	1.7	15	—
,5-anhydro-D-[^{3}H]glucitol (4.8 m*M*)	None	3	2.7		
	Plus 6-deoxyglucose	3	1.5	44.5	—
-deoxyglucose (0.48 m*M*)	None	3	1.8		
	Plus 1,5-anhydro-D-glucitol	3	1.3	27.8	—

compared closely with that calculated from the K_m values. With the pair, galactose and 1,5-anhydro-D-glucitol, a significant disparity was found. However, it may be calculated that this disparity is completely accounted for if there is an error in the relative K_m values of galactose and 1,5-anhydro-D-glucitol of about 2.

Inhibition of active transport by other sugars

Active sugar transport was not measureably influenced by the presence of compounds which are not actively transported when the relative concentration of the

latter was small. However, when high relative concentrations of the latter were used, some of them caused a significant degree of inhibition. For example, D-xylose at 47 mM inhibited the active transport of glucose, 3 mM, by 15.6 %. 1,5-anhydro-D-mannitol, on the other hand, at 47 mM had no effect. Further experiments were made with 1,5-anhydro-D-glucitol at 5 mM as the test sugar with the addition of non-actively transported sugars at a uniform concentration of 24 mM. The inhibitions observed were D-xylose, 23.4 %, D-ribose, 8.6 %, 2-deoxy-D-galactose, 1.0 % and 6-deoxy-L-galactose, 42.4 %. Collectively, these data indicate that some portion of the process of active transport is susceptible to inhibition by sugars which are not actively transported. There also seems to be some degree of specificity to the inhibition. Further studies along these lines are in progress in an effort to determine fully the specificity of inhibition by non-actively transported compounds. A tentative suggestion that may be made for this inhibition is that these compounds which lack one or more of the structural features necessary for active transport[2] combine with the same site or structure as the actively transported compounds but they cannot undergo the subsequent steps in the process which lead to accumulation against a concentration gradient.

DISCUSSION

Until the full details of the mechanism of the process of active transport of sugars by the small intestine are known, the precise value of studies of mutual inhibition cannot be decided. Were the system under study a simple one, such as purified single enzyme, however, it could be considered proved from the data presented above that only one process is involved in the active transport of all sugars. Although all known actively transported sugars have not been studied, those above, together with 3-O-methyl-D-glucose, reported by CSAKY[13] to inhibit competitively the active transport of glucose, represent a modification at each carbon atom of glucose except, of course, for carbon-2 which is specifically required for active transport[1,2].

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REFERENCES

1 R. K. CRANE AND S. M. KRANE, *Biochim. Biophys. Acta*, 20 (1956) 568.
2 T. H. WILSON AND R. K. CRANE, *Biochim. Biophys. Acta*, 29 (1958) 30.
3 R. K. CRANE AND S. M. KRANE, *Biochim. Biophys. Acta*, 31 (1959) 397.
4 T. H. WILSON AND B. R. LANDAU, *Am. J. Physiol.*, 198 (1960) 99.
5 R. K. CRANE AND P. MANDELSTAM, *Biochim. Biophys. Acta*, 45 (1960) 460.
6 L. MICHAELIS AND M. L. MENTEN, *Biochem. Z.*, 49 (1913) 333.
7 R. B. FISHER AND D. S. PARSONS, *J. Physiol. (London)*, 119 (1953) 210.
8 E. RIKLIS AND J. H. QUASTEL, *Can. J. Biochem. and Physiol.*, 36 (1958) 347.
9 R. B. FISHER AND D. S. PARSONS, *J. Physiol. (London)*, 119 (1953) 224.
10 R. K. CRANE AND T. H. WILSON, *J. Appl. Physiol.*, 12 (1958) 145.
11 H. LINEWEAVER AND D. BURK, *J. Am. Chem. Soc.*, 56 (1934) 658.
12 R. K. CRANE AND A. SOLS, *J. Biol. Chem.*, 210 (1954) 597.
13 T. Z. CSAKY, *Supplement to International Abstracts of Biological Sciences*, 6–79 (1958).