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FURTHER STUDIES ON INTESTINAL ACTIVE TRANSPORT DURING SEMISTARVATION

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SUMMARY

1. The effect of semistarvation (sufficient to produce a loss of 18–28% of initial body weight) on the active transport of D-glucose and L-histidine by the rat, the guinea-pig and the golden hamster has been investigated by the use of sacs of everted small intestine (from upper jejunum to lower ileum).

2. In the rat and the guinea-pig the dietary restriction resulted in increased active transport in all regions of the small intestine. In contrast, it caused no alteration in active transport in the hamster.

3. The response in the rat was most impressive in the middle-to-lower ileum during D-glucose uptake. Whereas normal sacs from this area appeared unable to move the sugar against its concentration gradient, sacs from semistarved rats did so quite well.

4. Although there was a considerable loss (24–29%) of intestinal dry weight in all three species when the food intake was reduced, shortening of the small intestine was not detectable in the guinea-pig or the hamster and was present to only a minor extent in the rat.

5. Evidence is presented indicating that the enhanced active transport is not merely a reflexion of the thinning of the intestinal wall and that it occurs during complete as well as in partial starvation.

INTRODUCTION

Semistarvation in young adult rats, sufficient to cause a loss of body weight of up to 20%, has been shown to enhance the ability of the small intestine to transport D-glucose and L-histidine against their concentration gradients *in vitro* (Neame & Wiseman, 1959) and to produce faster absorption of these substances *in vivo* (Kershaw, Neame & Wiseman, 1960). The food was deficient only in absolute amount (20–25% of normal intake) but not in its general basic composition. Within the limits of those experi-

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ments, the improvement in active transport was greater when the dietary restriction was more severe and there was a return to normal when the rats were refed. Since that report, Suda & Shimomura (1964) have also recorded augmentation of L-histidine active uptake by sacs of everted small intestine when rats were kept on an inadequate diet. The latter, in their case, contained either 5% casein or 20% gluten (in place of the normal 20% casein) and was given *ad libitum* for up to 15 days, during which time gain in body weight was brought almost to a stop.

In order to determine whether one part of the small intestine is affected more than another we have investigated the influence of semistarvation on the active movement of D-glucose and L-histidine at six equidistant points ranging from the upper jejunum to the lower ileum in the rat, the guinea-pig and the golden hamster. In the first two species the dietary restriction was found to be associated with better than normal transport in all parts of the intestine, while in the hamster no such change was induced in any region. In addition, measurement of the length and the dry weight of the small intestine indicated that variation in its total thickness could not account for the differences in active transport observed.

METHODS

Animals and diets. All the animals were young adult males, their weights being shown in Tables 1 and 2. They were kept in individual cages throughout the experimental period and for a week or so before. The control groups had an unlimited supply of water and food, the latter being diet 86 for rats and hamsters and diet S.G. 1 for guinea-pigs, purchased from Oxoid Ltd., Southwark Bridge Road, London, S.E. 1. The rats on a restricted diet were fed 4 g food/day for 1 week, the hamsters 1 g/day for 1 week and the guinea-pigs 6 g/day for 9 days. All were allowed water *ad libitum*.

The starting body weights of the restricted guinea-pigs (Tables 1 and 2) were obtained after a 24 hr fast as this species has a disproportionate gastrointestinal content.

Preparation of sacs. The animals were killed by a blow on the head, the abdomen and thorax opened by a midline 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. The intestine of the rat and the guinea-pig, which is fairly long, was divided into six equal lengths before being everted with the aid of a glass rod of about 1·5 mm diameter. In the case of the hamster, eversion was performed before the division of the intestine into six equal parts. The technique of preparing sacs was that originally described by Wilson & Wiseman (1954*a*) (and in greater detail by Wiseman, 1961).

For the hamster, sacs were made from more or less the whole of each sixth of intestine, whereas for the rat and the guinea-pig sacs were made from the *middle* 4 *cm* or so of each sixth. The sacs were moderately distended with a known amount (0·4–0·5 ml.) of bicarbonate–saline (equilibrated with 5% CO_2, 95% O_2) in which was dissolved D-glucose (16·7 mM) and L-histidine (2 mM) for the rat and the hamster, but only the sugar for the guinea-pig. The volume of this *initial serosal fluid* was recorded from the 1 ml. tuberculin syringe employed. Each sac was then 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 5% CO_2, 95% O_2, and the stoppered flask shaken (60 oscillations/min for the guinea-pig, 80 for the others, amplitude

5 cm) for 1 hr in a Warburg bath kept at 37° C. At the end of this period each sac was removed from its flask, its surface drained, and its fluid contents (*final serosal fluid*) collected and weighed. A short length of thread ligature left at one end of the sac facilitates the removal of the sac from the flask. This method recovers about 96 % of the serosal fluid (Wiseman, 1955). Samples of initial and final mucosal and serosal fluids were analysed for reducing sugar and histidine (where appropriate) concentrations.

Sacs whose serosal fluid volume decreased during the incubation were rejected.

Dry weight. After being emptied 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. Their dry weights were 20–40 mg.

For the values in Table 3, the whole of each sixth of intestine was dried for 4 hr at 120° C, then extracted to constant weight with diethyl ether and redried.

Measurement of intestinal length. The small intestine was washed out and removed from the animal in exactly the same manner as when sacs were to be made and, minus the duodenum, was put into a long narrow trough containing bicarbonate–saline (equilibrated with 5 % CO_2, 95 % O_2) at room temperature. To enable its length to be measured it was stretched only enough to make it uniformly straight, the procedure always being carried out by the same worker.

D-*glucose and* L-*histidine monohydrochloride.* These were commercial samples of chemically pure grade. The sugar was estimated by the colorimetric method of Nelson (1944) and the amino acid by the colorimetric method of Macpherson (1946).

Concentration ratios. The *final concentration ratio* was the ratio of the D-glucose or L-histidine 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 both D-glucose and L-histidine was 1:1.

Rates of transport of D-*glucose,* L-*histidine and water.* The amounts of D-glucose and L-histidine transported into the serosal fluid during an experiment were calculated (the initial and final concentrations and serosal fluid volumes being known) and the transport rates expressed as μmole D-glucose or L-histidine entering the serosal fluid/100 mg dry weight of 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 of sac/hr.

RESULTS

Transport studies

As can be seen from Figs. 1 and 2, the final (1 hr) concentration ratios and the total amounts of D-glucose and L-histidine transported to the serosal fluid were much greater when sacs were prepared from the small intestine of semistarved rats than from control ones. The phenomenon was evident, for both test substances, throughout the length of the small intestine. In the case of the sugar, sac 5 from semistarved rats (Fig. 1) was able to produce a serosal/mucosal ratio of nearly 2, signifying active transport, whereas normal intestine from this region gave a value of less than 1·0, suggesting only passive transport. The final concentration ratios for D-glucose in sac 6 of the rat, although significantly higher in the experimental than in the control animals, remained below 1·0. For L-histidine (Fig. 2), all regions of the small intestine transported the amino acid

against its gradient. Concomitant with the improved final concentration ratios in the dietary restricted rats, water entry into the serosal fluid was augmented in sacs 2–5 and unchanged in the others. This, together with the raised final concentration ratios, caused a pronounced mid-intestinal peak in the absolute quantities of D-glucose and L-histidine transferred to the serosal fluid.

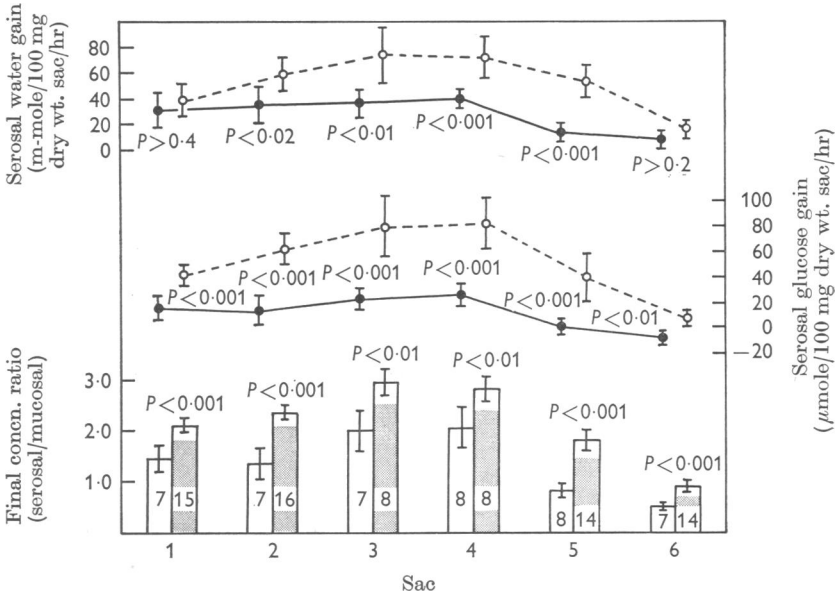

Fig. 1. Effect of dietary restriction on the active transport of D-glucose by sacs of everted small intestine of the rat. Control animals: open columns and continuous lines. Semistarved animals: stippled columns and interrupted lines. Histogram: ratio of D-glucose concentration in final serosal fluid to that in final mucosal fluid (initial ratio was 1:1). Lower two curves: D-glucose entry into serosal fluid. Upper two curves: water entry into serosal fluid. Initial mucosal and serosal fluid contained 16·7 mM D-glucose and 2 mM L-histidine; initial mucosal volume 20 ml.; initial serosal volume 0·4–0·5 ml.; length of sacs about 4 cm. Sac 1 = upper jejunum; sac 6 = lower ileum. Experimental period 1 hr. Temp. 37° C. Values are means $\pm 2 \times$ S.E.M., with number of sacs in each column.

As in the case of the rat, the small intestine of guinea-pigs on a diminished food intake showed enhanced active transport of D-glucose (Fig. 3), the final concentration ratios and the absolute amounts of the sugar entering the serosal fluid being increased in all sacs. Water movement, except in sac 1, was unaffected. Apart from sac 1, all regions of the guinea-pig small intestine seemed to be equally capable of actively taking up D-glucose.

In marked contrast to rats and guinea-pigs, semistarved hamsters displayed no variation in capacity to actively transport either D-glucose or L-histidine even after being allowed as little as 1 g food/day for 1 week

(Figs. 4 and 5). Nor was there any difference in water movement between control and experimental groups. As with the guinea-pig, no intestinal site was considered to be better than any other for active transport of the sugar or the amino acid.

It should be noted that the values for active transport of D-glucose in Figs. 1 and 4 would have been somewhat greater had the media been amino acid-free (Hindmarsh, Kilby & Wiseman, 1966*a*, *b*, *c*).

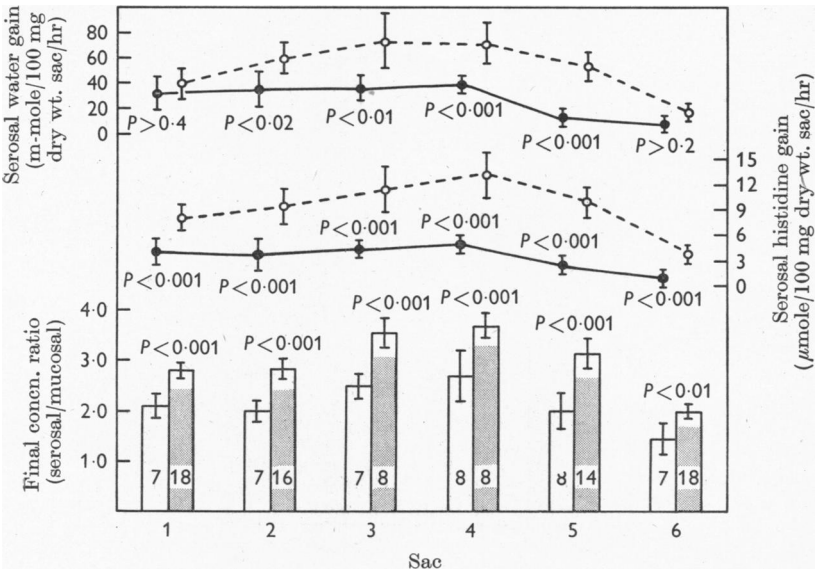

Fig. 2. Effect of dietary restriction on the active transport of L-histidine by sacs of everted small intestine of the rat. Control animals: open columns and continuous lines. Semistarved animals: stippled columns and interrupted lines. Histogram: ratio of L-histidine concentration in final serosal fluid to that in final mucosal fluid (initial ratio was 1:1). Lower two curves: L-histidine entry into serosal fluid. Upper two curves: water entry into serosal fluid. Values are means $\pm 2 \times$ S E.M., with number of sacs in each column. Other details as in Fig. 1.

The body weights of the control and restricted animals used for these transport studies are given in Table 1. The semistarved rats lost about 23% of their weight, the guinea-pigs about 18% and the hamsters about 28%.

Intestinal length and weight studies

The effect of the dietary regimen on the lengths and dry weights of the small intestines of rats, guinea-pigs and hamsters is recorded in Tables 2 and 3. These animals (Table 2) had similar body weights to those (Table 1) employed for the experiments depicted in Figs. 1–5, the restricted rats

losing about 27% of their initial body weight, the guinea-pigs about 18% and the hamsters about 27%.

The appreciable reduction in body weight induced by the dietary restriction was accompanied by a drop in intestinal dry weight, the overall decrease of the latter amounting to about 29% for the rats, 24% for the guinea-pigs and 29% for the hamsters (Table 3). The values for each sixth of intestine indicated that all regions suffered considerably. Despite

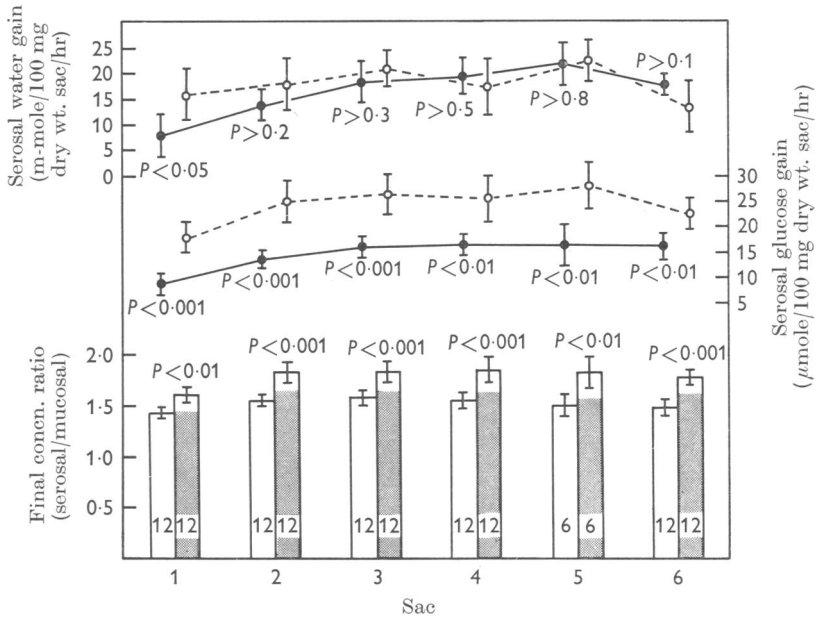

Fig. 3. Effect of dietary restriction on the active transport of D-glucose by sacs of everted small intestine of the guinea-pig. The mucosal and serosal fluids had no added amino acid. Control animals: open columns and continuous lines. Semi-starved animals: stippled columns and interrupted lines. Histogram: ratio of D-glucose concentration in final serosal fluid to that in final mucosal fluid (initial ratio was 1:1). Lower two curves: D-glucose entry into serosal fluid. Upper two curves: water entry into serosal fluid. Values are means $\pm 2 \times$ S.E.M., with number of sacs in each column. Other details as in Fig. 1.

the loss of substance, intestinal shortening occurred only to a small extent in the rats and was not detectable in the guinea-pigs or the hamsters (Table 2). The intestinal lengths in all three species showed remarkably little spread when one considers the relatively low accuracy of the technique utilized and corresponded well with those of Kershaw *et al.* (1960) for control (80 ± 1, S.E.M.) and semistarved (72 ± 1) rats.

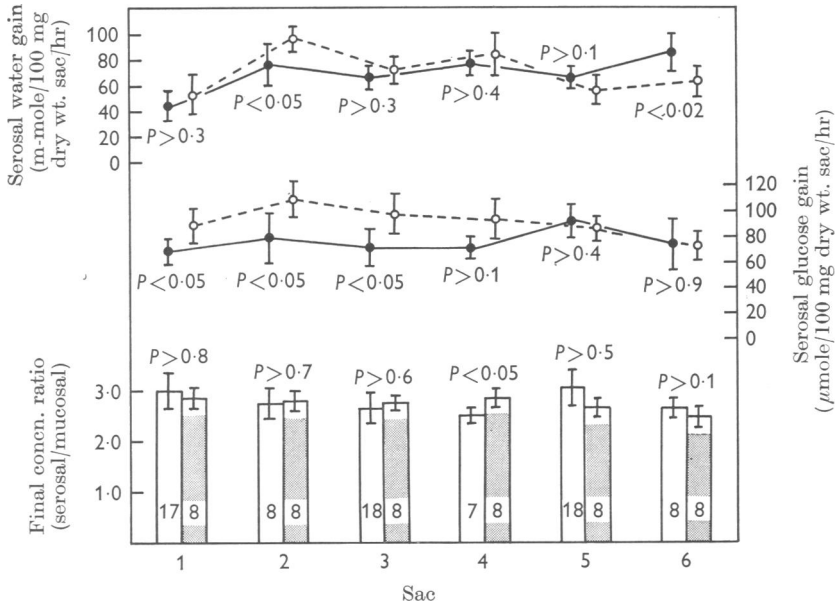

Fig. 4. Effect of dietary restriction on the active transport of D-glucose by sacs of everted small intestine of the hamster. Control animals: open columns and continuous lines. Semistarved animals: stippled columns and interrupted lines. Histogram: ratio of D-glucose concentration in final serosal fluid to that in final mucosal fluid (initial ratio was 1:1). Lower two curves: D-glucose entry into serosal fluid. Upper two curves: water entry into serosal fluid. Values are means $\pm 2 \times$ S.E.M., with number of sacs in each column. Other details as in Fig. 1.

DISCUSSION

In confirmation of earlier work (Neame & Wiseman, 1959; Kershaw *et al.* 1960; Suda & Shimomura, 1964), dietary restriction in the rat improved the active transport of D-glucose and L-histidine by sacs of everted small intestine. That the heightened final (1 hr) serosal/mucosal concentration ratios were not due to a depression of water uptake can be seen from the results in Figs. 1 and 2, the water entry into the serosal fluid being either unchanged or actually increased in the semistarved animals. In addition to the elevated ratios, therefore, the absolute amounts of sugar and amino acid transferred to the serosal fluid were greater. (It is of interest that whereas D-glucose is readily metabolized by the small intestine, L-histidine is not altered to any appreciable extent; Finch & Hird (1960) were able to recover from the intestinal wall 93% of the L-histidine which disappeared from the suspending medium.) Although the phenomenon affected the whole of the small intestine, it was most impressive in sac 5, where control rats gave no evidence of active transport of D-glucose (final ratio less than

1·0), yet restricted ones produced a ratio of nearly 2 (Fig. 1). Under the experimental conditions, sac 6 remained apparently unable to move D-glucose against the gradient but it did keep the ratio close to the original level. It is possible that more severe dietary restriction would unmask the terminal ileum's ability to transport D-glucose actively.

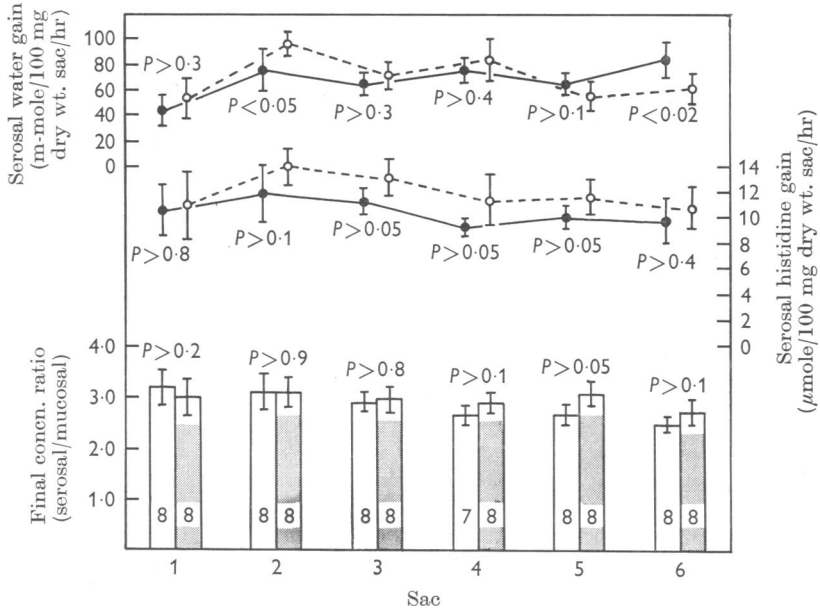

Fig. 5. Effect of dietary restriction on the active transport of L-histidine by sacs of everted small intestine of the hamster. Control animals: open columns and continuous lines. Semistarved animals: stippled columns and interrupted lines. Histogram: ratio of L-histidine concentration in final serosal fluid to that in final mucosal fluid (initial ratio was 1:1). Lower two curves: L-histidine entry into serosal fluid. Upper two curves: water entry into serosal fluid. Values are means $\pm 2 \times$ S.E.M., with number of sacs in each column. Other details as in Fig. 1.

There is good agreement between the results of Kershaw *et al.* (1960) and those of this investigation. The former group of workers made sacs from the proximal half of the small intestine and found that the average final (1 hr) concentration ratios for D-glucose and L-histidine in normal rats were $1{\cdot}44 \pm 0{\cdot}05$ (S.E.M.) and $1{\cdot}81 \pm 0{\cdot}05$ respectively. For semistarved rats of their group C (body weight loss about 20%) the values were $2{\cdot}63 \pm 0{\cdot}06$ (D-glucose) and $3{\cdot}17 \pm 0{\cdot}13$ (L-histidine). In the present report the final ratios for D-glucose and L-histidine in sacs 1–3 (upper half of the small intestine) averaged about 1·6 and 2·2 respectively in control rats, and 2·5 and 3·0 in those on the reduced diet. Likewise, Suda & Shimomura (1964), using sacs of everted small intestine, observed L-histidine concentration ratios of $1{\cdot}81 \pm 0{\cdot}17$ (S.E.M.) when rats had been fed a diet containing

TABLE 1. Body weights of the animals used in Figs. 1–5. Controls were fed *ad libitum*. For dietary restriction, rats were given 4 g food/day for 1 week, hamsters 1 g/day for 1 week and guinea-pigs 6 g/day for 9 days. All had free access to water. Values are means ±S.E.M.

Animal	Treatment	Number	Weight on day of experimentation (g)	Weight loss during dietary restriction before experimentation (g)
Rats	Control	8	209±7	—
	Experimental	25	162±2	47±2
Guinea-pigs	Control	12	317±7	—
	Experimental	11	250±9	55±3
Hamsters	Control	19	100±2	—
	Experimental	8	77±2	30±2

TABLE 2. Body weights and intestinal lengths of animals in Table 3.

Values are means ±S.E.M.

Animal	Treatment	Number	Weight on day of experimentation (g)	Weight loss during dietary restriction before experimentation (g)	Length of small intestine (cm)
Rats	Control	8	219±3	—	74·5±2·3
	Experimental	8	164±4	35±3	64·5±3·5
Guinea-pigs	Control	9	305±7	—	130±1·5
	Experimental	9	261±11	58±3	135±2·5
Hamsters	Control	8	111±3	—	31·0±1·0
	Experimental	9	80±2	29±2	28·4±1·1

TABLE 3. Effect of dietary restriction on the fat-free dry weight of the small intestines of rats, guinea-pigs and hamsters. Controls were fed *ad libitum*. For dietary restriction, rats were given 4 g food/day for 1 week, hamsters 1 g/day for 1 week and guinea-pigs 6 g/day for 9 days. All had free access to water. Each intestine was divided into six equal lengths (1 = upper jejunum; 6 = lower ileum). Body weights and intestinal lengths are shown in Table 2. Values are means ±S.E.M., with number of animals in parentheses

	mg fat-free dry weight/sixth of intestine					
	1	2	3	4	5	6
Control rats (8)	194±18	210±17	200±14	187±14	173±16	171±16
Restricted rats (8)	128±11	136±14	142±13	138±17	139±15	126±10
Percentage reduction	34·0	35·3	29·0	26·2	19·7	26·3
Control guinea-pigs (9)	332±14	301±8	285±10	261±10	234±8	218±5
Restricted guinea-pigs (9)	261±17	213±11	200±13	194±11	186±11	181±9
Percentage reduction	21·4	29·3	29·8	25·7	20·5	17·0
Control hamsters (8)	51·1±2·8	59·2±3·7	66·9±3·5	61·9±4·5	50·2±3·5	44·1±3·3
Restricted hamsters (9)	37·7±2·3	38·1±3·0	43·7±3·3	46·3±4·3	37·2±2·0	34·6±2·0
Percentage reduction	26·2	35·7	34·7	25·2	25·9	21·6

20% casein, but ratios of $3{\cdot}60 \pm 0{\cdot}84$ when the casein had been replaced by 20% gluten for 8 days. Under the latter dietary conditions the rats failed to gain weight.

The absorptive behaviour of fasted rats has also been studied by Levin, Newey & Smyth (1965), some of whose findings have been recalculated and are presented in Table 4. It can be seen that when allowance is made for the amount of glucose metabolized, each gram of fasted intestine took up more of the sugar (about 39 μmole/g wet weight of sac) than did each gram of control intestine (about 26 μmole/g wet weight of sac). Because

TABLE 4. Uptake of D-glucose and glycine from the mucosal fluid by sacs of everted mid-small intestine of normal, sham-operated (as if for adrenalectomy by lumbar approach) and 3-day fasted rats. A recalculation of the results of Levin *et al.* (1965)

	Normal	Fasted	Sham-operated
Water uptake (ml./g wet wt. of sac)	1·24	0·89	1·07
Total glucose uptake (μmole/g wet wt. of sac)	86	54	87
Glucose metabolized (μmole/g wet wt. of sac)	Probably about 60	15	60
Glucose uptake minus glucose metabolized (μmole/g wet wt. of sac)	Probably about 26	39	27
Glycine uptake (μmole/g wet wt. of sac)	53	49	46

water uptake by the fasted intestine was diminished, the final concentration of D-glucose in these sacs (intestinal wall plus serosal fluid) must have been considerably above that in control sacs. For glycine, although the quantity of the amino acid taken up per gram of intestine was much the same in all groups, the final sac concentration (wall plus serosal fluid) was, once again, higher in the starved rats owing to the smaller volume of water absorbed. The results for water absorption in the experiments of Levin *et al.* (1965) should not be compared with those in the present study as our values give only the water appearing in the serosal fluid, while the former workers recorded a composite figure for the water entering the serosal fluid plus that retained in the sac wall. It has been suggested (Wilson, 1962) that the response of the small intestine to semistarvation may be different from that found in complete starvation, active transport being enhanced by the former and depressed by the latter. However, in view of the results in Figs. 1–3, in which the animals were fed daily, and those in Table 4, where the rats were without food (but with free access to water) for 3 days, we believe that no such difference in adaptation occurs.

Guinea-pigs, as well as rats, responded to semistarvation by improved active transport of D-glucose, the final concentration ratios and the absolute amounts of the sugar gained by the serosal fluid being increased throughout the length of the small intestine (Fig. 3). Water entry into the

serosal fluid remained unchanged. In marked contrast to the findings in guinea-pigs and rats, active movement of D-glucose and L-histidine in hamsters was unaffected by the restricted diet (Figs. 4 and 5). This was despite the fact that the hamsters lost as much as 28% of their initial body weight and 29% of the dry weight of the small intestine. We have no explanation for this species variation. It seems improbable that reduced intestinal aerobic glycolysis during semistarvation in the rat could account for the improved active transport of D-glucose observed. First, the rate of aerobic glycolysis in the normal rat is much less in the lower ileum (about 3 μl./mg dry wt. hr) than in the upper jejunum (about 34 μl./mg dry wt. hr) (Wilson & Wiseman, 1954*b*), yet the latter region actively transports D-glucose much better than does the ileum (Fig. 1). A low rate of aerobic glycolysis, therefore, does not necessarily favour good active transport of D-glucose. This can be seen in the normal hamster, where although the distal ileum has a low rate of aerobic glycolysis (about 3 μl./mg dry wt. hr) compared with that of the proximal jejunum (about 11 μl./mg dry wt. hr) (Wilson & Wiseman, 1954*b*), all regions of the small intestine actively transport D-glucose to a similar extent. It is unlikely that reduced aerobic glycolysis due to semistarvation would improve active transport of D-glucose in the rat but do not do so in the hamster.

Although thinning of the intestinal wall during semistarvation (see Wiseman, Neame & Ghadially, 1959, for histological details) may allow more rapid diffusion from the subepithelial space to the serosal fluid, this cannot be the prime cause of the augmented active transport observed. In fact, Kershaw *et al.* (1960) found that the intramural concentration of L-histidine was considerably higher in semistarved sacs than in control ones. Table 3 shows that the fall in dry weight of the small intestine in semistarved hamsters (29%) was as extensive as that taking place in restricted rats (29%) and guinea-pigs (24%). In addition, there was no apparent alteration in intestinal length in the guinea-pigs or the hamsters and only a minor degree of shortening (which would, in fact, make the wall relatively thicker) in the rats. Thinning of about equal magnitude must have occurred, therefore, in the intestinal wall in each species, yet the hamsters showed no change in the final concentration ratios of D-glucose and L-histidine. Further, the dry weight per sixth of *normal* intestine was greater in the proximal part in the guinea-pig and in the middle third in the hamster, but nevertheless sacs from these regions produced high concentration ratios.

The results lead us to the conclusion that in some animals dietary restriction, whether partial or complete, produces better than normal active transport of sugar and amino acid and that the response is not merely a reflexion of the accompanying thinning of the intestinal wall.

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