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THE EFFECT OF SEMISTARVATION ON ABSORPTION BY THE RAT SMALL INTESTINE *IN VITRO* AND *IN VIVO*

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During a period of semistarvation there is a fall in the mass of body cells and a lowering of the basal metabolic rate (Wishart, 1934; Taylor & Keys, 1950; Grande, Anderson & Keys, 1958), the degree to which each change occurs depending upon the duration of the experiment and the severity of the dietary restriction. The loss of cellular mass and the fall in the metabolic rate depend upon the number of calories available, and provided that the caloric restriction is not too severe an equilibrium situation can be obtained. It was of interest therefore to investigate the absorptive ability of the small intestine during semistarvation, as it seemed a vulnerable organ in view of its great demands on the metabolic pool for the frequent replacement of its epithelial lining (Leblond & Stevens, 1948; McMinn, 1954).

We have found that after a period of semistarvation sufficient to cause a loss of about 20% of the initial body weight, there is an enhanced rate of disappearance of both glucose and L-histidine from the small intestine of the rat *in vivo*, and that *in vitro* the small intestine from semistarved animals can transport both these substances against a concentration gradient to a greater extent than can the small intestine of rats fed on an '*ad libitum*' diet. A preliminary report has been given by Neame & Wiseman (1959).

METHODS

Animals and diet. Male albino rats of an inbred strain were used, and were kept individually in separating cages with free access to water. The food used throughout was Diet 86, purchased from The North-Eastern Agricultural Cooperative Society, Ltd., Bannermill Place, Aberdeen, its composition being: soluble carbohydrate 53·4%; protein, 20·0%; fat, 3·8%; fibre, 3·3%; ash, 5·2%; moisture, 14·3%.

All animals were inspected every day and the dietary regimen was as follows:

Group *A* was fed *ad libitum*.
Group *B* was fed 5 g food per rat per day for 5 days.
Group *C* was fed 5 g food per rat per day for 9 days.
Group *D* was fed 5 g food per rat per day for 9 days, and then fed *ad libitum* for 24 hr.
Group *E* was fed 5 g food per rat per day for 9 days, and then fed *ad libitum* for 3 days.
Group *F* was fed 5 g food per rat per day for 9 days, and then fed *ad libitum* for 7 days.

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In vitro experiments

Animals from all groups were used.

Preparation of sacs. The animal was killed by a blow on the head, the abdomen and thorax immediately opened, and the heart incised. The small intestine was then removed and everted, and sacs prepared as described by Wilson & Wiseman (1954). Six sacs (3–4 cm in length) were obtained from each small intestine, and their initial and final volumes were measured as described by Wiseman (1957). Occasionally the serosal volume of a sac decreased: such sacs were discarded.

Experimental procedure. The sac, filled with a known volume of amino-acid-glucose solution (0·5–1·0 ml.), was placed in a 150 ml. Erlenmeyer flask containing 20 ml. of the same solution as that used for filling the sac. The air in the flask was then replaced with a gas mixture of 5 % CO_2, 95 % O_2 and the flask tightly stoppered. The flask and its contents were kept at 37° C and continuously shaken for 1 hr by the use of a Warburg bath (80 oscillations/min, amplitude 5 cm). The sac was then removed from the flask, its surface drained, and its fluid contents recovered and weighed. Samples of initial and final serosal and mucosal fluids were analysed for amino-acid and glucose concentrations. A short length of thread ligature left at one end of the sac greatly facilitates the removal of the sac from the flask.

Concentration ratios. The concentration ratio referred to in the results is the ratio of the concentration of L-histidine or glucose in the serosal fluid (inside the sac of everted intestine) to the concentration in the mucosal fluid (outside the sac).

Amounts of glucose and L-histidine transported. These are expressed as the amounts tranported into the serosal fluid per 100 mg dry weight of sac during the experimental period.

In vivo experiments

Animals from groups *A* (controls), *B* and *C* were used for the study of glucose absorption, and from groups *A* and *C* for L-histidine absorption. The animals were anaesthetized with pentobarbitone sodium B.P. 5 mg/100 g body wt., given intraperitoneally. As soon as anaesthesia had been induced the animal was placed on a warm operating table. The abdomen was opened by a mid-line incision and a transverse cut made in the lower duodenum and at the ileo-caecal junction, so that the whole of the small intestine could be washed out with bicarbonate saline solution (kept at 37° C). The duodenum was ligated about 1 cm distal to the transverse cut and the fluid contents of the intestine gently expressed. A loose ligature was placed around the lower ileum and a blunt needle, attached to a syringe containing glucose or L-histidine solution, was passed through the cut in the lower ileum and the ligature pulled tight over the needle shaft to prevent leakage of the injected 5 ml. test solution. As the needle was slowly withdrawn the ligature closed the intestine, which was then returned to the abdominal cavity. Absorption of the test solution was allowed to take place for a noted time, at the end of which the animal was killed by opening the chest and incising the heart. The small intestine was then removed, its surface carefully washed free from blood and its contents drained into a 50 ml. volumetric flask. The lumen was washed out with bicarbonate saline solution and the washings added to the 50 ml. flask, which was then filled up to the mark. The length of the small intestine was measured, care being taken to avoid stretching.

In some experiments the animals were killed as soon as the test solution had been injected into the intestine, and the latter washed out immediately.

Glucose and L-histidine solutions. D-glucose of Analar grade and L-histidine monohydrochloride of chemically pure grade were dissolved in bicarbonate saline solution (Krebs & Henseleit, 1932). In the *in vitro* experiments one solution was used containing 0·3 % glucose and 2 mM L-histidine. In the *in vivo* experiments either 0·4 % glucose was used or 1 mM

L-histidine with 0·3 % glucose. All solutions were gassed with 5 % CO_2 and 95 % O_2 before use.

Chemical estimations. Glucose was estimated by the colorimetric method of Nelson (1944), and L-histidine by the colorimetric method of Macpherson (1946).

RESULTS

In vitro experiments

Table 1 shows the effect of varying degrees of dietary restriction on the weights of the rats in the various groups. It should be noted that 5 g of food per rat per day represented about 20 % of that eaten per rat per day in the control group. The animals in groups *C–F* lost about 20 % of their starting weight during the period of dietary restriction, but remained otherwise apparently healthy. All the animals in groups *D*, *E* and *F* gained weight on being fed *ad libitum*, the animals in group *F* regaining their initial weight after 7 days.

TABLE 1. Effect of dietary regimen on body weight of rats used for *in vitro* experiments

Group	No. of animals	Nature of dietary regimen: No. of days restricted feeding (5 g/day)	Nature of dietary regimen: No. of days subsequent feeding *ad lib.*	Wt. at start of dietary restriction (g)	Wt. at end of dietary restriction (g)	Wt. after subsequent feeding *ad lib.* (g)
A	6	(Controls. Fed *ad libitum* throughout: final weight 258 ± 16)				
B	6	5	None	269 ± 10	235 ± 8	—
C	6	9	None	285 ± 12	227 ± 8	—
D	6	9	1	277 ± 19	221 ± 17	248 ± 17
E	6	9	3	296 ± 26	217 ± 20	263 ± 23
F	6	9	7	285 ± 18	230 ± 14	295 ± 22

Values are means ± standard deviations.

Table 2 shows the effect of dietary restriction on the ability of the rat small intestine to absorb glucose and L-histidine against a concentration gradient. After 9 days of dietary restriction it was found that the small intestine was able to develop concentration gradients considerably greater than those developed by the control group, and that the quantities of glucose and L-histidine which could be so transported were increased. The small intestine of animals fed on a restricted diet for 5 days was able to transport amounts of glucose and L-histidine, and to develop concentration gradients, intermediate between those of the control group and those of animals which had been fed on a restricted diet for 9 days.

In order to investigate the rate of return of the intestine to its normal state, some animals were fed *ad libitum* for 1, 3 or 7 days after being on the restricted diet for 9 days. It will be seen from Table 2 that there was a

return towards normal in the intestine's ability to concentrate both L-histidine and glucose, even after as short a period as 24 hr on an unrestricted diet (group *D*). After 7 days on an unrestricted diet (group *F*) absorption of glucose and L-histidine by the intestine was virtually normal, while group *E* gave intermediate values. The sudden return to an unrestricted diet had no apparent ill effect on any rat.

The serosal volumes of the sacs from control animals increased by $30 \pm 4\%$, from animals on a restricted diet for 5 days by $45 \pm 5\%$, and from animals on a restricted diet for 9 days by $37 \pm 4\%$ (mean $\pm$ standard error of mean).

Table 3 shows the final concentration of L-histidine in the mucosal and serosal fluids from the sacs of everted small intestine. It will be seen that

TABLE 2. Effect of semistarvation on transport of glucose and L-histidine by sacs of everted small intestine *in vitro*. Initial L-histidine concentration in mucosal and serosal fluid was 2 mM; initial mucosal volume 20 ml.; initial serosal volume 0·5–1·0 ml.; length of sac 3–4 cm. Experimental period 1 hr. Temp. 37° C

Group	No. of sacs	Concentration ratios developed (serosal concn./mucosal concn.)		Amount transported/100 mg dry wt. of sac	
		Glucose	L-histidine	Glucose (mg)	L- histidine (μ mole)
A	35	$1{\cdot}44 \pm 0{\cdot}05$	$1{\cdot}81 \pm 0{\cdot}05$	$0{\cdot}80 \pm 0{\cdot}23$	$2{\cdot}81 \pm 0{\cdot}25$
B	34	$2{\cdot}38 \pm 0{\cdot}07$	$2{\cdot}74 \pm 0{\cdot}14$	$3{\cdot}78 \pm 0{\cdot}32$	$5{\cdot}31 \pm 0{\cdot}60$
C	32	$2{\cdot}63 \pm 0{\cdot}06$	$3{\cdot}17 \pm 0{\cdot}13$	$6{\cdot}59 \pm 0{\cdot}43$	$8{\cdot}30 \pm 0{\cdot}50$
D	33	$1{\cdot}99 \pm 0{\cdot}05$	$2{\cdot}74 \pm 0{\cdot}08$	$3{\cdot}94 \pm 0{\cdot}34$	$6{\cdot}80 \pm 0{\cdot}40$
E	35	$1{\cdot}94 \pm 0{\cdot}05$	$2{\cdot}18 \pm 0{\cdot}07$	$3{\cdot}15 \pm 0{\cdot}28$	$4{\cdot}49 \pm 0{\cdot}33$
F	36	$1{\cdot}52 \pm 0{\cdot}07$	$1{\cdot}77 \pm 0{\cdot}06$	$1{\cdot}24 \pm 0{\cdot}27$	$2{\cdot}59 \pm 0{\cdot}23$

Values are means $\pm$ standard error of means.

in all groups the mucosal fluid showed a fall and the serosal fluid a rise in the concentration of L-histidine. The serosal volume was small compared with the mucosal volume and this enabled a large increase in its L-histidine content to occur without causing more than a relatively small change in the L-histidine concentration of the mucosal fluid. Not all the L-histidine which disappeared from the mucosal fluid was transported to the serosal fluid. If we assume that this fraction was not metabolized, it is possible to calculate its average concentration on the basis of even distribution throughout the intestinal wall, although the actual concentration of L-histidine is presumably higher in the subepithelial space than in the subserosal space and the latter concentration must be higher than that in the serosal fluid. On the assumption that the concentration of L-histidine in the subserosal space is in near equilibrium with that in the serosal fluid, the estimates shown in Table 3 suggest that the L-histidine concentration in the subepithelial space of the sacs from animals on a restricted diet must be considerably higher than that in sacs from control animals.

TABLE 3. Effect of semistarvation on concentration of L-histidine in final mucosal and serosal fluids from sacs of everted small intestine, and estimated concentration in intestinal wall after 1 hr. Initial L-histidine concentration in mucosal and serosal fluid was 2 mM; initial mucosal volume 20 ml.; initial serosal volume 0·5–1·0 ml. Length of sac 3–4 cm. Temp. 37° C

Group	Final mucosal concn. (mM)	Estimated mean concn. in intestinal wall (mM)	Final serosal concn. (mM)
A	1·92 ± 0·02	3·8	3·40 ± 0·08
B	1·72 ± 0·02	9·7	4·66 ± 0·20
C	1·68 ± 0·02	10·5	5·32 ± 0·17
D	1·73 ± 0·02	8·3	4·70 ± 0·11
E	1·72 ± 0·02	9·7	3·72 ± 0·10
F	1·87 ± 0·02	4·0	3·26 ± 0·10

Values are means ± standard error of means.

In vivo experiments

Table 4 shows the effects of dietary restriction on the body weight and the length of the small intestine of the rats used for these experiments. Measurement of intestinal length presents a problem due to muscular contraction and the ease with which the intestine can be stretched. However, under standard conditions the results for the various groups showed relatively little scatter. It will be seen that the degree of loss of weight in these animals was comparable with that found for the animals used in the *in vitro* experiments. It can also be seen that restricting the diet of the

TABLE 4. Effect of dietary regimen on body weight and length of small intestine of rats used for *in vivo* experiments

Group	Test substance	No. of animals	Wt. at start of dietary restriction (g)	Wt. at end of dietary restriction (g)	Length of small intestine (cm)
A	Glucose	38 (Controls. Fed *ad lib.* throughout: final wt. 272 ± 4)			80 ± 5
B	Glucose	39	272 ± 3	229 ± 7	73 ± 5
C	Glucose	37	272 ± 4	209 ± 8	71 ± 4
A	Histidine	34 (Controls. Fed *ad lib.* throughout: final wt. 274 ± 3)			81 ± 6
C	Histidine	34	274 ± 3	207 ± 8	72 ± 6

Values are means ± standard deviations.

animals to 5 g of food per day for either 5 days or 9 days caused a significant decrease in the length of the small intestine. It may also be noted here that the intestine of animals on a decreased diet had a smaller diameter and was considerably thinner than that of animals fed *ad libitum*.

The effect of dietary restriction on glucose and L-histidine absorption is shown in Figs. 1 and 2 respectively. The amount of glucose absorbed was measured for experimental periods of up to 30 min. In the case of L-histidine it was decided to measure the amount absorbed at zero time, and after 10, 20 and 30 min, to enable a statistical evaluation to be made

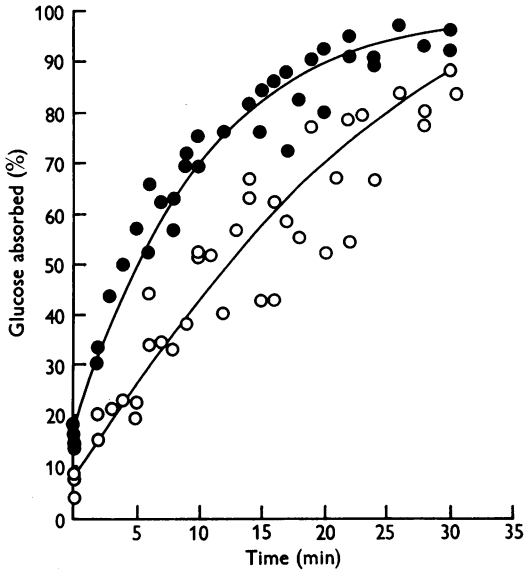

Fig. 1. Effect of dietary restriction on rate of absorption of glucose from whole of small intestine of rat *in vivo*. 5 ml. of 0·4 % glucose in bicarbonate saline solution introduced into each small intestine. ○, rats fed *ad. lib.*; ●, rats on restricted diet 9 days.

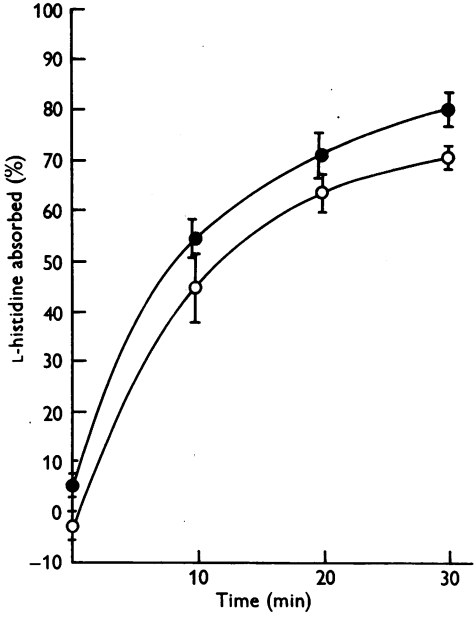

Fig. 2. Effect of dietary restriction on rate of absorption of L-histidine from whole of small intestine of rats *in vivo*. 5 ml. of 1 mM L-histidine with 0·3 % glucose in bicarbonate saline solution introduced into each small intestine. Ten animals in each group for estimation of absorption at zero time; eight animals in each group at 10, 20 and 30 min. Values shown are means and 95 % confidence intervals. Symbols as in Fig. 1.

of the results for each of these periods of absorption. The results plotted in Fig. 2 show means and 95% confidence intervals for each experimental period, i.e. it can be said with 95% certainty that the true mean lies within these limits. Testing the significance of the difference of the means, we found that at zero time $P < 0{\cdot}01$, at 10 min $P < 0{\cdot}02$, at 20 min $P < 0{\cdot}02$ and at 30 min $P < 0{\cdot}01$.

The semistarved animals absorbed better than control animals, the effect being more marked in the case of glucose. Animals on a restricted diet for 5 days absorbed glucose at about the same rate as those on a restricted diet for 9 days and have therefore been omitted from Fig. 1 for the sake of clarity. The amount of glucose absorbed by the semistarved animals after 10 min was about 60% more than that absorbed by the control animals, and after 20 min the corresponding value was about 27%. As almost all the glucose introduced into the intestinal lumen was absorbed in 30 min the difference between the two groups of animals became small at that time. For L-histidine the values at 10 and 20 min were 22 and 11% respectively. At zero time no L-histidine was absorbed by the control animals, whereas an immediate uptake of a small amount occurred in the restricted group. In the case of glucose, there was an immediate absorption of about 8% by the control animals and about 15% by the animals on a restricted diet.

DISCUSSION

Little critical work appears to have been done on the effect of semistarvation on intestinal absorption, although some incidental findings in rats deprived of food have been published. Thus Cori & Cori (1927), Horne, McDougal & Magee (1933), Marrazzi (1940), and Magee (1945) suggest that fasting causes a decrease in glucose absorption in rats *in vivo*, although Heller (1954) found that a 96 hr fast did not do so. However, these workers used very concentrated solutions of glucose and techniques which differed considerably from ours.

In the present paper the results of the *in vivo* experiments show that when 0·4% glucose solution is introduced into the lumen of the whole of the small intestine of semistarved rats, there is a disappearance of glucose at a rate greater than that found with rats fed *ad libitum*. Such an increase in the rate of disappearance of glucose could be due to an increased rate of utilization, increased storage by the intestinal wall, or increased absorption and transfer to the blood. The rate of glucose disappearance from the lumen in the *in vivo* experiments does not necessarily reflect the ability of the intestine to transfer glucose against a concentration gradient, but the latter can be easily measured by the use of sacs of everted small intestine *in vitro*. The results of such experiments show that the small intestine of

the semistarved animal can absorb glucose against its concentration gradient better than normal small intestine. An enhancement of absorption was also obtained when solutions of L-histidine were used, and although the increase in its rate of disappearance *in vivo* is less than that found for glucose the results will be seen to be statistically highly significant. It is interesting to note that the rate of disappearance of both glucose and L-histidine is enhanced *in vivo* in semistarved rats, despite the occurrence of a reduction in the length of the small intestine. From the *in vitro* experiments it is clear that the enhancement of transport of glucose and L-histidine is not due to a decrease in the ability of the intestine to absorb water. As was noted in the Results, the concentration of L-histidine in the subepithelial space must have been greater than that in the subserosal space of the sacs of everted small intestine. The rate of diffusion of L-histidine into the serosal fluid in such sacs will depend therefore on its subepithelial concentration and also on the barrier to diffusion presented by the intestinal wall. *In vitro*, therefore, the thinner intestinal wall found in the semistarved animals could conceivably exaggerate the difference between the absorptive powers of semistarved and control animals. In the case of absorption *in vivo*, however, the very close relationship between the villous epithelium and subepithelial capillaries is unaltered by dietary restriction and the enhanced rate of absorption of glucose and L-histidine in the semistarved animals *in vivo* cannot be exaggerated by a thinner intestinal wall. A more rapid rate of diffusion from the subepithelial space into the capillaries would, however, be brought about by the higher concentrations of glucose and L-histidine which are produced in this space in the animals on a restricted diet.

A sudden return to an unrestricted diet caused no ill effect after 9 days on a severely reduced diet. This is in agreement with the observation of Sun (1927) who found no ill effects on refeeding mice after starvation. These results are in contrast to the view that feeding *ad libitum* after a period of severe semistarvation may be fatal. Hehir (1922) has stated that in severe starvation there is a point of no return, despite provision of a diet adequate in calories. However, the results of the numerous investigations in Europe at the end of the 1939–45 war (Leyton, 1946; Mollison, 1946; Murray, 1947) would suggest that his view is incorrect in the absence of avitaminosis. The findings of Leyton (1946) are of interest in this respect as they show that in the absence of disease semistarved prisoners in German camps (who had lost at least 20% of their original weight) were able to eat immediately and without any ill effects an unrestricted diet provided that it had an adequate vitamin content.

SUMMARY

1. The effect of varying degrees of dietary restriction on the absorption of glucose and L-histidine by the small intestine of the rat has been studied *in vitro* and *in vivo*.

2. It was found that during a period of semistarvation some compensatory mechanism is brought into play which increases the ability of the small intestine to absorb glucose and L-histidine against a concentration gradient. The increase in active absorption disappeared after a few days on an '*ad libitum*' diet.

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