J. Physiol. (1969), **205**, *pp.* 159–178
Printed in Great Britain

THE USE OF DIETARY-RESTRICTED RAT INTESTINE FOR ACTIVE TRANSPORT STUDIES

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(*Received* 8 *May* 1969)

SUMMARY

1. The effect of dietary restriction (sufficient to produce a loss of about 32 % of initial body weight) on intestinal active transport has been studied in the rat by the use of sacs of everted mid-small intestine. Eight D-sugars, four L-sugars and two D-amino acids were employed.

2. Dietary restriction enhanced the normally occurring active transport of D-galactose, 3-*O*-methyl-D-glucose and D-methionine. In addition, sacs of dietary-restricted small intestine were able to concentrate in the serosal fluid D-fucose, D-xylose and D-histidine, which sacs of normal rat intestine could not do. The final (1 hr) serosal/mucosal concentration ratios produced for these actively transported substances were independent of net water movement.

3. Sugars which were not concentrated in the serosal fluid of sacs of fully fed or dietary-restricted intestine were D-arabinose, D-fructose, D-glucosamine, D-mannose, L-arabinose, L-fucose, L-sorbose and L-xylose.

4. The characteristics of D-fucose and D-xylose active transport suggest that they are transported by the mechanism which actively transports D-glucose. The comparatively low content of D-glucose in dietary-restricted intestine, compared with fully fed intestine, may be part of the explanation for observable active transport of D-fucose and D-xylose by dietary-restricted sacs.

5. Thinning of the intestinal wall is believed not to be the cause of the enhanced active transport found during dietary restriction.

6. The results show that dietary-restricted rat small intestine may, at times, be more useful than fully fed rat small intestine in the study of intestinal active transport.

INTRODUCTION

In 1959, Neame & Wiseman published their observations on the active transport of D-glucose and L-histidine by sacs of everted upper small intestine of young adult rats fed a restricted diet. The food was deficient

only in absolute amount (20–25 % of normal intake) but not in its general basic composition; the animals lost about 20 % of their weight. It was found that the dietary-restricted intestine transported both the sugar and the amino acid against much greater concentration gradients than did normal intestine. In the following year, Kershaw, Neame & Wiseman (1960) demonstrated that D-glucose and L-histidine both disappeared faster from the lumen of the whole small intestine of anaesthetized dietary-restricted rats than from the intestine of fully fed rats. They also noted that the increased active absorption by dietary-restricted rats gradually returned to normal when the animals were given food *ad libitum*. Augmentation of intestinal active transport during dietary restriction was later confirmed by Hindmarsh, Kilby, Ross & Wiseman (1967) for the rat and the guinea-pig, although they were unable to show the phenomenon with the golden hamster. In the case of the rat, dietary restriction converted the lower (but not terminal) ileum from a region which does not normally actively transport D-glucose to one which can do so quite well. More recently, Neale & Wiseman (1968*a*, *b*, *c*) have obtained transport against its concentration gradient of L-glucose by sacs of everted mid-small intestine of dietary-restricted rats, whereas with fully fed rats, under those experimental conditions, intestinal sacs were unable to raise the L-glucose concentration in the serosal fluid above that in the fluid bathing the mucosal surface.

Other workers to observe enhanced active transport by the intestine of animals on an inadequate diet are Faelli, Esposito & Capraro (1966; D-glucose by the rat), Dowling, Riecken, Laws & Booth (1967; D-glucose and water by the rat), Esposito, Faelli & Capraro (1967; D-glucose and sodium by the rat), Bogner, Braham & McLain (1966; D-glucose by the chick), Suda & Shimomura (1964; L-histidine by the rat), and Ziemlański, Cieślak, Pliszka & Szczygiel (1967; serum protein hydrolysate by the rat).

We have now tested dietary-restricted rat intestine for its ability to transport some sugars which are normally transported against a concentration gradient, some sugars which are not normally actively transported, and in addition D-methionine (transported against its concentration gradient by normal intestine) and D-histidine (not transported against its concentration gradient by normal intestine). Of the sugars used, it was found that those transported against their concentration gradient (D-galactose and 3-*O*-methyl-D-glucose) by sacs of normal rat intestine were concentrated to an even greater extent by the dietary-restricted intestine. In addition, dietary-restricted intestine was able to concentrate D-fucose and D-xylose, which sacs of normal rat intestine did not do. For the two amino acids employed, the normally occurring active transport of D-methionine was enhanced by dietary restriction, while transport of

D-histidine against its concentration gradient was achieved by dietary-restricted but not by fully fed intestine. The differences in behaviour of dietary-restricted and fully fed intestine were not due to changes in net water transport. The results show that for a number of substances dietary-restricted intestine has greater powers of active transport than has normal intestine. For many studies, therefore, dietary-restricted rat intestine may be more useful than fully fed rat intestine.

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. The control group were fed *ad libitum* and they weighed about 250 g at the time of experimentation. In addition to rats, some fully fed young adult male golden hamsters (*Mesocricetus auratus*), of body weight about 100 g, were used for D-fucose transport studies. All animals had free access to water at all times. 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 animals 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. The mesentery was then stripped off the small intestine and the duodenum removed. To overcome the problem of variation in transport activity of different regions (Hindmarsh *et al.* 1967), all the small intestine was everted but only the middle fifth of rat intestine, or the middle 9 cm of hamster intestine, was utilized for the making of two sacs. In the case of rats, each sac was about 9 cm in length; for hamsters, each sac was about 4 cm long. The general technique of preparing sacs was that originally described by Wilson & Wiseman (1954*a*) (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. For rats, this serosal fluid volume was about 0·8 ml. for a standard sac and about 2 ml. for an extra-distended one. For hamsters, the initial serosal volume was about 0·5 ml. 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 usually lost serosal fluid during incubation.

Experimental procedure for transport 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, and the stoppered flask shaken (80 oscillations/min, amplitude 5 cm) for 1 hr in a Warburg bath kept at 37° 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 or amino acid concentrations after deproteinization. For sugar estimations, the samples were deproteinized with $ZnSO_4$ and $Ba(OH)_2$. For

amino acid estimations, samples were deproteinized with equal volumes of 0·6 N perchloric acid, centrifuged at 3000 rev/min for 10 min, and the supernatant analysed without neutralization for D-methionine but with neutralization with KOH for D-histidine.

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. The dry weights of the sacs were derived from the initial wet weights by application of a wet weight/dry weight factor of 19·1. This factor was obtained by measuring the wet and dry weights of 12 sacs of normal and dietary-restricted intestine after 1 hr incubation in Krebs–Henseleit (1932) bicarbonate-saline under standard conditions.

For fully fed rats the dry weights were in the range 80–130 mg; for dietary-restricted rats the range was 50–75 mg; and for fully fed hamsters the range was 30–45 mg.

Chemical estimations. When sugars were used separately they were estimated by the colorimetric method of Nelson (1944). When D-glucose plus another sugar were present, the total reducing sugar content was measured by the Nelson (1944) technique and the D-glucose itself was measured by the specific D-glucose oxidase method of Huggett & Nixon (1957), enabling the amount of the second sugar to be obtained by difference. It should be borne in mind that different sugars may reduce the Nelson (1944) reagent to different extents and allowance for such variation must be made.

Methionine was estimated by the colorimetric method of McCarthy & Sullivan (1941) and histidine by the colorimetric method of Macpherson (1946).

In order to determine how much endogenous histidine-reacting material was released by dietary-restricted and normal intestine into the serosal and mucosal fluids during incubation, sacs were shaken for 1 hr in media to which no histidine had been added. The mucosal fluid at the end of the incubation period contained no detectable histidine-reacting material. In the case of the final serosal fluid, fully fed sacs released histidine-reacting substance which amounted to 8%, and dietary-restricted sacs 6%, of the total colour producing material present in experiments in which 2 mM-D-histidine had been added initially.

In experiments with D-methionine, 5 mM amino acid was added to the initial serosal and mucosal solutions, which was sufficient to make any endogenous methionine-reacting substance liberated by the intestine of no consequence.

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

Rates of transport. The amount of test substance 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 μ-mole entering the serosal fluid/100 mg dry weight sac.hr.

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

RESULTS

Fully fed intestine

Transport of sugars. Of the eight D- and four L-sugars employed in this series of experiments, only D-galactose and 3-*O*-methyl-D-glucose were transported against their concentration gradients by sacs of everted mid-small intestine of fully fed rats (Tables 1 and 2). Even when sacs were extra-distended at the start of incubation (initial serosal volume about 2 ml. instead of about 0·8 ml.) to cause some net loss of serosal (inner) fluid (Table 3), the concentrations of D-arabinose, D-fructose, D-fucose, D-glucosamine, D-mannose, D-xylose, L-arabinose, L-fucose, L-sorbose and L-xylose in the final serosal fluid always remained below their final mucosal concentrations. The inability of fully fed intestine to transport these sugars into the serosal fluid sufficiently to yield final serosal/mucosal concentration ratios greater than 1·0 (the starting value) was, therefore, clearly not due to net movement of water into the serosal fluid compartment. Likewise, prevention of net movement of water into the serosal fluid compartment did not increase the final serosal/mucosal concentration ratios achieved when D-galactose and 3-*O*-methyl-D-glucose were being actively transported (Table 1), the concentrative ability being no greater for extra-distended sacs than for standard ones.

The final serosal fluid concentrations and final serosal/mucosal concentration ratios for D-mannose, D-fructose, L-sorbose and D-glucosamine with standard sacs, especially with the first of these, were very low (Table 2). This was due in part to water entry into the serosal fluid, and in part to the metabolism of these sugars by the intestinal tissue. In the case of D-mannose, L-sorbose and D-glucosamine, poor downhill movement across the sac wall from the mucosal fluid helped to keep the serosal sugar concentrations low. For D-fructose, downhill movement from mucosal to serosal solution was easier, so that the concentration of D-fructose in the mucosal fluid fell from an initial 8·33 mM to 7·73 mM, whereas D-mannose, L-sorbose and D-glucosamine remained unchanged in the mucosal solution, their final concentrations being 8·24 mM, 8·31 mM and 8·39 mM respectively. When these sugars were used with extra-distended sacs (Table 3) there was less of a fall in their concentrations in the final serosal fluid because more of each sugar was introduced into the sacs initially (2 ml. instead of 0·8 ml.) and there was no net entry of water into the serosal fluid compartment.

In contrast to the lack of effect of extra-distension on sugars which were transported against their concentration gradients (Table 1), extra-distension caused an appreciable rise in the final serosal/mucosal concentration ratios of the non-actively transported sugars apart from D-fucose

TABLE 1. Sugars transported against their concentration gradients by sacs of everted mid-small intestine of fully fed 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 sugar (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. Extra-distended sacs contained about 2 ml. initial serosal volume. Values are means ± S.E. of the means, with number of sacs in parentheses

	Final concn. of sugar in serosal fluid (mM)	Final concn. of sugar in mucosal fluid (mM)	Transport of sugar into serosal fluid (μ-mole/ 100 mg dry wt. sac.hr)	Gain in serosal fluid water (m-mole/ 100 mg dry wt. sac.hr)	Sugar final concn. ratio (serosal/mucosal)
		Standard sacs			
D-galactose	26·6 ± 0·75	6·70 ± 0·11	18·4 ± 1·1	13·3 ± 1·3	4·01 ± 0·17 (16)
3-*O*-methyl-D-glucose	19·8 ± 0·84	7·25 ± 0·13	14·1 ± 1·5	16·2 ± 2·0	2·78 ± 0·17 (16)
		Extra-distended sacs			
D-galactose	22·2 ± 0·29	6·45 ± 0·10	29·1 ± 1·3	− 3·03 ± 1·08	3·46 ± 0·08 (20)
3-*O*-methyl-D-glucose	17·6 ± 0·67	6·78 ± 0·11	18·7 ± 1·5	− 5·40 ± 1·92	2·61 ± 0·13 (14)

TABLE 2. Sugars not transported against their concentration gradients by sacs of everted mid-small intestine of fully fed rats. Initial serosal (inner) volume 0·8 ml. Other details as in Table 1

	Final concn. of sugar in serosal fluid (mM)	Final concn. of sugar in mucosal fluid (mM)	Transport of sugar into serosal fluid (μ-mole/ 100 mg dry wt. sac.hr)	Gain in serosal fluid water (m-mole/ 100 mg dry wt. sac.hr)	Sugar final concn. ratio (serosal/mucosal)
D-arabinose	5·36 ± 0·14	8·45 ± 0·07	− 0·71 ± 0·13	16·4 ± 2·1	0·64 ± 0·02 (8)
D-fructose	2·67 ± 0·11	7·73 ± 0·06	− 3·12 ± 0·15	11·8 ± 1·6	0·35 ± 0·01 (8)
D-fucose	7·41 ± 0·08	8·16 ± 0·08	1·16 ± 0·14	13·7 ± 1·1	0·91 ± 0·01 (14)
D-glucosamine	4·06 ± 0·06	8·39 ± 0·03	− 2·20 ± 0·08	12·8 ± 0·5	0·48 ± 0·01 (8)
D-mannose	0·68 ± 0·13	8·24 ± 0·06	− 5·39 ± 0·36	11·9 ± 2·6	0·08 ± 0·02 (8)
D-xylose	6·47 ± 0·09	8·48 ± 0·08	− 0·14 ± 0·20	11·2 ± 1·7	0·76 ± 0·01 (8)
L-arabinose	5·50 ± 0·16	8·22 ± 0·04	− 1·46 ± 0·13	6·6 ± 1·2	0·67 ± 0·02 (8)
L-fucose	4·62 ± 0·12	8·31 ± 0·04	− 1·24 ± 0·21	15·6 ± 1·3	0·56 ± 0·01 (8)
L-glucose*	6·54 ± 0·19	8·19 ± 0·06	0·43 ± 0·20	17·4 ± 1·1	0·80 ± 0·02 (13)
L-sorbose	3·65 ± 0·13	8·31 ± 0·05	− 2·49 ± 0·12	11·1 ± 1·3	0·44 ± 0·01 (16)
L-xylose	5·35 ± 0·11	8·38 ± 0·11	− 1·06 ± 0·11	12·8 ± 2·0	0·64 ± 0·02 (8)

* From Neale & Wiseman (1968*c*)

TABLE 3. Sugars not transported against their concentration gradients by sacs of everted fully fed mid-small intestine distended to prevent net water transport. Initial serosal (inner) volume about 2 ml. Other details as in Table 1

	Final concn. of sugar in serosal fluid (mM)	Final concn. of sugar in mucosal fluid (mM)	Transport of sugar into serosal fluid (μ-mole/ 100 mg dry wt. sac.hr)	Gain in serosal fluid water (m-mole/ 100 mg dry wt. sac.hr)	Sugar final concn. ratio (serosal/mucosal)
D-arabinose	7·06 ± 0·09	8·31 ± 0·04	− 3·94 ± 0·23	− 6·55 ± 0·43	0·85 ± 0·01 (16)
D-fructose	4·80 ± 0·16	7·68 ± 0·07	− 7·58 ± 0·39	− 0·70 ± 0·97	0·62 ± 0·02 (8)
D-fucose	7·74 ± 0·13	8·29 ± 0·06	− 1·79 ± 0·31	− 3·69 ± 1·60	0·94 ± 0·02 (12)
D-glucosamine	6·30 ± 0·24	8·39 ± 0·04	− 5·71 ± 0·50	− 7·85 ± 1·64	0·75 ± 0·03 (8)
D-mannose	4·32 ± 0·09	8·20 ± 0·11	− 10·10 ± 0·25	− 9·89 ± 1·87	0·53 ± 0·01 (8)
D-xylose	8·19 ± 0·34	8·38 ± 0·12	− 1·81 ± 0·45	− 9·97 ± 3·70	0·98 ± 0·05 (6)
L-arabinose	7·34 ± 0·14	8·36 ± 0·04	− 3·46 ± 0·23	− 8·52 ± 1·15	0·88 ± 0·02 (16)
L-fucose	6·84 ± 0·13	8·18 ± 0·08	− 4·23 ± 0·22	− 6·35 ± 1·02	0·84 ± 0·02 (16)
L-glucose*	7·89 ± 0·11	8·27 ± 0·06	− 1·95 ± 0·31	− 6·50 ± 0·87	0·95 ± 0·02 (8)
L-sorbose	5·88 ± 0·22	8·28 ± 0·06	− 6·27 ± 0·37	− 6·91 ± 1·13	0·71 ± 0·03 (8)
L-xylose	7·04 ± 0·11	8·40 ± 0·05	− 3·89 ± 0·18	− 6·08 ± 0·93	0·84 ± 0·02 (16)

* From Neale & Wiseman (1968*c*)

(Tables 2 and 3). The latter sugar's final serosal/mucosal concentration ratio was, however, as high as 0·91 even with standard sacs.

As the failure of normal rat intestine to transport D-fucose against its concentration gradient was unexpected, because normal hamster small intestine has been reported by Hindmarsh, Kilby & Wiseman (1966) to do so readily, we repeated these workers experiments using standard sacs of everted mid-small intestine of fully fed golden hamsters. When the initial concentration of D-fucose in the serosal and mucosal solutions was 8·33 mM, the hamster intestine achieved a D-fucose final serosal/mucosal concentration ratio of $1{\cdot}54 \pm 0{\cdot}06$ (S.E. of mean; $n = 8$). For these sacs, the final serosal concentration of D-fucose was $11{\cdot}70 \pm 0{\cdot}45$ mM, the final mucosal concentration was $7{\cdot}61 \pm 0{\cdot}15$ mM, the sugar entry into the serosal fluid was $7{\cdot}99 \pm 0{\cdot}51$ μ-mole/100 mg dry wt. sac.hr, and the water entry into the serosal fluid was $15{\cdot}5 \pm 3{\cdot}4$ m-mole/100 mg dry wt. sac.hr.

Transport of amino acids. Sacs of everted fully fed rat intestine easily transported D-methionine against its concentration gradient, its initial concentration in the mucosal and serosal fluids being purposely kept at a comparatively low level (5 mM, Table 4). That small intestine can actively transport D-methionine, when present at a low initial concentration, has previously been shown by Lin, Hagihira & Wilson (1962), who used sacs of fully fed golden hamster small intestine. Unlike D-methionine, however, we found that the final serosal/mucosal concentration ratio for D-histidine remained at its starting value with fully fed intestine, even though its initial concentration in the incubating media was only 2 mM.

Dietary-restricted intestine

Transport of sugars. Table 5 shows that sacs of everted small intestine of dietary-restricted rats were able to concentrate D-galactose and 3-*O*-methyl-D-glucose to a greater extent than could normal rat intestine. In addition, intestine of dietary-restricted rats transported D-fucose and D-xylose against their concentration gradients, which normal rat intestine (unlike normal hamster intestine, Hindmarsh *et al.* 1966) could not do. Extra-distension of sacs to prevent net water transport did not improve the final serosal/mucosal concentration ratios achieved for these four sugars (Table 5), as was found for D-galactose and 3-*O*-methyl-D-glucose with fully fed intestine (Table 1).

Under our experimental conditions, dietary-restricted intestine failed to transport D-arabinose, D-fructose, D-glucosamine, D-mannose, L-arabinose, L-fucose, L-sorbose or L-xylose against their concentration gradients when either standard sacs (Table 6) or extra-distended ones (Table 7) were used. Extra-distension, however, kept the final serosal/

TABLE 4. Transport of D-methionine and D-histidine by sacs of everted mid-small intestine of fully fed and dietary-restricted rats. Initial serosal (inner) volume 0·8 ml. Initial serosal and mucosal fluids contained equal concentrations of amino acid (initial concentration ratio 1·0). Other details as in Table 1

	Final concn. of amino acid in serosal fluid (mM)	Final concn. of amino acid in mucosal fluid (mM)	Transport of amino acid into serosal fluid (μ-mole/ 100 mg dry wt. sac.hr)	Gain in serosal fluid water (m-mole/ 100 mg dry wt. sac.hr)	Amino acid final concn. ratio (serosal/mucosal)
5 mM-D-methionine and fully fed intestine	9·66 ± 0·35	4·25 ± 0·04	7·78 ± 0·44	20·4 ± 0·7	2·28 ± 0·09 (12)
5 mM-D-methionine and dietary-restricted intestine	16·10 ± 0·40	3·64 ± 0·08	22·40 ± 1·20	23·2 ± 4·9	4·45 ± 0·16 (12)
2 mM-D-histidine and fully fed intestine	2·05 ± 0·04	1·99 ± 0·04	0·78 ± 0·07	19·8 ± 1·1	1·04 ± 0·03 (14)
2 mM-D-histidine and dietary-restricted intestine	2·79 ± 0·09	1·96 ± 0·03	1·74 ± 0·13	11·6 ± 3·2	1·43 ± 0·06 (17)

TABLE 5. Sugars transported against their concentration gradients by sacs of everted mid-small intestine of dietary-restricted rats. Other details as in Table 1

	Final concn. of sugar in serosal fluid (mM)	Final concn. of sugar in mucosal fluid (mM)	Transport of sugar into serosal fluid (μ-mole/ 100 mg dry wt. sac.hr)	Gain in serosal fluid water (m-mole/ 100 mg dry wt. sac.hr)	Sugar final concn. ratio (serosal/mucosal)
		Standard sacs			
D-fucose	10·2 ± 0·48	8·18 ± 0·09	4·4 ± 0·4	13·0 ± 3·5	1·25 ± 0·06 (16)
D-galactose	39·0 ± 1·80	5·87 ± 0·13	47·4 ± 2·9	20·4 ± 3·4	6·77 ± 0·42 (16)
3-*O*-methyl-D-glucose	33·3 ± 1·60	6·60 ± 0·14	37·5 ± 2·1	15·5 ± 3·3	5·06 ± 0·21 (18)
D-xylose	9·8 ± 0·45	8·14 ± 0·03	3·1 ± 0·4	8·8 ± 3·4	1·21 ± 0·06 (12)
L-glucose*	10·5 ± 0·28	8·03 ± 0·06	6·1 ± 0·5	11·7 ± 2·7	1·32 ± 0·04 (15)
		Extra-distended sacs			
D-fucose	10·6 ± 0·37	7·64 ± 0·08	1·5 ± 0·6	− 25·7 ± 4·1	1·39 ± 0·05 (14)
D-galactose	34·1 ± 1·80	5·39 ± 0·16	79·4 ± 8·6	− 22·4 ± 2·5	6·48 ± 0·49 (14)
3-*O*-methyl-D-glucose	27·7 ± 0·85	5·80 ± 0·08	50·2 ± 2·2	− 16·8 ± 1·4	4·80 ± 0·20 (14)
D-xylose	11·1 ± 0·27	8·10 ± 0·06	2·3 ± 0·5	− 30·2 ± 3·7	1·37 ± 0·04 (6)
L-glucose*	11·0 ± 0·20	8·10 ± 0·06	5·6 ± 1·3	− 24·2 ± 4·0	1·36 ± 0·03 (10)

* From Neale & Wiseman (1968c)

TABLE 6. Sugars not transported against their concentration gradients by sacs of everted mid-small intestine of dietary-restricted rats. Initial serosal (inner) volume 0·8 ml. Other details as in Table 1

	Final concn. of sugar in serosal fluid (mM)	Final concn. of sugar in mucosal fluid (mM)	Transport of sugar into serosal fluid (μ-mole/ 100 mg dry wt. sac. hr)	Gain in serosal fluid water (m-mole/ 100 mg dry wt. sac. hr)	Sugar final concn. ratio (serosal/mucosal)
D-arabinose	6·71 ± 0·56	8·35 ± 0·07	− 1·32 ± 0·35	6·28 ± 4·10	0·81 ± 0·07 (8)
D-fructose	4·58 ± 0·30	8·03 ± 0·05	− 2·81 ± 0·45	19·20 ± 4·10	0·57 ± 0·04 (8)
D-glucosamine	5·83 ± 0·44	8·41 ± 0·03	− 2·49 ± 0·53	9·63 ± 3·18	0·70 ± 0·05 (8)
D-mannose	5·90 ± 0·40	8·05 ± 0·08	− 2·89 ± 0·30	2·26 ± 4·07	0·73 ± 0·05 (8)
L-arabinose	6·62 ± 0·50	8·33 ± 0·07	− 1·87 ± 0·19	1·68 ± 3·78	0·80 ± 0·07 (8)
L-fucose	6·48 ± 0·31	7·98 ± 0·11	− 1·63 ± 0·25	4·91 ± 3·28	0·81 ± 0·04 (8)
L-sorbose	4·13 ± 0·14	8·29 ± 0·04	− 3·98 ± 0·15	14·70 ± 1·80	0·50 ± 0·02 (16)
L-xylose	6·16 ± 0·23	8·46 ± 0·04	− 1·02 ± 0·34	14·50 ± 3·70	0·73 ± 0·03 (8)

TABLE 7. Sugars not transported against their concentration gradients by sacs of everted dietary-restricted mid-small intestine distended to prevent net water transport. Initial serosal (inner) volume about 2 ml. Other details as in Table 1

	Final concn. of sugar in serosal fluid (mM)	Final concn. of sugar in mucosal fluid (mM)	Transport of sugar into serosal fluid (μ-mole/ 100 mg dry wt. sac. hr)	Gain in serosal fluid water (m-mole/ 100 mg dry wt. sac. hr)	Sugar final concn. ratio (serosal/mucosal)
D-arabinose	8·44 ± 0·19	8·28 ± 0·02	− 2·78 ± 0·19	− 20·7 ± 2·3	1·02 ± 0·02 (8)
D-fructose	6·96 ± 0·10	7·85 ± 0·12	− 7·89 ± 0·52	− 26·8 ± 3·6	0·89 ± 0·02 (8)
D-glucosamine	7·76 ± 0·26	8·43 ± 0·04	− 5·62 ± 0·34	− 26·6 ± 2·7	0·92 ± 0·03 (8)
D-mannose	8·22 ± 0·23	8·25 ± 0·08	− 4·99 ± 0·54	− 33·2 ± 4·7	1·00 ± 0·03 (8)
L-arabinose	8·60 ± 0·18	8·40 ± 0·05	− 3·27 ± 0·04	− 27·2 ± 1·6	1·03 ± 0·02 (16)
L-fucose	8·04 ± 0·35	8·13 ± 0·10	− 3·83 ± 0·83	− 21·9 ± 1·5	0·99 ± 0·05 (8)
L-sorbose	6·73 ± 0·32	8·15 ± 0·06	− 6·56 ± 0·64	− 17·4 ± 6·0	0·83 ± 0·04 (8)
L-xylose	8·29 ± 0·19	8·28 ± 0·06	− 3·05 ± 0·39	− 19·2 ± 3·0	1·00 ± 0·03 (14)

mucosal concentration ratios for these eight sugars near 1·0, as was noted above for sacs of normal intestine when non-actively transported sugars were employed.

The final serosal concentrations of D-fructose, D-glucosamine, D-mannose and L-sorbose in these sacs of dietary-restricted intestine (Table 6) did not fall as dramatically as with standard sacs of fully fed intestine (Table 2).

Some of the characteristics of D-fucose and D-xylose active transport by dietary-restricted rat intestine are given in Tables 8 and 9. Active transport of both sugars could be completely inhibited by 10^{-6} M phlorrhizin or by replacing the NaCl of the incubating media by mannitol. Also, the presence of 8·33 mM-D-glucose in the initial mucosal and serosal fluids completely stopped the active transport of D-fucose, and probably of D-xylose.

Transport of amino acids. Under the same experimental conditions, dietary restriction enabled sacs of everted intestine to produce a D-methionine final serosal/mucosal concentration ratio of as much as 4·45, compared with a value of 2·28 for fully fed rat intestine (Table 4). Furthermore, dietary-restricted intestine, unlike fully fed intestine, was able to actively transport D-histidine, yielding a final serosal/mucosal concentration ratio of 1·43.

DISCUSSION

In confirmation of earlier reports (Neame & Wiseman, 1959; Kershaw *et al.* 1960; Suda & Shimomura, 1964; Faelli *et al.* 1966; Hindmarsh *et al.* 1967; Dowling *et al.* 1967; Esposito *et al.* 1967; Ziemlański *et al.* 1967; Neale & Wiseman, 1968*a*, *b*, *c*) we have found that when rats were kept on a restricted (but balanced) diet their small intestine developed enhanced ability for active transport. This augmentation was demonstrable not only for substances which were transported against their concentration gradients by normal rat intestine (D-galactose, 3-*O*-methyl-D-glucose and D-methionine) but also for some materials (D-fucose, D-xylose and D-histidine) which, under the experimental conditions, were not concentrated in the serosal fluid of sacs of normal rat intestine. A similar phenomenon has been observed by Neale & Wiseman (1968*a*–*c*) for L-glucose, which was concentrated in the serosal fluid when sacs of intestine from dietary-restricted rats were used but not when the rats had been fed *ad libitum*.

Although fully fed sacs did not actively transport D-xylose under our experimental conditions, as measured by entry of the sugar into the serosal fluid, dietary-restricted sacs were able to achieve a final serosal/mucosal concentration ratio greater than 1·0. This was not unexpected as there are several reports indicating that the absorption of this sugar is in

TABLE 8. Transport of D-fucose by sacs of everted mid-small intestine of dietary-restricted rats. Other details as in Table 1

	Final concn. of D-fucose in serosal fluid (mM)	Final concn. of D-fucose in mucosal fluid (mM)	Transport of D-fucose into serosal fluid (μ-mole/ 100 mg dry wt. sac.hr)	Gain in serosal fluid water (m-mole/ 100 mg dry wt. sac.hr)	D-fucose final concn. ratio (serosal/mucosal)
Under standard conditions	10·20 ± 0·48	8·18 ± 0·09	4·40 ± 0·37	13·00 ± 3·52	1·25 ± 0·06 (16)
10^{-6} M phlorrhizin present	7·16 ± 0·52	8·44 ± 0·11	− 1·33 ± 0·21	1·92 ± 4·59	0·86 ± 0·07 (8)
NaCl replaced by mannitol*	8·05 ± 0·12	8·53 ± 0·04	− 0·20 ± 0·16	0·94 ± 1·25	0·95 ± 0·01 (8)
Initial serosal vol. 2 ml.	10·59 ± 0·37	7·64 ± 0·08	1·47 ± 0·59	− 25·70 ± 4·10	1·39 ± 0·05 (14)
Initial serosal vol. 2 ml. and 8·33 mM-D-glucose present	7·57 ± 0·38	8·51 ± 0·04	− 3·16 ± 1·12	− 8·93 ± 2·34	0·89 ± 0·05 (16)

* 25 mM-$NaHCO_3$ still present.

TABLE 9. Transport of D-xylose by sacs of everted mid-small intestine of dietary-restricted rats. Other details as in Table 1

	Final concn. of D-xylose in serosal fluid (mM)	Final concn. of D-xylose in mucosal fluid (mM)	Transport of D-xylose into serosal fluid (μ-mole/ 100 mg dry wt. sac. hr)	Gain in serosal fluid water (m-mole/ 100 mg dry wt. sac. hr)	D-xylose final concn. ratio (serosal/mucosal)
Under standard conditions	9·81 ± 0·45	8·14 ± 0·03	3·08 ± 0·38	8·82 ± 3·40	1·21 ± 0·06 (12)
10^{-6} M phlorrhizin present	6·00 ± 0·26	8·43 ± 0·02	− 1·09 ± 0·30	17·10 ± 5·60	0·71 ± 0·03 (8)
NaCl replaced by mannitol*	7·16 ± 0·10	8·43 ± 0·02	− 1·12 ± 0·22	2·51 ± 1·53	0·85 ± 0·01 (6)
Initial serosal vol. 2 ml.	11·07 ± 0·27	8·10 ± 0·06	2·34 ± 0·46	− 30·20 ± 3·70	1·37 ± 0·04 (6)
Initial serosal vol. 2 ml. and 8·33 mM-D-glucose present	9·50 ± 0·30	8·69 ± 0·04	1·68 ± 1·00	− 13·30 ± 2·20	1·09 ± 0·04 (16)
Initial serosal vol. 2 ml. and 16·7 mM-D-glucose present	8·55 ± 0·42	8·89 ± 0·11	− 0·88 ± 0·99	− 9·02 ± 2·05	0·96 ± 0·05 (8)

* 25 mM-$NaHCO_3$ still present.

some way facilitated even in the normal animal. In 1940 Larson, Blatherwick, Bradshaw, Ewing & Sawyer stated that D-xylose disappeared from the normal rat intestinal lumen 10 times more rapidly than did L-xylose, and since then absorption by some special (possibly carrier-linked) mechanism has been indicated by the work (using normal animals) of Salomon, Allums & Smith (guinea-pig, 1961), Csáky & Lassen (frog and rat, 1964), Csáky & Ho (rat, 1965), Duthie & Hindmarsh (golden hamster, 1966), Alvarado (golden hamster, 1966) and Lassen & Csáky (bullfrog, 1966). For D-fucose, the inability of normal rat sacs to concentrate this sugar was somewhat surprising, as sacs from the normal golden hamster do so quite well, as was demonstrated by Hindmarsh *et al.* (1966) and has been confirmed in the present report. Dietary restriction, however, enabled rat small intestine to transport D-fucose into the serosal fluid against its concentration gradient, producing a final serosal/mucosal concentration ratio of 1·25.

We believe this to be the first demonstration of transport of D-histidine against its concentration gradient by sacs of intestine. It is of interest that Jervis & Smyth (1959), who examined the behaviour of D-histidine and L-methionine when the amino acids were present either singly or together in the intestinal lumen of anaesthetized rats, recorded that L-methionine decreased the rate of luminal disappearance of D-histidine. More recently, Hindmarsh *et al.* (1966) argued that D-histidine was actively transported by normal hamster intestine, because they found that it acted as an inhibitor of sugar transport, as did amino acids which were known to be actively transported, whereas passively transported amino acids had no such effect.

Extra-distension did not alter the serosal/mucosal concentration ratios at 1 hr for sugars which were transported against their concentration gradients (Tables 1 and 5) and the results indicated that the ratios must have been the maximum (or steady-state) values for the conditions employed. These ratios were, as the results clearly show, independent of whether water was gained or lost by the serosal fluid. The serosal/mucosal concentration ratios at 1 hr for the passively transported sugars, on the other hand, were influenced by extra-distension (Tables 2, 3, 6 and 7), prevention of net water entry into the serosal fluid by extra-distension keeping the ratios comparatively near to the original value of 1·0 (Tables 3 and 7). When net water transport was allowed (Tables 2 and 6), by using standard sacs (initial serosal volume 0·8 ml. instead of 2 ml.), the more rapid uptake of water than of passively transported sugars diluted the latter and caused the serosal/mucosal concentration ratios to fall considerably. Presumably the steady-state concentration ratio for a non-metabolized passively transported sugar is 1·0, although a 1 hr incubation

period was insufficient for this to be attained. It should be emphasized that even though extra-distension caused net loss of water from the serosal fluid, it had neither deleterious nor beneficial effect on the movement of actively transported substances as measured by the final serosal/mucosal concentration ratio. In addition, despite extra-distension causing water to leave sacs, it did not produce a final serosal/mucosal concentration ratio greater than 1·0 for substances which were not actively transported under standard conditions (when sacs gained water), the final ratios for such sugars (Tables 3 and 7) not exceeding 1·0. Thus extra-distension is not a procedure enabling passively transported sugars to appear to be actively transported. The value of the procedure is that it highlights the fact that the final serosal/mucosal concentration ratios for actively transported sugars are independent of whether sacs gain or lose water.

The very low final serosal/mucosal concentration ratios seen for D-mannose, D-fructose, L-sorbose and D-glucosamine were probably due, in part, to metabolism of these sugars by the intestinal wall. Metabolism of D-fructose by intestine is well known (Wiseman, 1964), and according to Duerdoth, Newey, Sanford & Smyth (1965), D-mannose added to the serosal fluid (but not the mucosal fluid) of sacs of fully fed rat small intestine was metabolized sufficiently well to support a water uptake which was comparable to the water uptake noted when D-glucose was used. With regard to D-glucosamine and L-sorbose, Sols (1956) has shown phosphorylation of both sugars by rat intestinal hexokinase, the rate for D-glucosamine being much faster than that for L-sorbose and about three quarters that for D-glucose. Poor rates of passage of D-fructose, D-mannose, D-glucosamine and L-sorbose from mucosal to serosal fluids would, of course, help to keep the concentrations of these sugars low in the final serosal fluid, especially when standard sacs, which gave net water transport, were employed.

Some of the characteristics of D-fucose and D-xylose active transport by rat small intestine are given in Tables 8 and 9. For both sugars, 10^{-6} M phlorrhizin completely inhibited active transport, which was also stopped when the NaCl of the media (but not the $NaHCO_3$, equivalent to 25 mM-Na) was replaced by mannitol. This sensitivity to phlorrhizin was similar to that found by Neale & Wiseman (1968*a*–*c*) for L-glucose and was appreciably greater than that found by Parsons, Smyth & Taylor (1958) for D-glucose, the latter sugar being actively transported by rat intestine in the presence of up to 10^{-5} M phlorrhizin. The active transport of D-fucose and D-xylose was also prevented by D-glucose. The results support the view that all three sugars are transported by the same mechanism (Salomon *et al.* 1961; Alvarado, 1964, 1966; Csáky & Ho, 1965).

It seems likely that the enhanced active transport of D-galactose, 3-*O*-

methyl-D-glucose, D-fucose and D-xylose by dietary-restricted rats was due, at least partially, to the reduced endogenous D-glucose content of the intestinal wall of such animals. Neale & Wiseman (1968*a–c*) found that the endogenous D-glucose content of the mid-small intestine (whole wall) of dietary-restricted rats was only 10·8 mg D-glucose/100 g wet weight of tissue, whereas the value was 37·8 mg D-glucose/100 g wet weight when the animals had been fed *ad libitum*. It can be seen from Tables 8 and 9 that added D-glucose was a powerful inhibitor of the active transport of D-fucose and D-xylose, and Farrar, Small, Bullard & Ingelfinger (1956) have reported that the rate of absorption of D-xylose by the rat *in vivo* was greatly suppressed in the presence of D-fructose and D-glucose. Absorption of D-galactose (Cori, 1926; Fisher & Parsons, 1953), 3-*O*-methyl-D-glucose (Csáky, 1958) and L-glucose (Neale & Wiseman, 1968*a–c*) has also been shown to be lowered by added D-glucose. It is not yet known how endogenous D-glucose affects other actively transported sugars, but it could presumably act by preventing entry into the epithelial cell or exit from the cell (or both). In either case active transport across the epithelial membrane would be reduced.

Even though thinning of the intestinal wall, which occurred during dietary restriction, may allow more rapid downhill passage of substances from the subepithelial space to the serosal fluid, there are a number of reasons for not believing that such thinning was the cause of the augmented active transport. First, Dowling & Booth (1967) have described enhanced glucose absorption by rat small intestine which had actually hypertrophied as a response to extensive intestinal resection. In another study, Dowling *et al.* (1967) observed that stressing the small intestine by feeding a high-bulk low-calorie diet (expanded by the addition of kaolin) resulted in better glucose and water absorption by the jejunum without change in its thickness. In addition, Hindmarsh *et al.* (1967) were unable to demonstrate better active transport by golden hamster small intestine despite considerable thinning brought about by dietary restriction. It is also of interest that on passing down the small intestine of the rat, active transport of D-glucose gets poorer (Hindmarsh *et al.* 1967) even though the intestinal wall gets thinner (Wilson & Wiseman, 1954*b*).

The results show that dietary restriction is accompanied by enhanced intestinal active transport for a number of substances, and for studies on active transport the small intestine of rats kept on a restricted diet can be more useful than normal intestine.

We wish to thank Mr D. Hutson for skilled technical assistance. One of us (R.J.N.) is indebted to the M.R.C. for a Research Scholarship.

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