

Absorption of D-Glucose and L-Leucine by Isolated Bowel Segments in Rats[†]

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= Abstract = For clinical application of the technique for creation of an isolated bowel segment (IBS) for lengthening the intestine, the absorptive function of the IBS was measured in rats. An IBS is a segment of the intestine which is devoid of its mesentery and nourished solely by the collaterals from the abdominal wall. The technique for creation of the IBS has been established, but its absorptive function has yet to be explored. Absorptive clearance of D-glucose and L-leucine was compared between the control loop and the IBS. Water flux, capacity and the intraluminal pressure of the bowel segment were also measured, using the single-perfusion technique. This study revealed that the IBS maintained substantial absorptive function and that the loss of external innervation might have influenced water flux in the IBS.

Key Words: Isolated bowel segment, Absorption, D-Glucose, L-Leucine

INTRODUCTION

An isolated bowel segment (IBS) is a Thiry-Vella loop without its mesentery. The IBS is sutured to the muscle of the abdominal wall after creating a fresh wound on the peritoneal side of the anterior abdominal wall while at the same time a Thiry-Vella loop is created thus insuring revascularization. The mesentery is divided at the appropriate time (Ienaga *et al.* 1990). Kimura and Soper (1990) developed this technique for lengthening the bowel horizontally. Vertical bisection of the intestine has recently been used clinically for lengthening the

bowel in cases of short bowel syndrome (Bianchi 1980, Thompson 1991). Although the technique of creating the IBS is established, the absorptive function of the IBS has to be explored. Because, contrary to Bianchi's method which doubles the length of the divided intestine with its own mesentery, the horizontally divided IBS has no mesentery.

To study the absorptive function of the IBS, absorptive clearance of D-glucose and L-leucine was tested in rats. Because the D-glucose absorption study provides a standard test of the integrity of the transport function of the gut (Alpers 1987). Another index of absorptive activity is to examine absorption of an amino acid. The most important of the several transport systems for amino acids is that for neutral amino acid. Function of this systems can be assessed by measuring absorption of L-leucine because it has a high affinity for uptake (Miller *et al.* 1984).

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MATERIALS AND METHODS

Animals and diet

Thirty male Sprague-Dawley rats of the same age weighing 340-360g were used. The animals received a standard pellet diet and water ad libitum.

Operations

Creation of a myoenteropexied Thiry-Vella loop: The animals were fasted 16-20 hr before operation but were allowed free access to water. The rats were anesthetized with an intraperitoneal injection of chloral hydrate (350mg per kg). Midline laparotomy was performed. Entering the abdomen a segment of jejunum with an intact blood supply was isolated by transecting at a point 10cm distal to the Treitz ligament and about 7cm aboral to that. Intestinal continuity was restored by one layer continuous 5-0, black silk suture. A 10 French rubber catheter (10cm long) was inserted in the isolated loop. With the tube in situ the antimesenteric side (2mm wide) of the isolated loop was scrubbed gently with sandpaper (fine-50, Household Product Division/3M, St Paul, MN) until fresh bleeding was observed then the tube was removed. A longitudinal wound (5cm long) was made on the peritoneal surface of the anterior abdominal wall with a scalpel 1 mm deep, 1 cm right to the midline beginning 1.5cm caudal to the costal margin, and the wound was spread 2 mm wide. After confirming that the mesentery of the isolated loop was not twisted the lateral margin of the fresh wound of the loop was approximated to the lateral margin of the abdominal wall wound by continuous mattress 5-0 Vicryl suture. The medial margin was approximated in the same manner. After myoenteropexy both ends of the isolated loop were enterostomized and matured by 4-0 interrupted black silk suture. Warm normal saline 10 cc and ampicillin 100 mg were given intraperitoneally before closure of the abdominal wall by 3-0 continuous polypropylene suture. After operation the animals were kept

NPO for 24 hours, then 5% dextrose was given for a day. Rat chow and water were given 2 days after operation

Creation of the IBS: Animals were not fasted for division of the mesentery which was performed immediately after the absorption study of the control loop done 8 weeks after creation of the IBS. This time the dosage of intraperitoneal injection of chloral hydrate was reduced to 300mg per Kg. A small midline incision was made on the previous operation scar. The abdomen was entered, and the mesentery of the isolated loop was identified, double ligated and divided. Ampicillin 100 mg was given intraperitoneally before closure of the abdominal wall

Absorption study

Perfusion solution: The concentration of D-glucose and L-leucine was decided for its maximal uptake (Diamond *et al.* 1984, Karasov *et al.* 1986). The perfusate was prepared by adding 10 mmol D-glucose (Sigma chemical Co., St Louis, Mo.) and 3 mmol L-leucine (Sigma chemical Co., St Louis, Mo.) to 1000 ml of Ringer's solution. $1.0\mu\text{Ci } ^{14}\text{C-PEG}$ (specific activity, 0.36 mCi/gm, Mol Wt 4,000, New England Nuclear, Boston, MA) and $5.0\mu\text{Ci } ^3\text{H-L-leucine}$ (specific activity 5.0 Ci/mmol, New England Nuclear, Boston, MA) were added to the above mixture. The pH of the perfusate was 6.3 and osmolarity

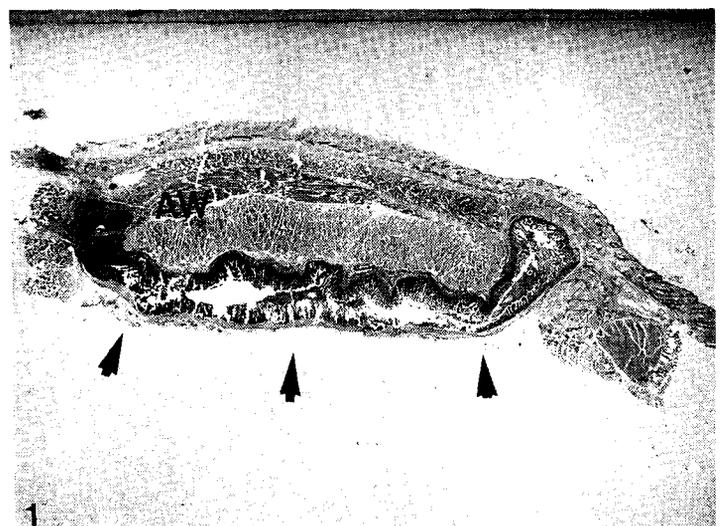


Fig. 1. Longitudinal view of the IBS. H & E X1. AW: abdominal wall, arrowhead: IBS

was 290 mOsm. The perfusate was stored in a refrigerator and an aliquot of perfusate was warmed to 37°C before each perfusion

Perfusion Procedure: Animals were not fasted for perfusion. Perfusion of the control loop was done just before division of the mesentery under the same anesthesia. Three weeks after division of the mesentery, perfusion of the IBS was done under chloral hydrate anesthesia (250mg per Kg).

After anesthesia, a polyethylene catheter (2 mm i. d., 3 mm o. d.) was inserted through the cephalic stoma. The loop was flushed with perfusate until the affluent fluid was clear and the segment was free of waste and debris. Then a tube was inserted through the caudal stoma serving as an outlet. The inlet and the outlet were tested for leakage after a purse-string suture was applied around the stoma. Then the loop was cleared of perfusate by injecting air (Fig 2.). The capacity of the loop was measured by perfusate injection. Then perfusion was performed with a syringe pump at a rate of 5 ml per hour for minimizing variability in the in-situ absorption study (Savina *et al.* 1981) and by

single-pass method which showed the most constant absorption rate and lowest variance (Schurgers *et al.* 1986). Sample collection was achieved by gravity. After 30 minutes to attain a steady-state (Garrido *et al.* 1979), a 30-minute collection was made.

After completion of perfusion, intraluminal pressure was measured by manometry.

Assays

To determine the radioactivity of ¹⁴C-PEG and ³H-L-leucine, 0.1 ml of sample was added to 2 ml of scintillation fluid (Budget-Solve, Research Product International Corp., Mount Pleasant, IL) and the radioactivity was measured in a Beckman LS 3501 liquid scintillation counter with automatic quench compensation. The concentration of glucose was measured by Beckman Glucose Analyzer 2.

Calculation

The absorption rate or net rate of disappearance of D-glucose and L-leucine during perfusion was calculated as

$$DR = (T - S \frac{C_t}{C_s}) R$$

Where DR is disappearance rate of test material, T is concentration of D-glucose or radioactivity of ³H-L-leucine in the test solution, S is concentration of D-glucose or radioactivity of ¹⁴C-PEG in the sample, C_t is radioactivity of ¹⁴C-PEG in the test solution, C_s is radioactivity of ¹⁴C-PEG in the sample and R is the rate of infusion. Water flux was calculated as

$$\text{Water flux} = \frac{C_s - C_t}{C_t} R$$

Where (+) means absorption of water by the loop and (–) means excretion of water by the intestinal segment.

The absorptive function of the IBS was expressed as a percentage value of the absorption rate of the IBS compared to that of the control. The bowel segment before division of the mesentery is the control loop in each rat respectively.

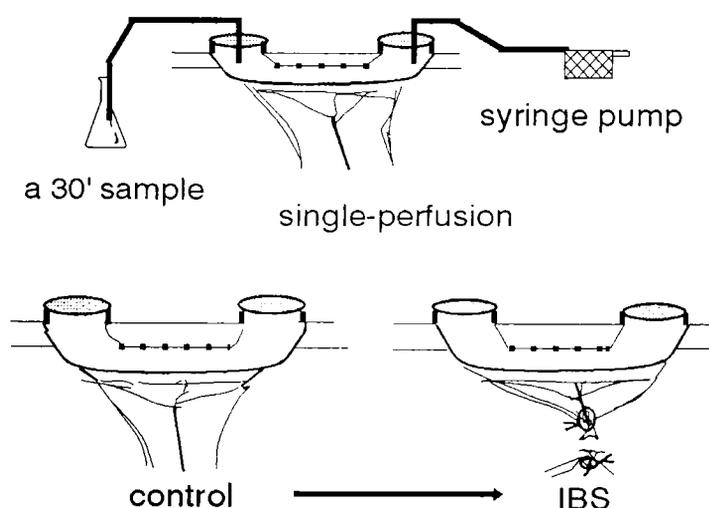


Fig. 2. Creation of an IBS and perfusion method. An IBS was created 8 weeks after myoenteropexy by division of the mesentery. Division of the mesentery was performed immediately after control run. Experimental run was performed three weeks after creation of the IBS.

RESULTS

Among 30 rats only four rats survived the whole procedure; *i. e.*, 2 operations, three anesthesia.

Intraluminal pressure measured at the end of perfusion had showed an average 4.9% increase in the IBSs and the capacity of the bowel segment decreased an average 4.6% compared to the control (Table 1). The loops with larger capacity had dilatation due to impacted intestinal succus. There was no correlation between intraluminal pressure and capacity of the loop.

Water shift calculated by the radiocativity

Table 1. Intraluminal pressure and capacity of bowel segment

Rat serial No.	Pressure (cm H ₂ O)		Capacity (ml)	
	Control	IBS	Control	IBS
1	7.0	7.5	1.2	1.3
2	9.3	8.5	0.8	0.7
3	10.8	11.4	1.4	1.2
4	8.2	8.8	0.5	0.5

Control; before ligation of the mesentery, IBS; isolated bowel segment

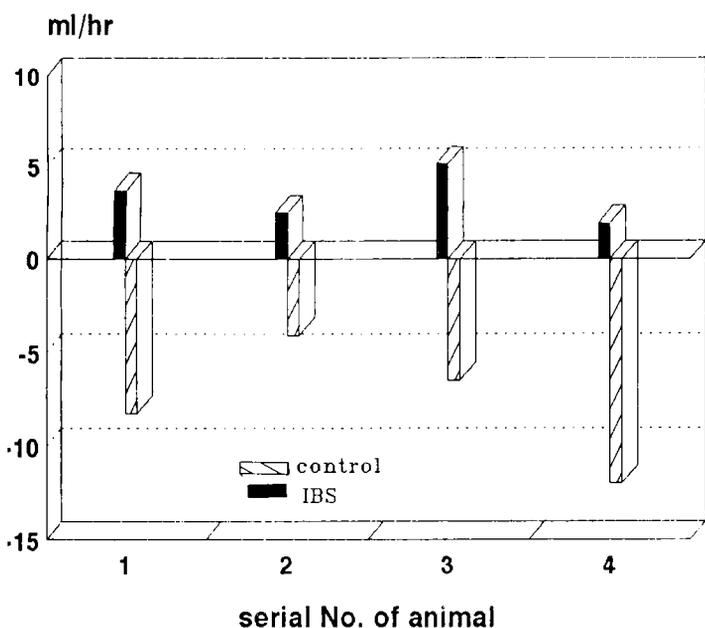


Fig. 3. Water shift measured by C-PEG.

of the impermeable indicator ¹⁴C-PEG revealed excretion of water by control loops and absorption of water by the IBSs (Fig. 3).

The absorption of D-glucose by the IBS was 109.7 ± 50.6 (SEM)% of control. When rat #2 which had a very low control value was excluded the average D-glucose absorption was 84.5% of the control (Table 2, Fig. 4).

The absorption of L-leucine calculated by radioactivity of ³H-L-leucine showed 94.4 ± 19.8 (SEM)% of the control loop in the IBS, Excluding #2 the absorption rate was 83.3% of the control (Table. 2, Fig. 4).

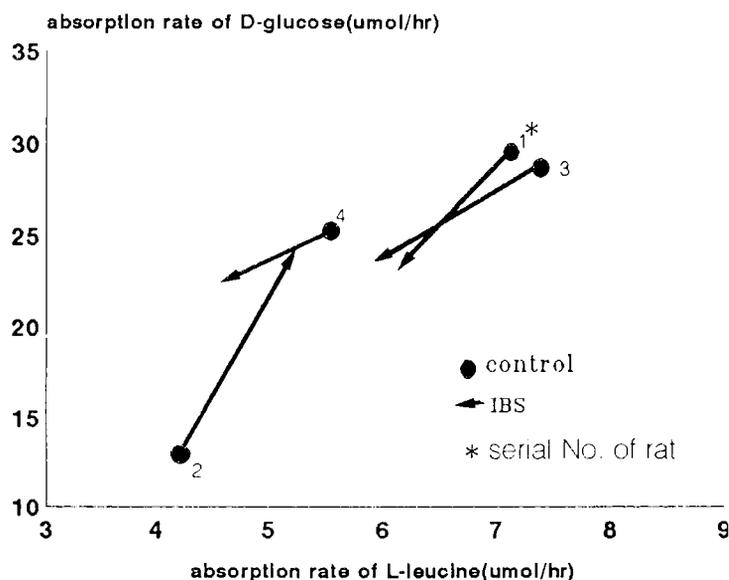


Fig. 4. Absorption of D-glucose and L-leucine by control and IBS.

Table 2. Absorptive disappearance rate percentage of D-glucose and L-leucine in the IBS compared to the control

Rat serial No.	D-glucose	L-leucine
1	80.3	87.3
2	185.3	123.8
3	82.4	81.1
4	90.8	85.5

DISCUSSION

The rats succumbed easily after repeated anesthesia and operation. The control value of #2 was very low compared to the other rats.

This may be due to poor circulation and hypoxia during anesthesia. This rat was almost moribund during the absorption study for control value. It would be proper to exclude this rat.

Because the length of each IBS can not be the same and the operative trauma is different the absorption is compared within each rat. The interesting trend is that all the control loops secreted water and all the IBSs absorbed water. Swaab *et al.* (1982) described how an increase in intraluminal pressure for over 30 min and a pressure level of over 20 cm H₂O resulted in an irreversible stimulation of secretion. But intraluminal pressure was under 11.5 cm H₂O in this study. Sjovall *et al.* (1983) have documented how intraluminal glucose stimulates vagal afferent that ends in the nodose ganglion. The extrinsic innervation of the IBS was cut off during division of the mesentery. This may explain why water influx occurs in the IBS because passive water transport by the difference of osmolarity will dominate the net water flux. Because the control study was done 3 weeks earlier the disuse atrophy of the bowel segment may have contributed to the decrease in absorptive function. From the clinical aspect the IBS can be constructed in short bowel syndrome children whose only candidate for intestinal lengthening is the duodenum. Although the sample size is very small in this study, with this magnitude of absorptive function retained in the IBS it will offer another option for handling the short bowel syndrome. The motility of the IBS requires further study.

REFERENCES

- Alpers, DH. Digestion and absorption of carbohydrate and protein. In Johnson LR, Christensen J, Jackson ED, Walsh JH. (Ed) *Physiology of the gastrointestinal tract*. New York, Raven Press, 1987:pp. 1469-87
- Bianchi A. Intestinal loop lengthening- A technique for increasing small intestinal length. *J Pediatr Surg* 1980; 15:145-51
- Diamond JM, Karasov WH. Effect of dietary carbohydrate on monosaccharide uptake by mouse intestine in vitro. *J Physiol* 1984; 349:419-40
- Garrido AB, Freeman HJ, Chung YC, Kim YS. Amino acid and peptide absorption after proximal small intestinal resection in the rat. *Gut* 1979; 20:114-20
- Ienaga T, Kimura K, Hashimoto K, Lee sc, Brakstad M, Soper RT. Isolated Bowel Segment (Iowa model I): Technique and histologic study. *J Pediatr Surg* 1990; 25:902-4
- Karasov W, Solberg D, Carter S, Hughes M, Phan D, Diamond J. Uptake pathways for amino acids in mouse intestine. *Am J Physiol* 1986; 251:G501-8
- Kimura K, Soper RT. Isolated bowel segment (Model I): Creation by myoenteropexy. *J Pediatr Surg* 1990; 25:512-3
- Miller PM, Burston D, Brueton MJ, Matthews DM. Kinetics of uptake of L-leucine and glycylsarcosine into normal and protein malnourished young rat jejunum. *Pediatr Res* 1984 18; 504-8
- Savina PM, Staubus AE, Gaginella TS, Smith DF. Intestinal absorption studies in rats. *J Pharm Sci* 1981; 70:239-43
- Schurgers N, Bidendik J, Tukker JJ, Crommelin DJ. Comparison of four experimental technique for studying drug absorption kinetics in the anesthetized rat in situ. *J Pharm Sci* 1981; 70:239-53
- Sjovall H, Redfors S, Jodal M, Lundgren O. On the mode of action of the sympathetic fibers on intestinal fluid transport: Evidence for the existence of a glucose-stimulated secretory nervous pathway in the intestinal wall. *Acta Physiol Scand* 1983; 119:29-48
- Swaab E, Hynes R, Marnane W, McNeil J, Decker R, Tai YH, Donowitz M. Intestinal filtration-secretion due to increased intraluminal pressure in rabbits. *Am J Physiol* 1982; 242:G65-75
- Thompson JS, Pinch LW, Murray N, Vanderhoof, Schultz LR. Experience with intestinal lengthening for the short bowel syndrome. *J Pediatr Surg* 1991; 26:721-4