Absorption of L-histidine and glucose from the jejunum segment of the pig and its diurnal fluctuation

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(*Received 2 January 1975 – Accepted 21 March 1975*)

1. Flow rate of digesta and its components in the upper jejunum, and the absorption of L-histidine and glucose from the jejunum segment were measured in pigs fitted with three simple cannulas. The pigs were fed once daily at 08.30 hours.

2. A maximum flow of digesta was obtained in the period 10.00–10.30 hours; the flow rate decreased with time after feeding, reaching a minimum in the period 22.00–22.30 hours.

3. The absorption rate for L-histidine and glucose increased in a hyperbolic manner with increasing concentrations of infused test material, which ranged from 2·5 to 20 g/l for each material.

4. L-histidine and glucose were absorbed nearly independently when perfused in combination. The absorption rates for glucose were significantly ($P < 0{\cdot}01$) greater than the corresponding rates for L-histidine at each concentration of infusate.

5. The absorption of both L-histidine and glucose expressed as a percentage of the amounts in the perfusate decreased with increasing flow rate of perfusate, from 400 to 800 ml/h. The increase in flow rate from 400 to 800 ml/h was associated with a 20 % increase in L-histidine absorption rate; there was a 30 % increase in glucose absorption rate when the flow rate was increased to 600 ml/h, but no further increase at 800 ml/h.

6. The absorption of both L-histidine and glucose decreased with time after feeding; the absorption rates for L-histidine and glucose measured for the period 10.00–10.30 hours were 126 and 133 %, respectively, of those measured for the period 22.00–22.30 hours.

Some rhythmic phenomena in intestinal metabolism are known to be related to periodicity of food intake. For example, the daily rhythmic changes in the level of [^{14}C]cycloleucine injected subcutaneously (Baril & Potter, 1968) and in leucylnaphthylamidase and α-glucosidase (*EC* 3.2.1.20) activities for rat intestine are attributable to the pattern of food intake (Saito, 1972). Also, daily rhythmic changes in the active transport of L-histidine by everted sacs of rat small intestine (Furuya & Yugari, 1971) and in the in vivo absorption of L-histidine and glucose through the intestine of anaesthetized rats (Furuya & Yugari, 1974) have been shown to be related to cyclic food intake. Similar phenomena may be expected to occur in other species, but in the published studies on intestinal absorption in man and other species little emphasis has been placed on the daily rhythmic change.

Although re-entrant cannulation of the small intestine has been used extensively in ruminants to yield quantitative information about intestinal absorption (e.g. Coombe & Smith, 1973), little information appears to have been obtained for the pig. This paper reports studies on some quantitative aspects of L-histidine and glucose absorption from the jejunum segment of the pig fitted with simple cannulas, and also on the diurnal fluctuations in L-histidine and glucose absorption with time after feeding.

Table 1. *Composition of the experimental diet fed to pigs (g/kg)*

Ingredient	
Ground maize	435·5
Sorghum grain	100
Wheat meal	150
Soya-bean meal	140
White fish meal	60
Skim milk	50
Dried brewer's yeast	20
Sucrose	20
Calcium carbonate	6
Dicalcium phosphate	8
Sodium chloride	4·5
Vitamin and mineral mixture*	5
DL-methionine	1
Proximate composition	
Dry matter	884
Crude protein (nitrogen × 6·25)	199

* Providing (/kg diet): retinol 22·5 mg, cholecalciferol 25 μg, thiamin 1·5 mg, riboflavin 5 mg, cyanocobalamin 12 μg, pantothenic acid 15 mg, nicotinic acid 30 mg, choline 500 mg, manganese 50 mg, zinc 150 mg, copper 200 mg, iron 100 mg, iodine 1 mg.

EXPERIMENTAL

Animals

Three female pigs, 10–15 weeks old and 25–30 kg live weight at the time of operation, were used. They were fitted with three simple cannulas in the upper jejunum (cannula 1, 1·0 m beyond the pylorus; cannula 2, 0·20 m distal to cannula 1; cannula 3, 1·0 m distal to cannula 2). Lengths of polyethylene tubing (8 mm i.d., 40 mm long) were used to construct the tubular stems of the cannulas. Each stem was fitted with a gutter-type flange prepared from a 50 mm section of polyethylene tubing (8 mm i.d.). The surgical technique was similar to that of Furuya, Takahashi & Omori (1974) which was modelled on that of Markowitz (1954). Each cannula was exteriorized through the body wall in the region of the left flank; cannula 2 was exteriorized dorsal to cannulas 1 and 3 so that the test solutions infused through cannula 2 could flow by gravity toward cannula 3.

Within 1 week, all the pigs had recovered from surgery and had returned to their pre-surgery dietary intakes. The pigs were kept individually in metabolism cages and were housed in an animal room with the temperature controlled at 23–25°.

Diet and feeding regimen

The pigs were given 40 g food/kg body-weight once daily at 08.30 hours. The pigs were weighed once/week and the amount of food given was adjusted. The composition of the diet is shown in Table 1. Each meal was given as a mash, mixed with twice its weight of water. Drinking water was freely available.

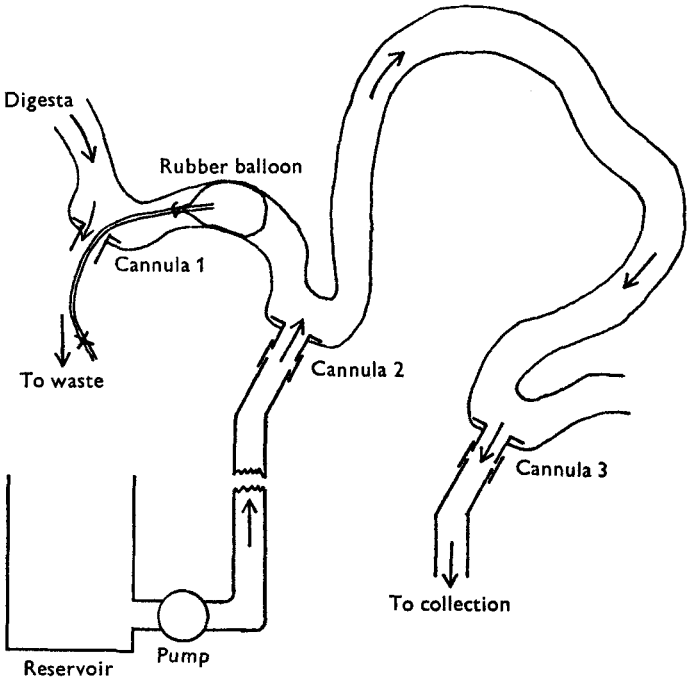

Fig. 1. Perfusion system for a jejunum segment, used in Expts 2, 3 and 4 to study the absorption of L-histidine and glucose in pigs fitted with three simple cannulas.

Experimental procedures

Expt 1. *Digesta flow and rate of passage of nutrients in the upper jejunum.* Three pigs (nos. 1, 2 and 3, weighing 28·0, 27·5 and 26·0 kg respectively) were used in this experiment. Digesta were collected three times/d, 10.00–10.30, 16.00–16.30 and 22.00–22.30 hours, by removing the rubber stopper from cannula 1. Passage of digesta beyond cannula 1 was prevented by placing a rubber balloon inflated with 15 ml water between cannula 1 and cannula 2. This procedure was based on the intubation technique used in man (Blankenhorn, Hirsch & Ahrens, 1955; Cummins & Jussila, 1955). Intestinal contents were allowed to flow from cannula 1 through a soft rubber tube (20 mm i.d., 500 mm long) and collected for 30 min in a graduated measuring cylinder. Samples were stored at $-5°$. The remainder of the digesta was not returned to the pigs. The amount of dry matter flowing from cannula 1 in three measurements/d did not exceed 10% of that ingested daily. Three collections were made on alternate days and combined samples for 3 d for each time of measurement were used for analysis of dry matter, total nitrogen, non-protein-N and free reducing sugars.

Expt 2. *Effect of concentration of* L-*histidine and of glucose solutions on their absorption from the jejunum.* Two pigs (nos. 2 and 3) were used in this experiment after they had been used in Expt 1. The perfusion system used by Freeman, Noakes, Annison & Hill (1968) for pigs prepared with double re-entrant fistulas, was modified in the present study for the pigs fitted with three simple cannulas (Fig. 1).

Test solutions used for this experiment contained (g/l): 2·5, 5, 10 or 20 L-histidine (in the form of L-histidine HCl); or 2·5, 5, 10 or 20 glucose; or a mixture of 2·5, 5 or 10 g each L-histidine and glucose, in sodium phosphate buffer (43·3 mM-Na_2HPO_4, 18·2 mM-NaH_2PO_4; pH 7·15–7·20) with 2 g polyethylene glycol (PEG; molecular weight about 4000)/l, and a sufficient amount of NaCl to adjust the total osmolarity to about 320 mosmol/l. For the test solution containing 20 g L-histidine/l, the buffer was diluted to obtain isotonicity with the other solutions.

The solutions were infused sequentially through cannula 2 at a flow rate of 600 ml/h for 30 min, using a porportioning pump. The infused solutions were passed through a vial heated in a water bath maintained at 38°. To prevent intestinal contents entering the intestine caudal to cannula 2, a rubber balloon was inserted into the intestine between cannula 1 and cannula 2 and distended with about 15 ml water. Effluent was collected from cannula 3 in a graduated measuring cylinder for the last 15 min of each perfusion period. Preliminary experiments had shown that a steady-state was achieved at this time. Occasionally the effluent ceased to flow from cannula 3, and the perfusion period was then prolonged until normal flow was restored.

There was little contamination of the effluent by intestinal contents when the rubber balloon was correctly positioned between cannulas 1 and 2. A control perfusion was done each day, with a solution which did not contain L-histidine or glucose, and these compounds were hardly detected (less than 0·1 g of each compound/l) in the control perfusate.

The digesta which flowed from cannula 1 during the perfusion period were not returned to the animal. Apart from the perfusion periods, normal flow of digesta was maintained by taking out the rubber balloon and putting a stopper into each cannula.

Four of the eleven test solutions were used in random order each day between 13.00 and 16.00 hours, and the four solutions were perfused one after the other. Experiments with the same solutions were repeated at least three times on different days. Absorption showed a tendency to decrease with perfusion order, but this was not statistically significant.

The mucus contained in the effluent was removed by centrifuging for 10 min at 1250 *g*, and the samples were stored at $-5°$ for analysis.

Expt 3. *Effect of flow rate on the absorption of* L-*histidine and glucose from the jejunum.* The same pigs as those used in Expt 2 were used. A solution containing 10 g each of L-histidine and glucose/l sodium phosphate buffer containing 2 g PEG/l and adjusted to about 320 mosmol/l with NaCl, was infused at flow rates of 400, 600 and 800 ml/h for 30 min. The other perfusion conditions were identical to those of Expt 2. Three different flow rates were tested between 14·00 and 16·00 hours, in randomized order. Experiments with each flow rate were repeated at least three times on different days. No significant correlation between perfusion order and rate of absorption was found.

Expt 4. *Diurnal fluctuations with time after feeding in absorption of* L-*histidine and glucose from the jejunum.* Three pigs (nos. 1, 2 and 3, weighing 38·1, 33·4 and 31·2 kg respectively) were used in this experiment. Absorption of L-histidine and glucose was measured simultaneously three times/d, at 10.00–10.30, 16.00–16.30 and 22.00–22.30 hours, using the solution used in Expt 3. The flow rate of the test solution was

600 ml/h. The other perfusion conditions were the same as those described in Expt 2. For each pig, absorption of L-histidine and glucose was measured on alternate days on two occasions.

Calculation of absorption of L-*histidine and glucose from the jejunum.* Disappearance of the test material from the jejunum segment (1 m long at the time of operation) was considered to represent absorption, and was calculated as described by Freeman *et al.* (1968). The absorption (A_x; %) of test material (x) is given by the equation:

$$A_x = \left(1 - \frac{PEG_1}{PEG_2} \times \frac{C_2}{C_1}\right) \times 100,$$

where PEG_1 and PEG_2 (g/l) are the concentrations of PEG entering and leaving the jejunum respectively; C_1 and C_2 (g/l) are the concentrations of test material entering and leaving the jejunum respectively. The absorption rate (R_x; g/h) for x is given by the equation:

$$R_x = \frac{A_x}{100} \times R_1 \times C_1,$$

where R_1 (l/h) is the rate of entry of perfusate.

Average recovery for PEG was $0{\cdot}989 \pm 0{\cdot}017$ (SEM) for 117 perfusion experiments done in the present study.

Analytical methods

Analysis of the intestinal contents

Moisture content and total N. Moisture content was determined by drying at 105° for 6 h and total N was estimated using the Kjeldahl method (Association of Official Analytical Chemists, 1970).

Non-protein-N. The intestinal samples were centrifuged for 10 min at 1250 *g*, and 3 ml supernatant fraction was added to 15 ml picric acid (10 g/l), shaken for 2 min, and centrifuged for 10 min at 1250 *g*. The supernatant fraction was passed through a column of Dowex 2 (× 8, Cl^--form) to retain the picric acid, and the effluent was used for N determination by the Kjeldahl method.

Free reducing sugars. A portion of the supernatant fraction from the intestinal samples was deproteinized by the addition of 2 ml each of zinc sulphate (50 g/l) and 0·15 M-barium hydroxide, and the free reducing-sugar content was determined by a modification of Hoffman's (1937) method adapted for an AutoAnalyzer system (Technicon Instruments Corporation, 1967), assuming all free reducing sugar in the intestinal contents was glucose. Results of the analysis for sugars by ion-exchange chromatography (Model 034 general-purpose liquid chromatograph, with 2630 resin; Hitachi, Tokyo, Japan), for a typical sample of intestinal contents taken approximately 5 h after feeding indicated that more than 90% of the total free reducing sugar determined by the modification of Hoffman's (1937) method was glucose.

Table 2. *Expt* 1. *Rate of flow of digesta, and rate of passage and concentration of dry matter* (DM), *total nitrogen, non-protein-N and free reducing sugars for digesta from the upper jejunum of pigs fitted with three simple cannulas*

(Mean values with their standard errors for three pigs. Pig nos. 1, 2 and 3 were given 1120, 1100 and 1040 g diet, respectively, once daily at 08.30 hours)

Period of measurement (hours)	Rate of flow of digesta (g/h)		Concentration in digesta (g/kg)							
			DM		Total N		Non-protein-N		Free reducing sugars	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
10.00–10.30	958	241	100·0	9·9	4·72	0·07	1·88	0·15	14·3	1·4
16.00–16.30	405	45	74·1	3·7	4·24	0·57	2·22	0·04	12·7	0·7
22.00–22.30	286	31	59·5	16·4	2·75	0·47	1·64	0·11	10·2	1·3

Period of measurement (hours)	Rate of passage in digesta (g/h)							
	DM		Total N		Non-protein-N		Free reducing sugars	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
10.00–10.30	93·5	14·7	4·51	1·07	1·83	0·59	13·4	2·09
16.00–16.30	30·2	4·9	1·74	0·42	0·90	0·12	5·11	0·29
22.00–22.30	17·5	6·6	0·80	0·22	0·47	0·08	2·94	0·67

Analysis of the input and effluent solutions

PEG content was determined using the method of Smith (1958), L-histidine content was estimated by a modification (Furuya & Yugari, 1971) of Macpherson's (1946) method. The glucose content was estimated from the total reducing-sugar content as measured by the modification of Hoffman's (1937) method (Technicon Instruments Corporation, 1967).

Statistical analysis

The significance of differences between the mean values was assessed using the paired *t* test (Snedecor, 1956).

RESULTS

Expt 1. *Diurnal fluctuations in flow rate of digesta and its components along the upper jejunum.* The results are given in Table 2. A maximum flow of digesta along the upper jejunum (958 g/h) was obtained in the period 10.00–10.30 hours; the flow rate of digesta decreased with time after feeding, to 286 g/h for the period 22.00–22.30 hours. Also, concentrations of the components in digesta decreased with time, except that for non-protein-N, for which the maximum value was obtained in the period 16.00–16·30 hours. Accordingly, flow rates for each component were found to have a diurnal fluctuation; flow rates for dry matter, total N, non-protein-N and free reducing sugars for the period 22.00–22.30 hours were 19, 18, 26 and 22% respectively, of those for the period 10.00–10.30 hours.

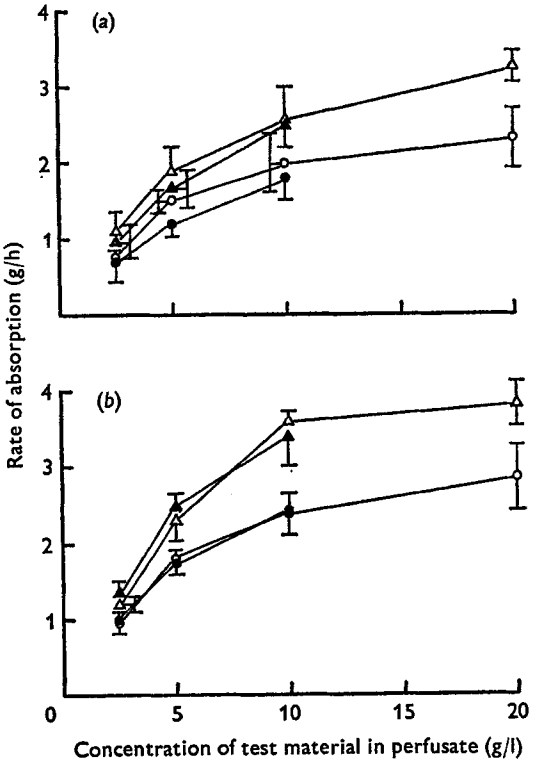

Fig. 2. Expt 2. Rates of absorption for L-histidine and glucose for pig no. 2 (*a*) and pig no. 3 (*b*) when these test materials were perfused alone (L-histidine (○), glucose (△)) or in combination (L-histidine (●), glucose (▲)) at a rate of 600 ml/h through a jejunum segment (1 m long). Mean values for three or more measurements, with their standard errors represented by vertical bars. For details of perfusion system, see Fig. 1 and p. 270.

Expt 2. *The effect of concentration on the absorption of* L-*histidine and glucose from the jejunum.* The results obtained for pig nos. 2 and 3 are given in Fig. 2*a* and *b* respectively. The results show that, when L-histidine and glucose were perfused separately or in combination through the jejunum, the absorption rate for these compounds increased in hyperbolic manner as the concentration of solutions perfused was increased.

Perfusion with a mixture of L-histidine and glucose was also studied to determine whether the materials were absorbed independently or whether there was competition between these materials for absorption. The results (Fig. 2) suggested that, within the range of concentrations used, L-histidine and glucose did not compete for absorption sites.

The absorption rates for glucose were significantly ($P < 0{\cdot}01$) greater than the corresponding rates for L-histidine at each concentration of infusate.

Expt 3. *The effect of flow rate on the absorption of* L-*histidine and glucose from the jejunum.* As the flow rate was increased from 400 to 800 ml/h, the absorption (%) of both materials decreased proportionately (Table 3). The rate of absorption of L-

Table 3. *Expt 3. Effect of flow rate on the absorption of* L-*histidine and glucose from a test solution* containing* 10 *g of each per l, perfused† through a jejunum segment of pigs fitted with three simple cannulas*

(Mean values for two pigs)

Flow rate of test solution (ml/h)	Absorption (%)		Rate of absorption (g/h)	
	L-histidine	Glucose	L-histidine	Glucose
400	46·3	68·2	1·85	2·73
600	32·1	59·2	1·93	3·55
800	28·0	44·3	2·24	3·54

* Sodium phosphate buffer (43·3 mM-Na_2HPO_4, 18·2 mM-NaH_2PO_4; pH 7·15–7·20), containing 2 g polyethylene glycol (molecular weight about 4000)/l and adjusted to 320 mosmol/l with NaCl.
† For details of perfusion system, see Fig. 1 and p. 270.

Table 4. *Expt 4. Effect of period of measurement on the absorption of* L-*histidine and glucose from a test solution* containing* 10 *g of each per l, perfused† at a rate of* 600 *ml/h through a jejunum segment of pigs fitted with three simple cannulas*

(Mean values with their standard errors for three pigs)

Period of measurement (hours)	Absorption (%)				Rate of absorption (g/h)			
	L-histidine		Glucose		L-histidine		Glucose	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
10.00–10.30	36·7	2·2[a]	60·2	5·3[a]	2·20	0·10[a]	3·61	0·19[a]
16.00–16.30	34·3	2·0[a]	52·7	5·5[a]	2·06	0·10[a]	3·16	0·29[a]
22.00–22.30	29·2	1·8[b]	45·2	5·1[b]	1·75	0·09[b]	2·71	0·26[b]

[a, b] Values in the same column with different superscripts are significantly different ($P < 0·01$).
* Sodium phosphate buffer (43·3 mM-Na_2HPO_4, 18·2 mM-NaH_2PO_4; pH 7·15–7·20), containing 2 g polyethylene glycol (molecular weight about 4000)/l and adjusted to 320 mosmol/l with NaCl.
† For details of perfusion system, see Fig. 1 and p. 270.

histidine increased slightly with increasing flow rates, but no increase in the rate of absorption of glucose occurred when the flow rate was increased from 600 to 800 ml/h.

Expt 4. Diurnal fluctuations in the absorption of L-*histidine and glucose from the jejunum.* When a test solution containing 10 g each of L-histidine and glucose/l was perfused through the jejunum segment, their absorption fluctuated; it was high in the morning (10.00–10.30 hours) and low at night (22.00–22.30 hours) (Table 4). The patterns for L-histidine and glucose absorption were synchronous, and the absorption rates for L-histidine and glucose measured in the morning were 126 and 133% respectively, of those at night. Absorption (%) and absorption rates (g/h) for L-histidine and glucose measured at night were significantly ($P < 0·01$) lower than those for the periods 10·00–10·30 and 16·00–16.30 hours.

DISCUSSION

The evidence for the carrier-mediated transport of hexoses from the small intestine in vivo have been derived from the finding of a non-linear relationship between the concentrations of infused test materials and the increase in their absorption rates, for the dog (Annegers, 1964), for man (Holdsworth & Dawson, 1964), for the rat (Lifshitz, Hawkins, Diaz-Bensussen & Wapnir, 1972) and for the calf (Coombe & Smith, 1973), Also, a non-linear relationship for lysine has been found for man (Hellier, Perrett & Holdsworth, 1970).

The results of the present study with pigs have indicated that the absorption rates for L-histidine and glucose from a solution perfused through the jejunum segment of the pig increase in a hyperbolic manner with increasing concentration in the perfusate, and thus support the hypothesis for carrier-mediated transport of L-histidine and glucose.

However, since complete saturation of the absorption process was not achieved in this experiment (Fig. 2), simple diffusion may also take place at high concentrations of the perfused test materials because of the very high lumen-to-blood gradient, as suggested for the calf (Siddons, Smith, Henschel, Hill & Porter, 1969; Coombe & Smith, 1973). The extent of simple diffusion processes in the absorption of L-histidine and glucose remains obscure.

Interaction of sugars and amino acids during intestinal transport has recently attracted much attention; many hypotheses for the mechanism have been put forward, as reviewed by Semenza (1971). In in vitro studies, the effects of glucose on amino acid transport vary from inhibition to stimulation or even no effect (Hardcastle, Newey & Smyth, 1968). Newey & Smyth (1964) have suggested that sugar and amino acid transport systems use energy from a common source of limited availability in vitro, and that the variable effects of glucose on amino acid transport are explained by the differences in the metabolic state of glucose.

The results of the present in vivo study with pigs suggested that L-histidine and glucose have little or no inter-reaction during absorption from the jejunum segment. This agrees with the hypothesis that there is no competition for energy for intestinal transport; in vivo a rich supply of energy to the epithelial cells is available through the mesenteric blood. The present result is in agreement with the in vivo findings for rats (Bingham, Newey & Smyth, 1966), but differ from those for man (Cook, 1971) where it was found that glucose and glycine each significantly inhibited the other's absorption, although the experimental techniques were different from those in the present study.

Diurnal changes in the active transport of L-histidine from the everted sacs of rat intestine (Furuya & Yugari, 1971) and of L-histidine and glucose absorption in vivo in rat intestine (Furuya & Yugari, 1974) have been reported previously; the same phenomenon was found in the present study with conscious pigs.

However, the extent of the diurnal fluctuation in the in vitro rat experiment (Furuya & Yugari, 1971) was greater than that found in the present experiment; in the latter, the absorption of L-histidine and glucose in the morning was respectively only 26 and

33% higher than that at night when pigs were fed once daily at 08.30 hours, while in the former, the L-histidine concentration ratio (serosal:mucosal) fluctuated from 1·9 at night to 4·3 in the morning when rats were trained to eat between 09.00 and 15.00 hours. This difference may be due to the mechanisms participating in the absorption process, since in the present study, with high concentrations of test material in the perfusate, a simple diffusion process would also be involved in the absorption.

The results of Expt 2 in the present study indicated that L-histidine and glucose were absorbed by a process which involved some rate-limiting factor, and so this process may participate in the diurnal fluctuation in absorption; but the possibility that the decrease in the surface area of the lumen, or the decrease in mesenteric blood flow with time after feeding, is responsible for the decrease in L-histidine and glucose absorption through the diffusion process cannot be excluded. In the instance of glucose there is the additional complication that it is metabolized and supplies part of the energy for its own transport (Sanford, Smyth & Watling, 1965).

It was found in the present study that the absorption (%) of L-histidine and glucose from the jejunum segment decreased with increasing concentration of infused test materials and with the flow rate of infused solutions. Therefore, if there is a similar situation for absorption from digesta in the intestine of the intact animal, the percentage absorption may be reduced immediately after feeding because of an intensive flow of digesta and a high concentration of its component for that time, as found for the period 10.00–10.30 hours in Expt 1.

If the capacity of glucose absorption is assumed to be 3·61 g/h per m intestine, from the results obtained for the period 10.00–10.30 hours in Expt 4, and the value is extrapolated to the whole small intestine (approximately 16 m in length, as measured during cannulation for each pig), an approximate value of 57·76 g/h for the absorptive capacity for glucose from the whole small intestine can be obtained. The amount of dry matter passing through the upper jejunum was 93·5 g/h for the period 10.00–10.30 hours (Table 2). Assuming that the carbohydrate content of digesta is 60% of the dry matter content, the flow rate for carbohydrate may be estimated as 56·2 g/h. This calculated value for carbohydrate flow rate through the upper jejunum for the period 10.00–10.30 hours is below the estimated capacity of the whole small intestine to absorb glucose. Thus, even during periods of intensive digesta flow, i.e. the period 10.00–10.30 hours in the present study, the influx of carbohydrate to the small intestine does not exceed the absorptive capacity. The decrease in absorption with time after feeding obtained in Expt 4 will be covered by the decrease in digesta flow and that of the concentration of its component with time after feeding.

There seems to be little possibility, therefore, that the influx of glucose will exceed the absorptive capacity in the physiological state. The present conclusion that intestinal absorptive capacity is not the limiting factor in carbohydrate assimilation is similar to that of Kvasnitskii (1951, quoted by Freeman *et al.* 1968) and of Freeman *et al.* (1968) in relation to carbohydrate absorption and lipid absorption respectively, from the small intestine of the pig.

The authors thank Mrs F. Usui for the care of the experimental animals, Mr S. Masaki for the analysis of sugars by liquid chromatography, and Drs K. Kameoka and M. Saitoh for their helpful discussions. They also thank Dr Y. Yugari, Research Institute for Life Sciences, Ajinomoto Co. Inc., for his continuous encouragement during this work.

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Printed in Great Britain