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EFFECT OF AMINO ACIDS ON SUGAR ABSORPTION

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SUMMARY

1. Sacs of everted mid-small intestine of the hamster have been used to study the effect of amino acids on sugar absorption.

2. The sugars employed were D-glucose, D-galactose, 3-O-methyl-D-glucose, D-fucose, L-glucose, α-glucoheptose, L-fucose, D-mannose and L-sorbose. The amino acids were L- and D-histidine, L- and D-methionine, L- and D-alanine, L- and D-valine, L- and D-glutamic acid, L-leucine, L-proline, L-ornithine and L-aspartic acid.

3. Actively absorbed amino acids considerably inhibit the transport of actively absorbed sugars. The results give support for the view that D-histidine and L-glucose are actively transferred. Passively absorbed amino acids and sugars are not involved.

4. As L-glutamic and L-aspartic acids in the mucosal fluid have no inhibitory effect on D-glucose absorption, although mucosal fluid L-alanine is quite potent, the step at which the latter exerts its inhibitory action must be before that at which the intracellular transamination of L-glutamic and L-aspartic acids occurs. It would seem likely, therefore, that L-alanine interferes with the process by which epithelial cells capture and concentrate sugars at the luminal border.

5. More than one active transfer system may exist for D-glucose.

6. The influence of actively absorbed L-amino acids on D-glucose active transport seems to be in some way related to the efficiency with which the amino acids are themselves concentrated.

7. Inhibition of D-glucose active absorption by an amino acid may be a simple test of an amino acid's participation in an active transport system.

INTRODUCTION

Although much effort has been expended in recent years on the study of active absorption of sugars, little consideration has been given to the influence that amino acids might have on the sugar transport process. This is despite the fact that both amino acids and sugars have often been

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present, sometimes at high concentrations, in the nutrient media. The series of experiments here described followed our initial observation that L-histidine had an appreciable inhibitory effect on the active transport of D-glucose by the small intestine of the hamster. Accordingly, a number of L- and D-amino acids have been examined for their action on the absorption of actively and passively transported sugars. Preliminary reports of this work have been given by Hindmarsh, Kilby & Wiseman (1966*a*, *b*).

METHODS

Preparation of sacs. The method used for preparing and filling sacs of everted small intestine of the hamster (*Mesocricetus auratus*) was that described by Wilson & Wiseman (1954) (and in greater detail by Wiseman, 1961). The animals (young adult males), 80–100 g body weight, were killed by a blow on the head. They had been allowed food and water *ad libitum*. To overcome the problem of variation in absorptive activity of different regions, only the middle third of the small intestine was employed and of this two sacs were made, each 3–4 cm in length. At least six animals were utilized for investigation of a sugar or sugar-amino acid combination.

Measurement of initial and final volumes. The initial volume of fluid (serosal) introduced into the carefully drained sac of everted intestine was recorded from the 1 ml. tuberculin syringe which was used for the introduction of the fluid. This serosal fluid volume was initially about 0·40 ml. The final volume of the serosal fluid was estimated by draining the sac of its contents and weighing the fluid obtained. This technique enables about 96 % of the serosal fluid to be recovered (Wiseman, 1955). The volume of fluid (mucosal) into which the sac was placed at the beginning of the experimental period was 20 ml.

Occasionally the serosal volume of a sac decreased during the experimental period: such sacs were discarded.

Experimental procedure. The sac, filled with a known volume of the appropriate solution, was put into a 150 ml. Erlenmeyer flask containing 20 ml. of outer (mucosal) fluid. The air in the flask was then replaced by a gas mixture of 5 % CO_2, 95 % O_2 and the flask tightly stoppered. The flask and its contents were then kept at 37° C and continuously shaken for 1 hr in a Warburg bath (rate of shaking 80 oscillations/min, amplitude 5 cm). At the end of the hour the sac was removed, its surface drained, and its fluid contents collected and weighed. Samples of initial and final mucosal and serosal fluids were analysed for reducing sugar concentrations. A short length of thread ligature left at one end of the sac facilitates the removal of the sac from the flask.

When an *actively transported* sugar was being studied it was present initially in both the *mucosal and serosal* fluids at equal concentration. For a *passively transported* sugar, *only the mucosal* fluid contained the sugar at the start of the experiment. When the effect of an amino acid (L- or D-form) was investigated it was added in equal concentration to both the mucosal and serosal fluids.

Sugar and amino acid solutions. The sugars and amino acids were commercial samples of chemically pure grade and were employed without further purification. The glutamic and aspartic acids were used as the mono-sodium salts and the histidine and ornithine as the mono-hydrochlorides. The test substances were dissolved in bicarbonate-saline (Krebs & Henseleit, 1932) and all solutions were gassed with 5 % CO_2, 95 % O_2 for at least 30 min.

The concentrations of the sugars and the amino acids in the various experiments are given in the tables.

Dry weight. After removal of the serosal fluid the sacs were laid on Whatman No. 50 filter paper, and the tissue beyond the ligatures, together with the ligature thread, cut off

and discarded. Excess surface fluid was then blotted and the empty sacs dried for 2 hr at 120° C and the weights determined. The dry weights were of the order of 20–35 mg.

Chemical estimations. The sugars were estimated by the colorimetric method of Nelson (1944). It was found that the amino acids studied did not interfere with this reaction.

Concentration ratios. The *final concentration ratio* was the ratio of the sugar concentration in the *serosal* (inner) fluid to that in the *mucosal* (outer) fluid at the end of the 1 hr experimental period. The *initial concentration ratio* for actively transported sugars was 1:1; for passively transported sugars it was 0:1.

Rates of transport of sugar and water. The amount of sugar absorbed into the serosal fluid during an experiment was calculated (the initial and final sugar concentrations and serosal fluid volumes being known) and its transport rate expressed as μmole sugar entering serosal fluid/100 mg dry wt. of sac/hr.

The rate of absorption of water into the serosal fluid during an experiment is given in m-mole water/100 mg dry wt. of sac/hr.

The standard errors of the means were obtained using the formula for small samples.

RESULTS

Effect of L-*amino acids on* D-*glucose active absorption*

The influence of different concentrations of L-histidine and L-methionine on the active absorption of D-glucose is shown in Tables 1 and 2. Both amino acids inhibited the transport of the sugar against its gradient, and the lowered final (1 hr) D-glucose concentration ratios (serosal to mucosal) were accompanied by slower rates of entry of D-glucose into the serosal fluid. The movement of water was unaffected by weak solutions of the amino acids and depressed by stronger ones, so that the subnormal ratios could not be attributed to enhanced gain of water by the serosal fluid. In the case of L-histidine, the maximum effect was achieved, under the experimental conditions, by a concentration falling between 5 and 10 mM, beyond which extra L-histidine had no further action. For L-methionine, the D-glucose concentration ratio was maximally reduced when the amino acid was present at the 5 mM level and increasing this to 20 mM gave no additional response.

In view of the results obtained with L-histidine and L-methionine, a concentration of 20 mM was chosen for the other L-amino acids employed. As can be seen from Table 3, only those L-amino acids which can be actively absorbed (by one mechanism or another) caused a fall in the D-glucose final concentration ratio and in the rate of entry of the sugar into the serosal fluid. On the other hand, both L-glutamic and L-aspartic acids, for which extensive research has failed to indicate the presence of an active transfer system, were without effect on the uptake of D-glucose. The presence of these L-amino acids had no influence on water absorption, except for L-histidine, L-methionine and L-ornithine which reduced it. The depressed final concentration ratios, therefore, were not directly related to water movement.

When the L-amino acids were listed (Table 3) in order of their effectiveness as inhibitors of D-glucose active absorption, it was observed that, in general, those with a high K_t had a greater action on D-glucose active uptake than had those with a low K_t. The term K_t refers to the initial concentration of an amino acid at which its active transport proceeds at half-maximum velocity and is analogous to K_m of enzyme kinetics. The lower the value of K_t, the greater is the affinity of the amino acid for the

TABLE 1. Effect of L-histidine on D-glucose active absorption by sacs of everted mid-small intestine of the hamster. Initial mucosal and serosal fluid contained 16·7 mM D-glucose; initial mucosal volume 20 ml.; initial serosal volume about 0·4 ml.; length of sacs 3–4 cm. Experimental period 1 hr. Temp. 37° C. Values are means ± S.E. of the means, with number of sacs in parentheses

L-histidine initial concn. in mucosal and serosal fluid (mM)	D-glucose final concn. ratio (serosal/mucosal)	Transport of glucose into serosal fluid (μmole/100 mg dry wt. sac/hr)	Gain in serosal fluid water (m-mole/100 mg dry wt. sac/hr)
0	3·23 ± 0·12 (19)	95·0 ± 5·0	61·9 ± 4·2
2	2·67 ± 0·15 (18)	72·1 ± 5·5	66·2 ± 4·6
5	2·35 ± 0·10 (18)	48·8 ± 3·2	37·3 ± 3·7
10	2·07 ± 0·05 (18)	44·5 ± 2·5	37·4 ± 3·0
20	2·04 ± 0·06 (17)	44·0 ± 3·0	44·9 ± 4·3

TABLE 2. Effect of L-methionine on D-glucose active absorption by sacs of everted mid-small intestine of the hamster. Other details as in Table 1

L-methionine initial concn. in mucosal and serosal fluid (mM)	D-glucose final concn. ratio (serosal/mucosal)	Transport of glucose into serosal fluid (μmole/100 mg dry wt. sac/hr)	Gain in serosal fluid water (m-mole/100 mg dry wt. sac/hr)
0	3·23 ± 0·12 (19)	95·0 ± 5·0	61·9 ± 4·2
5	2·65 ± 0·12 (11)	66·6 ± 3·9	52·5 ± 3·8
10	2·68 ± 0·07 (18)	67·9 ± 3·0	47·4 ± 2·8
20	2·54 ± 0·07 (12)	66·8 ± 4·5	44·4 ± 3·5

absorbing mechanism, which means that it will, at any particular concentration, occupy more active sites than will another amino acid which has a higher K_t. Because various approximations and assumptions have to be made when measuring the K_t of an amino acid, the term 'apparent K_t' has been used in Table 3.

Effect of histidine and methionine on actively absorbed sugars

As well as decreasing the D-glucose transporting activity of the small intestine, histidine and methionine diminished the final concentration ratios and the rates of uptake of D-galactose, 3-O-methyl-D-glucose and D-fucose (6-deoxy-D-galactose) (Table 4). Once again, the observed reductions could not be explained on a basis of change in water absorption,

which remained unaltered by the presence of the amino acids. The rate of water entry into the serosal fluid in these experiments was 20–30 m-mole/100 mg dry wt. sac/hr, in contrast to 62 m-mole/100 mg dry wt. sac/hr when D-glucose was being absorbed. The active movement of these sugars was inhibited by the D-forms as well as by the L-forms of the amino acids, the unnatural isomers being, under the conditions of the experiments, about as effective as their respective L-forms.

TABLE 3. Relation between the inhibitory action of 20 mM L-amino acids on D-glucose active absorption and their apparent K_t values. Other details as in Table 1

Amino acid in mucosal and serosal fluid	Apparent K_t of amino acid (mM)	D-glucose final concn. ratio (serosal/mucosal)	Transport of glucose into serosal fluid (μmole/100 mg dry wt. sac/hr)
None	—	3·23 ± 0·12 (19)	95·0 ± 5·0
	Passively absorbed		
L-glutamic acid	—	3·24 ± 0·06 (12)	91·2 ± 2·6
L-aspartic acid	—	2·90 ± 0·05 (12)	88·0 ± 3·7
	Actively absorbed		
L-methionine	0·9*; 5·3†	2·54 ± 0·07 (12)	66·8 ± 4·5
L-ornithine	0·7†	2·49 ± 0·07 (12)	54·7 ± 1·9
L-leucine	0·7*; 1·6‡; 2·2†	2·41 ± 0·07 (12)	60·2 ± 3·9
L-valine	2·0‡; 2·1*; 3·3†	2·27 ± 0·09 (12)	64·2 ± 5·4
L-proline	10*	2·12 ± 0·06 (18)	55·1 ± 2·1
L-histidine	6·0†; 10·0*	2·04 ± 0·06 (17)	44·0 ± 3·0
L-alanine	5·0*; 6·3†; 7·5‡	2·03 ± 0·03 (12)	44·6 ± 2·6

* Finch & Hird (1960); † Larsen, Ross & Tapley (1964); ‡ Matthews & Laster (1965).

Effect of L-*histidine on passively absorbed sugars*

The response of the small intestine to 10 mM L-histidine during the absorption (*down* the gradient) of passively transported sugars is detailed in Table 5. Of these five materials, only L-glucose had its rate of accumulation in the serosal fluid and its final concentration ratio lowered. For the others, there was no alteration in their uptake. Water movement was unchanged when the amino acid was used with any of these sugars, and was about 30 m-mole/100 mg dry wt. sac/hr. The final concentration ratios for α-glucoheptose, L-fucose (6-deoxy-L-galactose), D-mannose and L-sorbose were remarkably similar (0·37–0·40) and considerably below that for L-glucose (0·93).

Effect of D-*amino acids on* D-*glucose active absorption*

Of the D-amino acids tested, whereas 20 mM D-valine, 20 mM D-glutamic acid and probably 20 mM D-alanine had no influence on the active transport of D-glucose, the action of 10 mM D-methionine and 10 mM D-histidine was to decrease substantially the sugar's movement against its gradient (Table

TABLE 4. Effect of histidine and methionine on the absorption of actively transported sugars by sacs of everted mid-small intestine of the hamster. Values for D-glucose are given in Tables 1–3. Initial mucosal and serosal fluid contained either 16·7 mM D-galactose, or 16·7 mM 3-O-methyl-D-glucose, or 18·3 mM D-fucose. Other details as in Table 1

Amino acid in mucosal and serosal fluid	Final concn. ratio (serosal/mucosal)			Transport of sugar into serosal fluid (μmole/100 mg dry wt. sac/hr)		
	D-galactose	3-O-methyl-D-glucose	D-fucose	D-galactose	3-O-methyl-D-glucose	D-fucose
None	2·96 ± 0·07 (16)	2·48 ± 0·11 (12)	1·40 ± 0·02 (18)	66·9 ± 5·7 (16)	47·7 ± 3·4 (12)	24·5 ± 2·0 (18)
L-histidine (10 mM)	2·27 ± 0·12 (12)	1·95 ± 0·05 (11)	1·23 ± 0·02 (18)	45·1 ± 4·7 (12)	32·9 ± 1·8 (11)	14·6 ± 0·9 (18)
L-histidine (20 mM)	1·88 ± 0·04 (12)	—	1·20 ± 0·02 (12)	27·6 ± 1·8 (12)	—	10·8 ± 1·3 (12)
D-histidine (10 mM)	2·27 ± 0·09 (12)	2·09 ± 0·10 (12)	1·27 ± 0·03 (12)	47·2 ± 3·1 (12)	45·1 ± 2·7 (12)	15·7 ± 1·1 (12)
L-methionine (10 mM)	2·65 ± 0·10 (24)	—	—	54·0 ± 3·2 (24)	—	—
L-methionine (20 mM)	2·19 ± 0·07 (12)	—	1·22 ± 0·03 (12)	42·1 ± 2·8 (12)	—	15·3 ± 1·8 (12)
D-methionine (10 mM)	2·44 ± 0·18 (10)	—	1·27 ± 0·03 (11)	39·6 ± 7·9 (10)	—	16·5 ± 1·5 (11)

TABLE 5. Effect of 10 mM L-histidine on the absorption of passively transported sugars by sacs of everted mid-small intestine of the hamster. Initial serosal fluid contained 10 mM L-histidine but no sugar; initial mucosal fluid contained 10 mM L-histidine and either 8·35 mM L-glucose, or 7·16 mM α-glucoheptose, or 18·3 mM L-fucose, or 16·7 mM D-mannose, or 16·7 mM L-sorbose. Other details as in Table 1

Sugar added to mucosal fluid only	No histidine present		In presence of histidine	
	Sugar final concn. ratio (serosal/mucosal)	Transport of sugar into serosal fluid (μmole/100 mg dry wt. sac/hr)	Sugar final concn. ratio (serosal/mucosal)	Transport of sugar into serosal fluid (μmole/100 mg dry wt. sac/hr)
L-glucose	0·93 ± 0·03 (11)	14·7 ± 0·4	0·63 ± 0·02 (12)	10·5 ± 0·4
α-glucoheptose	0·40 ± 0·03 (11)	6·3 ± 0·6	0·38 ± 0·02 (12)	5·7 ± 0·4
L-fucose	0·39 ± 0·03 (11)	13·8 ± 1·0	0·41 ± 0·02 (12)	15·0 ± 0·8
D-mannose	0·38 ± 0·02 (18)	13·0 ± 1·0	0·32 ± 0·04 (12)	11·5 ± 1·7
L-sorbose	0·37 ± 0·03 (12)	11·6 ± 0·8	0·34 ± 0·14 (12)	10·0 ± 0·6

6). The D-form of histidine was as potent as the L-form, while 10 mM D-methionine seemed somewhat more inhibitory than 10 mM L-methionine. The D-amino acids (histidine and methionine) which caused a marked reduction in D-glucose absorption also depressed water entry into the sacs.

TABLE 6. Effect of D-amino acids on D-glucose active absorption by sacs of everted mid-small intestine of the hamster. Other details as in Table 1

Amino acid in mucosal and serosal fluid	D-glucose final concn. ratio (serosal/mucosal)	Transport of glucose into serosal fluid (μmole/100 mg dry wt. sac/hr)	Gain in serosal fluid water (m-mole/100 mg dry wt. sac/hr)
None	3·23 ± 0·12 (19)	95·0 ± 5·0	61·9 ± 4·2
D-valine (20 mM)	3·53 ± 0·12 (12)	100·1 ± 4·7	51·3 ± 3·0
D-glutamic acid (20 mM)	2·88 ± 0·13 (12)	81·5 ± 4·6	54·7 ± 4·6
D-alanine (20 mM)	2·83 ± 0·08 (12)	89·0 ± 4·6	63·0 ± 3·9
D-methionine (10 mM)	2·36 ± 0·08 (18)	47·0 ± 3·0	34·6 ± 4·2
D-histidine (10 mM)	2·13 ± 0·09 (12)	42·0 ± 2·6	36·4 ± 4·0

DISCUSSION

The results show quite clearly that some amino acids have a considerable inhibitory effect on the active transport mechanism of some sugars. The phenomenon is obvious when D-glucose is being moved against its concentration gradient in the presence of actively absorbed L-amino acids (including the basic L-ornithine), but does not occur with the passively absorbed L-glutamic and L-aspartic acids (Table 3). It is also seen when D-methionine and D-histidine are used, but not with D-valine or D-glutamic acid, and probably not with D-alanine (Table 6). Of these D-amino acids, D-methionine can be transported against its concentration gradient by both hamster (Lin, Hagihira & Wilson, 1962) and rat (Jervis & Smyth, 1960) small intestine, and although this has not yet been demonstrated for D-histidine, Jervis & Smyth (1959) have reported that it may reduce intestinal uptake of L-histidine and that its own absorption rate is lowered by L-histidine and also by L-methionine. Hence, there is reason to accept that D-histidine is involved in an amino acid active transport system, and its behaviour in the present series of experiments would add support for such an opinion. On the other hand, D-alanine (Finch & Hird, 1960; Randall & Evered, 1964), D-valine (Finch & Hird, 1960) and D-glutamic acid have no affinity for such a mechanism. The amino acids which influence sugar active absorption, therefore, seem to be those which are themselves actively transported.

When we consider the sugars which are affected by the presence of amino acids, we find that, apart from L-glucose, they are the ones known to be actively absorbed (D-glucose, D-galactose, 3-O-methyl-D-glucose and

D-fucose), but not those passively taken up (α-glucoheptose, L-fucose, D-mannose and L-sorbose). Although there was no direct evidence of active transfer of L-glucose in the present experiments (final concentration ratio always less than 1·0), and none could be found by Wilson (1958) in the hamster or by Csáky & Fernald (1960) in the frog, the rate of uptake of L-glucose was very much faster than that of the other passively absorbed sugars. Further, although the final concentration ratios for α-glucoheptose, L-fucose, D-mannose and L-sorbose lay in the region of 0·4, that for L-glucose was very close to 1·0. It is not improbable, then, that L-glucose movement across the intestine is in some way facilitated and the appreciable reduction in its absorption in the presence of 10 mM L-histidine leads to that conclusion. If this interpretation is correct, only sugars which participate in a special absorption mechanism are affected by actively transported L- or D-amino acids.

It is of especial interest that L-glutamic and L-aspartic acids have no inhibitory effect on the active movement of D-glucose, because these amino acids give rise to L-alanine (which is then concentrated in the serosal fluid or blood) during their absorption (Matthews & Wiseman, 1953; Neame & Wiseman, 1956, 1957*a*, *b*, 1958). As L-alanine added to the mucosal fluid greatly depresses the transference of D-glucose against its gradient (Table 3), it appears that actively absorbed amino acid exerts its effect during the active uptake of sugar by the epithelial cell (presumably at its luminal border) and that once this step has been passed, intracellular amino acid has no further influence.

Among the amino acids most capable of diminishing active transport of D-glucose is L-histidine, which at 10 mM brought the final concentration ratio to about 2·0 from a control value of 3·2. Raising the concentration of L-histidine to 20 mM gave no additional inhibition, suggesting that either the active mechanism possesses a certain amount of absolute preference for D-glucose or that there is available an alternative active pathway. The results obtained with L-methionine can be interpreted similarly.

The extent to which D-glucose active transport is decreased by an amino acid seems to be related to the acid's affinity for active absorption (Table 3). Those amino acids with a low over-all affinity (high K_t) have, at the 20 mM level, more effect on D-glucose than do amino acids with a high affinity (low K_t). At 20 mM, the amino acids with low affinity are themselves better concentrated than are the high affinity amino acids (Wiseman, 1955). Whether or not these results signify the sharing of a limited energy supply for active transport is, as yet, unknown. It should be noted that in the case of the D-glucose experiments there is ample metabolizable sugar in the mucosal and serosal fluids at the end of the incubation period, showing that availability of energy-yielding substrate *per se* is not the

determining factor. The latter may, of course, be the rate of turnover of some intermediate in the energy cycle. It must be borne in mind that these amino acids do not all utilize just one mechanism, and that the results in Tables 1 and 2 indicate that amino acids and sugars may compete for more than one pathway.

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