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ACTIVE TRANSPORT OF L-GLUCOSE BY ISOLATED SMALL INTESTINE OF THE DIETARY-RESTRICTED RAT

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SUMMARY

1. The effect of semistarvation and complete starvation (sufficient to produce a loss of about 32 and 25 % respectively of initial body weight) on the active transport of L-glucose has been studied by the use of sacs of everted mid-small intestine of rats. The animals were allowed free access to water.

2. Sacs from animals on a restricted diet transported L-glucose against its concentration gradient, but sacs from fully fed rats did not. Even when sacs from fully fed rats were distended sufficiently to cause them to lose serosal volume, the L-glucose concentration in the final serosal fluid was never greater than that in the final mucosal fluid.

3. The L-glucose active transport was independent of net water movement, needed oxygen, was not demonstrable at 27° C, and required Na ions at a concentration of 83 mM or greater. It could be completely inhibited by 10^{-6} M phlorrhizin, or 10 mM L-histidine, or 1·39 mM D-glucose. Phlorrhizin at a concentration of 10^{-8} M reduced, but did not prevent, L-glucose active transport.

4. It seems probable that L-glucose active transport is mediated by the mechanism that actively transports D-glucose.

5. Un-incubated mid-small intestine of fully fed rats contained 37·8 mg D-glucose/100 g wet wt. of tissue, whereas semistarved intestine had only 10·8 mg D-glucose/100 g. The lack of demonstrable active transport of L-glucose by normal intestine may possibly have been caused, at least in part, by inhibition of the process by endogenous D-glucose.

6. There appeared to be no metabolism of L-glucose by rat intestine, nor conversion to the D-form.

7. The hypothesis that sugars require the D-pyranose ring structure for active absorption is no longer tenable.

INTRODUCTION

In their investigation of the effect of amino acids on sugar transport by sacs of everted small intestine of the normal golden hamster, Hindmarsh, Kilby & Wiseman (1966*a*, *b*) demonstrated that the transport of L-glucose down its concentration gradient was substantially reduced by the presence of L-histidine. The amino acid had no such action on the downhill movement of α-glucoheptose, L-fucose, D-mannose or L-sorbose, which are believed to be passively absorbed, but it did act as an inhibitor of the active (uphill) transport of D-glucose, D-galactose, D-fucose and 3-O-methyl-D-glucose. In addition, it was observed that the ratio of the sugar concentration in the final serosal fluid to that in the final mucosal fluid (when no amino acid was present) was 0·93 (initial ratio 0:1) for L-glucose whereas it was only about 0·40 for α-glucoheptose, L-fucose, D-mannose and L-sorbose. The results suggested that although L-glucose was not transported against its concentration gradient under those conditions, its movement across the intestine was in some way facilitated.

In studying this problem further, we have used sacs of everted small intestine of rats on a restricted diet as this species shows enhanced active transport of D-glucose when semistarved (Neame & Wiseman, 1959; Kershaw, Neame & Wiseman, 1960; Hindmarsh, Kilby, Ross & Wiseman, 1967). We have found that, in contrast to fully fed rats, semistarved and fully starved ones can transport L-glucose against its concentration gradient quite well, and the characteristics of the transport indicate that the mechanism is probably one which is shared with D-glucose. This is the first direct demonstration of intestinal active transport of L-glucose. The current hypothesis (Wilson, 1962; Wiseman, 1964) that sugars require the D-pyranose ring structure for active absorption is, therefore, no longer tenable.

Preliminary reports of this work have been given by Neale & Wiseman (1968*a*, *b*).

METHODS

Animals and diets. All the rats were young adult males and were kept in individual cages throughout the experimental period. Those on the restricted diet were fed 6–10 g food/day for 9 days, which caused their body weight to fall from about 250 g to about 170 g. Some rats were given no food at all for 4–5 days, causing their body weight to fall from about 240 g to about 180 g. The control group were fed *ad libitum* and they weighed about 250 g at the time of experimentation. All were allowed free access to water. The food was diet 86, purchased from Oxoid Ltd., Southwark Bridge Road, London, S.E. 1.

Preparation of sacs. The animals were anaesthetized with 'Nembutal' (pentobarbitone sodium) given intraperitoneally (0·15 ml. for dietary-restricted rats and 0·40 ml. for controls); the abdomen was opened by a mid line incision, and the small intestine washed out with bicarbonate-saline (Krebs & Henseleit, 1932) equilibrated with 5 % CO_2, 95 % O_2 (or 5 % CO_2,

95 % N_2 in the anoxia experiments). The mesentery was then stripped off the small intestine and the duodenum removed. To overcome the problem of variation in absorptive activity of different regions (Hindmarsh *et al.* 1967), all the small intestine was everted but only the middle fifth was used, and of this two sacs were made, each about 9 cm in length. The general technique of preparing sacs was that originally described by Wilson & Wiseman (1954) (and in greater detail by Wiseman, 1961).

Measurement of initial and final volumes. The initial volume of fluid (serosal) introduced into the carefully drained sac of everted intestine was determined by weighing the sac before and after filling it. This serosal fluid volume was about 0·8 ml. for a standard sac and about 2 ml. for an extra-distended one. The final volume of the serosal fluid was estimated by draining the sac of its contents and weighing the fluid collected. This latter technique enabled about 96 % of introduced fluid to be recovered from unincubated sacs. The volume of fluid (mucosal) into which the sac was placed at the beginning of the experimental period was 20 ml.

Almost all standard sacs (initial serosal volume about 0·8 ml.) gained serosal fluid, whereas extra-distended sacs often lost serosal fluid during incubation.

Experimental procedure for absorption experiments. The sac, filled with a known volume (initial serosal fluid) of the appropriate solution, was put into a 150 ml. Erlenmeyer flask containing 20 ml. (initial mucosal fluid) of the same solution as was used for filling it, the air replaced by a gas mixture of 5 % CO_2, 95 % O_2 (or 5 % CO_2, 95 % N_2 in anoxia experiments), and the stoppered flask shaken (80 oscillations/min, amplitude 5 cm) for 1 hr in a Warburg bath kept at 37° C (or, in some experiments, 27° or 40° C). At the end of the hour the sac was removed, its surface drained, and its fluid contents collected. Samples of initial and final mucosal and serosal fluids were analysed for sugar concentrations after deproteinization with $ZnSO_4$ and $Ba(OH)_2$.

The serosal and mucosal fluids were usually Krebs–Henseleit (1932) bicarbonate-saline plus the necessary substrate; where the composition of the bicarbonate-saline was altered the details are given in the text and Tables. The latter also show the amount and type of substrate investigated.

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 to constant weight at 120° C. The dry weights varied from 25 to 100 mg.

Estimation of endogenous D-*glucose in intestinal wall.* For the estimation of endogenous D-glucose in un-incubated intestinal wall, the middle two fifths of the washed out (not everted) small intestine was employed. The tissue, rinsed free of blood, was plunged into 20 ml. boiling water and homogenized using a M.S.E. homogenizer with stainless-steel cutting blades operated at about 14,000 rev/min. for 3 min. The homogenate was deproteinized by the use of $ZnSO_4$ and $Ba(OH)_2$, and 1 ml. samples of filtrate from control rats and 2 ml. samples from semistarved rats were analysed by the D-glucose oxidase method.

L-*glucose recovery experiments.* To test whether L-glucose was lost from the system during incubation, sacs from fully fed or semistarved rats were made as for standard absorption experiments and filled with 0·8 ml. bicarbonate-saline solution with or without 8·33 mM L-glucose and shaken for 1 hr in 10 ml. of the same solution as was used for filling them. At the end of the incubation period, each sac was homogenized (as above) and the homogenate added to its pooled final serosal plus mucosal fluids, deproteinized with $ZnSO_4$ and $Ba(OH_2)$, made up to 200 ml., and filtered. One millilitre filtrate was analysed for total reducing sugar by the Nelson (1944) method. The amount of reducing sugar recovered (allowance being made for the initial wet weight of the sac) in experiments in which no L-glucose had been added ('blank' experiments) was used as a correction factor in determining the amount of L-glucose recovered in experiments in which the sugar had been added. Under these conditions the 'blank' values (no added L-glucose) were about 10 % of the 'experimental' values (L-glucose added to system) with fully fed rats and about 3 % with semistarved rats.

Analysis of samples with D-glucose oxidase gave no evidence of conversion of L-glucose to the D-form.

Chemical estimations. When only L-glucose was used, it was estimated by the colorimetric method of Nelson (1944) for reducing sugars. When both L- and D-glucose were employed, the total reducing sugar (L- plus D-glucose) was measured by the Nelson method and the D-glucose itself by the specific D-glucose oxidase colorimetric method of Huggett & Nixon (1957); the amount of L-glucose was then obtained by difference.

The L-glucose was found to be free of D-glucose.

Concentration ratios. The *final concentration ratio* was the ratio of the L-glucose concentration in the *serosal* (inner) fluid to that in the *mucosal* (outer) fluid at the end of the 1 hr incubation. The *initial concentration ratio* for L-glucose was 1:1.

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

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

RESULTS

It can be seen from Table 1 that sacs of everted mid-small intestine of semistarved rats were able to transfer L-glucose against its concentration gradient, the ratio of the concentration of the sugar in the final serosal fluid to that in the final mucosal fluid being 1·32 (initial ratio 1·0) under standard conditions. Even though water was also transported at the same time as the L-glucose, the concentration of the latter in the sac rose from 8·33 to 10·53 mM. The mucosal fluid concentration of L-glucose showed only a small fall as the volume of the mucosal fluid was relatively large. It was not possible to improve the final concentration ratio by preventing net gain of water by sacs by distending them with an initial serosal volume of about 2·0 ml. instead of about 0·8 ml.; the final concentration ratio remained unchanged (Table 1). In addition, the L-glucose final concentration ratio was the same when 2·78 mM or 8·33 mM L-glucose was used, and whether or not the serosal fluid gained or lost water.

In distinct contrast to the results with semistarved intestine, sacs made from intestine of rats fed *ad libitum* were unable to actively transport L-glucose (Table 2). The final concentration ratio of sugar for these sacs was 0·80 when the initial serosal volume was 0·8 ml., and it remained below 1·0 when the initial serosal volume was increased to 2·3 ml. in order to produce a loss of water from the sac. The difference between semistarved and normal intestine in L-glucose active transport was not caused, therefore, by net water movement. Attempts were made to obtain active transport of L-glucose by normal intestine by incubating it at 40° C, by using 2·78 mM or 16·67 mM L-glucose, and even by adding 8·33 mM D-glucose although this sugar inhibited active transport of L-glucose by semistarved intestine. In no case did the normal intestine

transport the L-glucose against its concentration gradient and in all these experiments its final concentration in the serosal fluid was below its initial concentration.

TABLE 1. Transport of L-glucose by sacs of everted mid-small intestine of dietary-restricted rats. Standard conditions were: sac length about 9 cm; initial serosal (inner) volume 0·8 ml.; initial mucosal (outer) volume 20 ml.; initial serosal and mucosal fluids contained 8·33 mM L-glucose (initial concentration ratio 1·0); gas phase 5% CO_2, 95% O_2; experimental period 1 hr; temperature 37° C; 80 oscillations/min, amplitude 5 cm. Values are means ± S.E. of the means, with number of sacs in parentheses

	Final concn. of L-glucose in serosal fluid (mM)	Final concn. of L-glucose in mucosal fluid (mM)	Transport of L-glucose into serosal fluid (μmole/100 mg dry wt. sac/hr)	Gain in serosal fluid water (m-mole/100 mg dry wt. sac/hr.)	L-glucose final concn. ratio (serosal/mucosal)
Under standard conditions	10·53 ± 0·28	8·03 ± 0·06	6·07 ± 0·49	11·68 ± 2·66	1·32 ± 0·04 (15)
Initial serosal volume 2 ml.	11·00 ± 0·20	8·10 ± 0·06	5·55 ± 1·30	−24·20 ± 3·95	1·36 ± 0·03 (10)
O_2 replaced by N_2	7·81 ± 0·08	8·30 ± 0·04	−1·40 ± 0·17	−3·41 ± 0·69	0·94 ± 0·01 (10)
Temp. 27° C	8·43 ± 0·18	8·23 ± 0·11	−0·18 ± 0·08	−3·28 ± 1·51	1·03 ± 0·02 (8)
10 mM L-histidine present	8·03 ± 0·13	8·23 ± 0·04	1·60 ± 0·16	14·39 ± 2·28	0·98 ± 0·02 (17)
L-glucose reduced to 2·78 mM	3·75 ± 0·10	2·70 ± 0·02	1·92 ± 0·20	−1·50 ± 2·92	1·39 ± 0·04 (20)
L-glucose reduced to 2·78 mM and initial serosal volume 2 ml.	3·84 ± 0·10	2·64 ± 0·03	2·11 ± 0·38	−30·90 ± 1·83	1·46 ± 0·05 (12)

TABLE 2. Transport of L-glucose by sacs of everted mid-small intestine of rats fed *ad libitum*. Other details as in Table 1

	Final concn. of L-glucose in serosal fluid (mM)	Final concn. of L-glucose in mucosal fluid (mM)	Transport of L-glucose into serosal fluid (μmole/100 mg dry wt. sac/hr)	Gain in serosal fluid water (m-mole/100 mg dry wt. sac/hr)	L- glucose final concn. ratio (serosal/mucosal)
Under standard conditions	6·54 ± 0·19	8·19 ± 0·06	0·43 ± 0·20	17·40 ± 1·12	0·80 ± 0·02 (13)
Initial serosal volume 2·3 ml.	7·89 ± 0·11	8·27 ± 0·06	−1·95 ± 0·31	−6·50 ± 0·87	0·95 ± 0·02 (8)
Temp. 40° C	7·20 ± 0·15	8·48 ± 0·05	0·49 ± 0·08	15·00 ± 1·91	0·85 ± 0·02 (8)
L-glucose reduced to 2·78 mM	2·23 ± 0·06	2·67 ± 0·02	0·35 ± 0·11	21·20 ± 2·08	0·84 ± 0·02 (16)
L-glucose increased to 16·67 mM	13·94 ± 0·18	16·66 ± 0·01	0·01 ± 0·37	9·61 ± 1·68	0·84 ± 0·01 (8)
8·33 mM D-glucose present and initial serosal volume 2 ml.	5·51 ± 0·54	8·80 ± 0·10	−8·87 ± 1·46	−9·62 ± 1·36	0·63 ± 0·07 (8)

The active transport of L-glucose by semistarved intestine was oxygen-dependent, was not demonstrable at 27° C, needed an adequate concentration of Na ions, and could be inhibited by L-histidine, phlorrhizin, and D-glucose (Tables 1, 3–7). When the NaCl (but not the $NaHCO_3$, equivalent to 25 mM Na) of the incubation media was entirely replaced by KCl, active

transport of L-glucose was lost, and it did not reappear until the total Na concentration was restored to between 113 and 131 mM (Table 3). In the experiments in which mannitol was used for replacing the NaCl, the necessary Na concentration for active transport of L-glucose was between

TABLE 3. Effect of replacing NaCl by KCl on the active transport of L-glucose by sacs of everted mid-small intestine of dietary-restricted rats. Other details as in Table 1

Na concn. in initial serosal and mucosal fluids (mM)	Final concn. of L-glucose in serosal fluid (mM)	Final concn. of L-glucose in mucosal fluid (mM)	Transport of L-glucose into serosal fluid (μmole/100 mg dry wt. sac/hr)	Gain in serosal fluid water (m-mole/100 mg dry wt. sac/hr)	L-glucose final concn. ratio (serosal/mucosal)
143 (standard conditions)	10·53 ± 0·28	8·03 ± 0·06	6·07 ± 0·49	11·68 ± 2·66	1·32 ± 0·04(15)
131	9·54 ± 0·14	8·05 ± 0·07	4·02 ± 0·31	10·25 ± 1·76	1·18 ± 0·02 (6)
113	8·43 ± 0·20	8·07 ± 0·12	4·12 ± 0·45	23·40 ± 4·94	1·05 ± 0·04 (6)
84	8·19 ± 0·05	8·11 ± 0·07	0·02 ± 0·09	0·59 ± 0·40	1·01 ± 0·01 (6)
25	7·22 ± 0·07	8·11 ± 0·09	−3·18 ± 0·27	−7·26 ± 1·18	0·89 ± 0·01 (6)

TABLE 4. Effect of replacing NaCl by mannitol on the active transport of L-glucose by sacs of everted mid-small intestine of dietary-restricted rats. Other details as in Table 1

Na concn. in initial serosal and mucosal fluids (mM)	Final concn. of L-glucose in serosal fluid (mM)	Final concn. of L-glucose in mucosal fluid (mM)	Transport of L-glucose into serosal fluid (μmole/100 mg dry wt. sac/hr)	Gain in serosal fluid water (m-mole/100 mg dry wt. sac/hr)	L-glucose final concn. ratio (serosal/mucosal)
143 (standard conditions)	10·53 ± 0·28	8·03 ± 0·06	6·07 ± 0·49	11·68 ± 2·66	1·32 ± 0·04 (15)
131	9·40 ± 0·11	7·81 ± 0·09	3·66 ± 0·36	9·75 ± 2·38	1·21 ± 0·02 (6)
113	9·31 ± 0·28	8·08 ± 0·04	3·19 ± 0·69	11·80 ± 4·00	1·15 ± 0·04 (6)
84	8·83 ± 0·25	8·30 ± 0·09	1·40 ± 0·58	3·48 ± 1·37	1·07 ± 0·04 (6)
25	7·47 ± 0·05	8·21 ± 0·07	−1·45 ± 0·14	0·04 ± 0·67	0·91 ± 0·01 (6)

TABLE 5. Effect of phlorrhizin on the active transport of L-glucose by sacs of everted mid-small intestine of dietary-restricted rats. Other details as in Table 1

Phlorrhizin concn. in initial serosal and mucosal fluids (M)	Final concn. of L-glucose in serosal fluid (mM)	Final concn. of L-glucose in mucosal fluid (mM)	Transport of L-glucose into serosal fluid (μmole/100 mg dry wt. sac/hr)	Gain in serosal fluid water (m-mole/100 mg dry wt. sac/hr)	L-glucose final concn. ratio (serosal/mucosal)
0 (standard conditions)	10·53 ± 0·28	8·03 ± 0·06	6·07 ± 0·49	11·68 ± 2·66	1·3 ± 0·04 (15)
10^{-8}	9·46 ± 0·48	8·13 ± 0·09	4·46 ± 0·69	15·13 ± 3·27	1·16 ± 0·06 (12)
10^{-6}	6·82 ± 0·21	8·23 ± 0·03	−0·55 ± 0·32	15·89 ± 3·06	0·82 ± 0·03 (12)
10^{-5}	5·33 ± 0·26	8·46 ± 0·03	−3·08 ± 0·13	24·79 ± 4·64	0·63 ± 0·03 (10)
5×10^{-5}	4·99 ± 0·20	8·21 ± 0·09	−2·79 ± 0·24	15·69 ± 2·32	0·61 ± 0·02 (12)

84 and 113 mM (Table 4). Phlorrhizin (present in both the serosal and mucosal fluids) at a concentration of 10^{-6} M acted as a complete inhibitor, while at 10^{-8} M there was partial inhibition (Table 5). With D-glucose, active transport of L-glucose was stopped (final concentration ratio 0·94) when the initial concentration of D-glucose in the serosal and mucoal fluids was only 1·39 mM. Increasing the initial D-glucose concentration

above 1·39 mM caused L-glucose to be lost from inside the sacs and its final concentration ratios to fall to quite low values (Table 6). This fall in L-glucose concentration in the serosal fluid could be only partially overcome by reducing the water gain by the sacs by distending them with an initial serosal volume of 1·6 ml. instead of 0·8 ml.; the efflux of L-glucose remained unaltered. There was good active transport of D-glucose in all the experiments in which it was present, the actual final concentrations of

TABLE 6. Effect of D-glucose on the active transport of L-glucose by sacs of everted mid-small intestine of dietary-restricted rats. Other details as in Table 1

D-glucose concn. in initial serosal and mucosal fluids (mM)	Final concn. of L-glucose in serosal fluid (mM)	Final concn. of L-glucose in mucosal fluid (mM)	Transport of L-glucose into serosal fluid (μmole/100 mg dry wt. sac/hr)	Gain in serosal fluid water (m-mole/100 mg dry wt. sac/hr)	L-glucose final concn. ratio (serosal/mucosal)
0 (standard conditions)	10·53 ± 0·28	8·03 ± 0·06	6·07 ± 0·49	11·68 ± 2·66	1·32 ± 0·04 (15)
1·39	7·68 ± 0·30	8·28 ± 0·15	4·54 ± 0·79	42·20 ± 7·40	0·94 ± 0·05 (13)
2·78	4·38 ± 0·64	8·35 ± 0·08	−5·00 ± 1·56	27·94 ± 7·18	0·53 ± 0·08 (12)
8·33	2·10 ± 0·28	8·95 ± 0·05	−7·67 ± 0·69	60·50 ± 5·33	0·24 ± 0·03 (16)
8·33 and initial serosal volume 1·6 ml.	4·47 ± 0·56	9·14 ± 0·08	−9·20 ± 2·03	18·16 ± 6·35	0·49 ± 0·06 (16)

TABLE 7. Final serosal and mucosal concentrations of L- and D-glucose in the experiments shown in Table 6

D-glucose concn. in initial serosal and mucosal fluids (mM)	Final total glucose (D+L) concn. (mM)		Final D-glucose concn. (mM)		Final L-glucose concn. (mM)	
	Serosal	Mucosal	Serosal	Mucosal	Serosal	Mucosal
0 (standard conditions)	10·53±0·28	8·03±0·06	0	0	10·53±0·28	8·03±0·06 (15)
1·39	17·21±1·35	8·28±0·15	9·53±1·18	0	7·68±0·30	8·28±0·15 (13)
2·78	24·09±2·19	8·35±0·08	19·71±2·45	0	4·38±0·64	8·35±0·08 (12)
8·33	38·91±1·86	12·29±0·10	36·81±2·00	3·34±0·14	2·10±0·28	8·95±0·05 (16)
8·33 and initial serosal volume 1·6 ml.	44·31±2·13	11·68±0·31	39·84±1·85	2·54±0·19	4·47±0·56	9·14±0·08 (16)

both D- and L-glucose in serosal and mucosal fluids being given in Table 7. The movement of L-glucose out of the sacs produced a small rise in its concentration in the final mucosal fluid.

As in the case of semistarved rats, there was active transport of L-glucose by sacs of everted mid-small intestine of rats given no food (but allowed water freely) for 4–5 days. The L-glucose final concentration ratio was 1·11 ± 0·01 (n = 12), the gain in serosal fluid water was 3·74 ± 2·10 m-mole/100 mg dry wt. sac/hr, the gain in serosal fluid L-glucose was 1·49 ± 0·40 μmole/100 mg dry wt. sac/hr, the final mucosal L-glucose concentration was 8·00 ± 0·03 mM, and the final serosal L-glucose concentration was 8·85 ± 0·09 mM. The body weight of these fully starved animals fell by about 25 %, compared with a fall of about 32 %, for the semistarved ones.

The amount of L-glucose that could be recovered from the final serosal and mucosal fluids plus that in the sac wall was 100% of the L-glucose added (16·2 mg) when semistarved intestine was used, and 95·6% when normal intestine was employed. Analysis of the recovered material with D-glucose oxidase indicated that there was no measurable conversion of the L-glucose to the D-form.

In order to determine how much endogenous reducing material was released by semistarved and normal intestine into the serosal and mucosal fluids during incubation, sacs were shaken for 1 hr in media to which no sugar had been added. The serosal and mucosal fluids at the end of the incubation period contained reducing material which amounted to less than 1% of that added in experiments with 8·33 mM L-glucose.

We found unincubated semistarved mid-small intestine (whole wall) to contain 10·8 mg D-glucose/100 g wet wt. tissue (D-glucose oxidase method), whereas normal intestine had 37·8 mg D-glucose/100 g.

DISCUSSION

The results show clearly, and for the first time, that L-glucose can be actively transported by the small intestine of the rat. For demonstration of the phenomenon, the animal must be in the semistarved or fully starved state.

The movement of the L-glucose against its concentration gradient was independent of net water transport, needed oxygen, was abolished by reducing the temperature to 27° C, required a Na ion concentration of about 84 mM or greater, and was completely inhibited by 10^{-6} M phlorrhizin or 10 mM L-histidine or 1·39 mM D-glucose. The sensitivity of the active transport to phlorrhizin was appreciably greater than that found by Parsons, Smyth & Taylor (1958) for D-glucose, the latter being actively transported by rat intestine in the presence of up to 10^{-5} M phlorrhizin. On the other hand, the requirement for Na ions by L-glucose was about the same as that found for D-glucose transport both in the rat (Clarkson & Rothstein, 1960) and the guinea-pig (Riklis & Quastel, 1958). The effect of D-glucose on the active transport of L-glucose was marked, the D-form behaving as an inhibitor when present in the initial serosal and mucosal fluids at a concentration (1·39 mM) one sixth of that of the L-form. In such experiments the final mucosal fluid contained no measurable D-glucose, but the serosal fluid D-glucose concentration was 9·53 mM (Table 7). Movement of L-glucose into the serosal fluid down its concentration gradient still occurred, so that the L-glucose final concentration ratio remained at 0·94 despite the large amount of water transported into the serosal fluid (Table 6). However, when D-glucose was used at an initial concentration

higher than 1·39 mM, L-glucose was actually lost from inside the sacs. The migration of L-glucose from serosal to mucosal fluid against its concentration gradient has also been recorded by Huang & Rout (1967), who employed sacs of non-everted intestine of the killifish (*Fundulus heteroclitus*). In their experiments no sugar other than L-glucose had been added to the incubation media. The characteristics of L-glucose active transport by the dietary-restricted rat, especially the relationship to D-glucose, phlorrhizin and Na ions, suggest that its movement across the intestinal epithelium is mediated by the mechanism that transports D-glucose. It is of considerable interest to note that Huang & Woosley (1968) have already postulated that the system in the proximal tubular cells which actively reabsorbs D-glucose is identical to that which secretes L-glucose into the proximal tubular fluid. They based their statement on the observations that, in steady-state experiments, dog and rat kidney tubules secreted L-glucose, that the secretion was augmented by increasing the loading of D-glucose, and that secretion of the L-glucose could be inhibited by phlorrhizin.

The inability of normal rat mid-small intestine to transport L-glucose against its concentration gradient was independent of net water movement, there being a final concentration ratio of less than 1·0 even when the sacs were distended sufficiently to cause them to lose serosal volume during incubation (Table 2). That normal intestine does not actively absorb L-glucose has also been reported for the hamster (Wilson, 1958; Wilson & Landau, 1960; Hindmarsh *et al.* 1966*a*, *b*), the frog (Csáky & Fernald, 1960), and the killifish (Huang & Rout, 1967).

Although we are unable to state definitely the reason for this difference in absorptive behaviour between normal and semistarved intestine, two possible explanations are available. First, semistarved intestine has an enhanced capacity for absorption of various substances. This has been shown for D-glucose (Neame & Wiseman, 1959; Kershaw *et al.* 1960; Faelli, Esposito & Capraro, 1966; Hindmarsh *et al.* 1967; Esposito, Faelli & Capraro, 1967), L-histidine (Neame & Wiseman, 1959; Kershaw *et al.* 1960; Suda & Shimomura, 1964), sodium (Esposito *et al.* 1967) and serum protein hydrolysate (Ziemlański, Cieślak, Pliszka & Szczygiel, 1967) in the rat; and for D-glucose in the chick (Bogner, Braham & McLain, 1966) and the guinea-pig (Hindmarsh *et al.* 1967). In the case of the rat, the effect of semistarvation on D-glucose active transport is particularly obvious in the lower (but not terminal) ileum. This region in the normal rat does not exhibit active transport of D-glucose, but it does so quite well when semistarved, which may be an unmasking of an inherent capability rather than acquisition of a new one (Hindmarsh *et al.* 1967). The second possible reason for demonstrable active transport of L-glucose by semistarved

intestine is that, under our experimental conditions, semistarved intestine contained only 10·8 mg D-glucose/100 g wet wt. of tissue, whereas normal intestine had 37·8 mg D-glucose/100 g. There might, therefore, be less inhibition of L-glucose active transport by endogenous D-glucose in the semistarved intestine.

There was no evidence that the mid-small intestine of the rat metabolized the L-glucose. In experiments with normal and semistarved intestine the amount of reducing sugar recoverable at the end of the incubation was 95·6 and 100% respectively of the quantity of L-glucose added (allowance being made for release of endogenous reducing material). Analysis with D-glucose oxidase indicated no measurable conversion of the L-glucose to the D-form.

The current hypothesis (Wilson, 1962; Wiseman, 1964) that sugars require the D-pyranose ring structure for active absorption is no longer tenable.

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