SUGAR TRANSPORT IN THE GUT OF A LIMPET

(Megathura crenulata)

A Thesis

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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Richard Oscar Wright III

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ABSTRACT

The movement of four monosaccharides (D-glucose, D-galactose, 3-O-methyl-D-glucopyranose, and D-fructose) across the gut of the marine gastropod Megathura crenulata was studied using the everted sac technique of Crane and Wilson (1958). Positive S/M ratios (evidence for active transport) were obtained for D-glucose and 3-O-methyl-D-glucopyranose, but not for D-galactose and D-fructose in the posterior esophagus. No evidence was obtained for active transport of any of the four tested monosaccharides by either the anterior intestine or the posterior intestine. The net accumulation of D-glucose against an apparent concentration gradient by the posterior esophagus was dependent upon metabolic energy from glycolysis but not the tricarboxylic acid cycle. Thus, it is concluded that D-glucose and 3-O-methyl-D-glucopyranose are actively transported by the posterior esophagus.

There appears to be no measurable water movement in the posterior esophagus during active transport of D-glucose or 3-O-methyl-D-glycopyranose.

D-Glucose and D-galactose appear to be metabolized by all regions of the gut tested. D-fructose and 3-O-methyl-D-glucopyranose did not appear to undergo any significant amount of metabolism.

Observed transmural potentials indicate active ion transport is present in all regions of the gut studied. Transmural potentials were significantly larger in the posterior esophagus than in the anterior or posterior intestine. The serosal surface was negative to the mucosal surface in all regions tested. The transmural potentials observed in the posterior esophagus were altered by the removal of oxygen and the presence of the glycolytic inhibitor, NaF.

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INTRODUCTION

Selective absorption is an important function of the intestine of animals. Active transport is a mechanism of a absorption used by many animals whereby specific compounds are moved against an electro-chemical gradient. Active transport of ions or organic compounds such as monosaccharides is characterized by (1) movement against an apparent concentration gradient, (2) the requirement of constant supply of metabolic energy, (3) the maintenance of molecular homogeneity of the substance moved across the membrane, and (4) some degree of specificity as to physical characteristics of molecules moved.

The active transport mechanisms for monosaccharides has been determined for many of the vertebrates and has been well characterized in mammals where at least fourteen monosaccharides have been shown to be actively transported (Crane, 1960; Wilson and Landau, 1962). Several excellent reviews have been published during the last decade summarizing the voluminous data on monosaccharide activé transport by the mammalian gut (Wilson, 1962; Wiseman, 1964; Crane, 1963; Benson and Rampone, 1966; Smyth, 1967).

Monosaccharide active transport mechanisms have also been studied in non-mammalian vertebrates, though not as extensively. Some of the more important studies using the

non-mammalian gut are those using the avian gut (Alvarado, 1967) the reptilian gut (Fox, 1965), the amphibian gut (Csaky and Thale, 1960; Csaky and Fernald, 1960; Lawrence, 1963), and the fish gut (Carlisky and Huang, 1962; Musacchia et al., 1964; Stokes and Fromm, 1964; Mussachia et al., 1966; Huang and Rout, 1967).

Only a few invertebrates have been studied in relation to intestinal active transport of monosaccharides. These studies have been restricted to two classes of Echinodermata, one class of Arthropods, and one class of Mollusca. Other studies using the invertebrate gut have been concerned with passive diffusion or other mechanisms of absorption.

Active transport of monosaccharides appeared to be absent in the holothurian, Stichopus parvimenis, (Lawrence et al., 1967) although active transport of amino acids was observed. Probable active transport of D-glucose has been observed in the gut of the asteroid Asterias forbesii (Ferguson, 1964a,b) and the polychaete Hermodice carunlata (Marsden, 1963a,b, 1966). Trehalose has been observed to be actively transported in the gut of the grasshopper, Melanopus differentialis (Randal and Derr, 1965) while D-glucose is thought to be moved by facilitated diffusion (Trehearne, 1958).

The only study directly concerned with active transport of monosaccharides by a molluscan gut is that of Lawrence and Lawrence (1967). This study showed that active transport of

monosaccharides was presented in the gut of the amphineuran, Cryptochiton stelleri, and that the nature of the mechanism present differed markedly from that of the vertebrate gut. D-Glucose and 3-0-methyl-D-glucopyranose were actively transported by the anterior intestine of the chiton, but D-galactose was actively transported only by the posterior intestine. D-Mannose and D-fructose were not actively absorbed by any region of the gut. Further, neither D-fructose, D-mannose, nor D-galactose were absorbed by being first converted to D-glucose by the anterior intestine. Also, active transport of D-glucose and 3-0-methyl-D-glucose were reversibly inhibited by anaerobic conditions while no significant inhibition of active transport of D-galactose by anaerobic conditions occurred. These data and the results of competitive inhibition studies (Lawrence and Lawrence, 1968) have led these investigators to conclude that two active mechanisms are present in this molluscan species rather than the one carrier mechanism postulated for the vertebrate gut. Thus the one study of active transport of monosaccharides in a single species of the 45,000 species of mollusca has shown that active transport of monosaccharides is present and is different from the active transport of monosaccharides seen in the verebrate gut. Consequently, it would be of interest to both general and comparative physiologists to examine representative member of another major class of the phylum Mollusca in terms of active transport of monosaccharides.

Megathura crenulata, the "giant" keyhole limpet, was chosen as the molluscan species to use for this study as it is a gastropod, the largest class of the phylum Mollusca, and because the morphological characteristics of the gut are such that study by in vitro techniques are possible. Further, as Megathura crenulata is a member of the family archegastropoda a group phylogenetically advanced to the amphineurans but less advanced than the higher gastropods comparisons can be made between the mechanisms present in the two related but distinctly different species and insight can be gained into the nature of the mechanism for active transport of monosaccharides by gastropods. Comparisons between active transport mechanisms in gastropods and vertebrates can then be made.

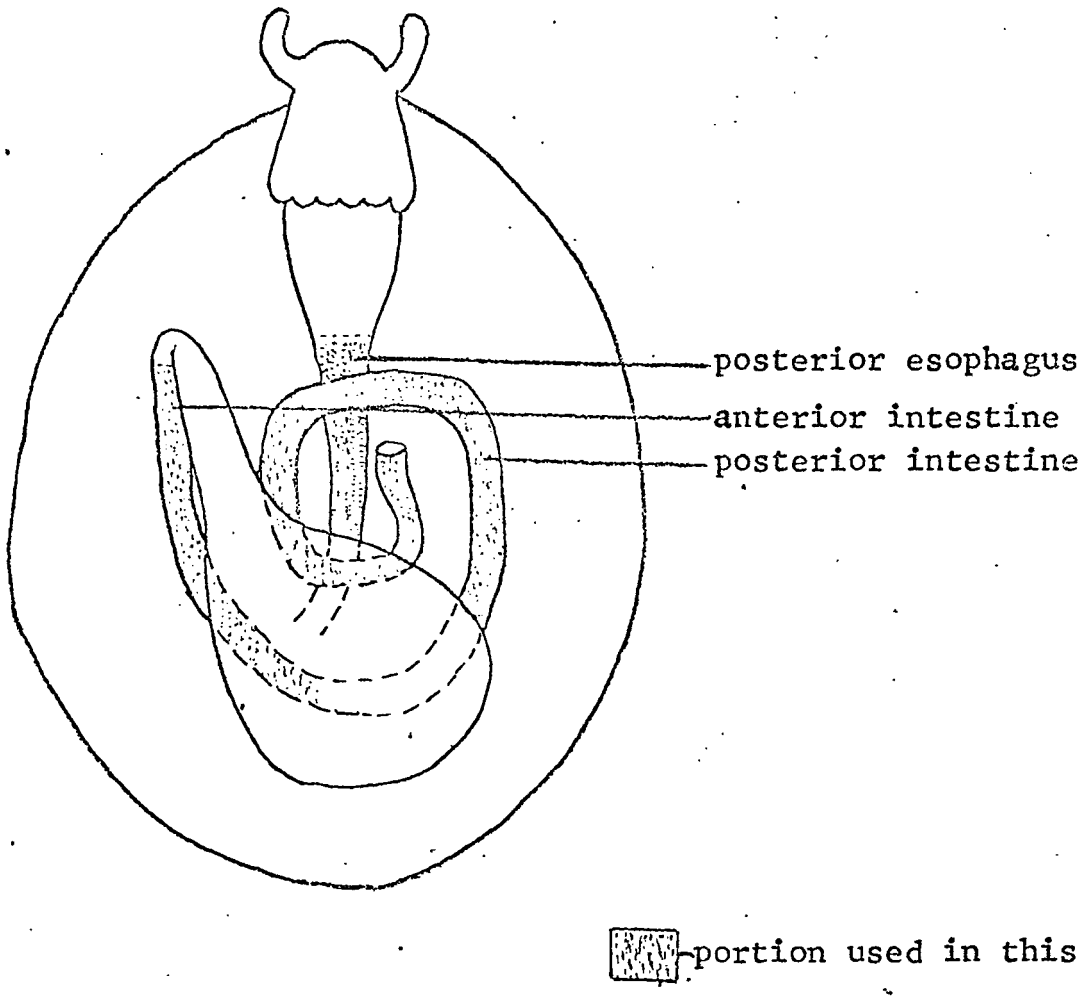
METHODS AND MATERIALS

Limpets (Megathura crenulata) of both sexes weighing approximately 250 grams were obtained from Pacific Bio-Marine Supply Company, Venice, California. These animals were maintained in 15°C aerated sea water under starved conditions until used.

IN VITRO METHOD

The in vitro method of Crane and Wilson (1958) was used in this study. Animals were sacrificed, the visceral mas removed, and the intestine separated from the surrounding tussue. Segments 2 to 6 cm in length were removed from the posterior esophagus, anterior intestine, and posterior intestine (see Figure 1 for a diagram of the regions used). The isolated segments were then everted using a glass rod. One end of the everted segment was secured to a small bore glass cannula embedded in a rubber stopper and the other end ligated to a glass weight. The gut segment was then placed in an incubation tube containing 32 ml. of the test monosaccharide-Ringer's solution. The cannula was then adjusted so that the entire everted segment was immersed. A solution which was quantitatively and qualitatively identical to the solution contained by the incubation tube was pipetted into the everted segment until the everted segment was filled and the cannula contained a column of fluid less than 2 cm in

FIGURE 1 DIGESTIVE SYSTEM OF MEGATHURA CRENULATA



(modified from Ward, 1966)

height above the level of fluid contained by the incubation tube. The inner solution contained in the everted segment and the cannula was referred to as the serosal solution and the solution contained by the incubation tube as the mucosal solution. Unless otherwise indicated, between 10 and 30 mls. of atmospheric air/min was passed through the mucosal solution, entering and leaving the solution by different hypodermic needles forced through the stopper. Figure 2 is a diagram of the in vitro experimental apparatus. All studies were performed at 15°C with constant temperature being maintained by a water bath (Magni-Whirl MR-3240C)

The composition of limpet Ringer's solution is shown in Table 1. This Ringer's solution was made to approximate gastropod body fluid ionic constituents (Biological Handbooks: Blood and Other Body Fluids).

The monosaccharides used in this study were D-glucose, 3-0-methyl-D-glucopyransoe (3-methylglucose), D-galactose, and D-fructose. Only the D form of these monosaccharides was studied. Carbon-14 labeled monosaccharides were used to follow the movement of the monosaccharides across the gut. D-Galactose-1-^{14}C, D-fructose- U-^{14}C, and 3-0-methyl-^{14}C-D-glucopyranose were obtained from New England Nuclear Corporation; D-glucose-U-^{14}C was obtained from the California Corporation for Biochemical Research. All other chemicals used were reagent grade.

FIGURE 2 APPARATUS USED IN THE IN VITRO METHOD FOR THE STUDY OF ABSORPTION OF SUGARS

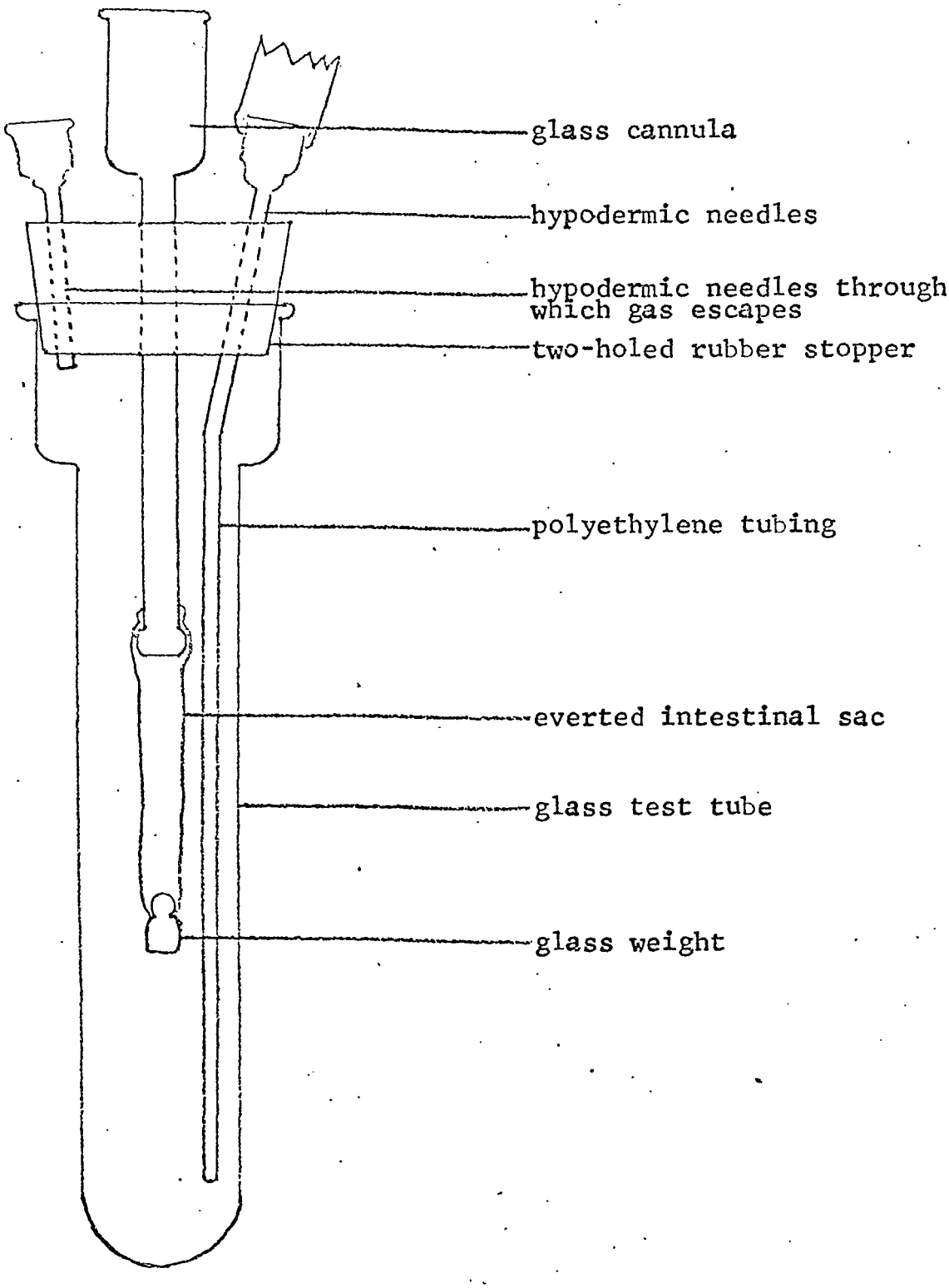


TABLE 1 COMPOSITION OF INVERTEBRATE RINGER'S SOLUTION USED IN TRANSPORT STUDIES

Compound	Grams/liter	mMolarity
NaCl	27.0	462.00
KCl	0.9	12.07
$MgCl_2$	2.0	9.83
$MgSO_4$	0.6	2.43
$NaHCO_3$	0.2	2.38
$CaCl_2$	1.5	13.32

The amount of radioactivity in serosal and mucosal solutions was determined by the following procedure. Samples (0.1 and 0.05 ml.) from the serosal and mucosal solutions plus and equal volume of rinse were placed in ringed aluminum planchets and evaporated to dryness by infrared light. The activity of these planchets was then determined by a thin window gas flow Geiger-Mueller counter (Nuclear-Chicago C110B tube with a Nuclear-Chicago 186 scalar) using 97.3% helium-1.7% butane as the ionizing gas. All samples were counted to an accuracy of one per cent standard deviation.

SERIES 1 Mapping Determinations

For the mapping determinations the previously described in vitro technique was employed. The initial concentration of each of the various monosaccharides tested was as follows: glucose, 1 μM/ml and 0.0045 μM/ml; galactose, 1 μM/ml and 0.081 μM/ml; 3-methylglucose, 1 μM/ml and 0.047 μM/ml; and fructose, 0.0021 μM/ml. The incubation periods were three hours in length and aerobic conditions were maintained throughout each incubation period. At the end of the incubation period, an aliquot of the serosal and the mucosal solutions was removed and its activity determined. The volume of the serosal solution also was determined to ascertain if a change in the volume of the serosal solution had taken place due to water movement.

SERIES II Anaerobic inhibition studies

The procedure for the anaerobic inhibition studies was identical to that of the mapping studies with the following differences. All incubation periods were two hours long. Each of the incubation periods was preceeded by a pre-incubation period. The initial pre-incubation was one hour followed by thirty minute pre-incubation periods prior to the second and third incubation period. The initial serosal volume was the same for all three pre-incubation periods and the three incubation periods. During the second pre-incubation period and the second incubation period the gut segments were placed in incubation tubes in which the test solution had been previously gassed with purified nitrogen gas. The atmospheric air aerating the mucosal solution for the first and third pre-incubation and incubation periods was replaced by purified nitrogen gas during the second pre-incubation and incubation periods.

At the end of each pre-incubation and incubation periods the serosal solution was removed and replaced by a fresh serosal solution. At this time the gut segment was placed in an incubation tube containing a fresh mucosal solution thus assuming constant initial conditions for each incubation period.

SERIES III Fluoride inhibition studies

The same procedure as in the anaerobic inhibition studies was followed except that during the second pre-incubation

and second incubation period the serosal and mucosal solutions also contained 7.5 mM NaF and atmospheric air was passed through the mucosal solution throughout all test periods.

SERIES IV Transmural potential studies

Transmural potential were determined for each of the three regions of the gut studied. These determinations were carried out during Series I experiments using glucose as the test monosaccharide. In Series II-III only the transmural potentials of the posterior esophagus were measured in the presence of glucose. The transmural potentials across the gut were measured by means of a high impedence electrometer (Keithley 610A). Salt bridges of 3% agar-limpet Ringer's solution were employed and calomel reference electrodes completed the circuit to the electrometer. The base line potentials were determined before each measurement. The difference between the base line potential and the measured gut potential was considered to represent the actual gut potential.

RESULTS

SERIES I Mapping determinations

As seen in Table 2 an increased serosal concentration of glucose is observed in the posterior esophagus. A positive serosal/mucosal (S/M) ratio was also observed. However, there was a decrease in the concentration in the mucosal and serosal compartments in the anterior and posterior intestine along with fractional S/M ratios. The accumulation of glucose against an apparent concentration by the posterior esophagus but not by the anterior or posterior intestine indicates that active transport of glucose is present in the posterior esophagus but not in the anterior or posterior intestine. An explanation for this interpretation will be presented in the "Discussion".

Fractional S/M ratios with a decrease in serosal and mucosal concentrations were obtained from all regions of the gut when incubated with a 1 μM/ml galactose solution (Table 2). This was also seen by incubation of segments from all three regions of the gut with a 1 μM/ml solution of 3-methylglucose (Table 2). These data suggest that galactose and 3-methylglucose are not actively transported by any region of the gut.

Concentrations of 0.0045 μM/ml glucose, 0.081 μM/ml galactose, 0.047 μM/ml 3-methylglucose, and 0.0021 μM/ml fructose were also tested. These very low concentrations

TABLE 2 MAPPING DETERMINATIONS OF M. CRENULATA GUT I.

Region of the gut	Number of observations	Final serosal concentration µM/ml ± S.E.M.	Final mucosal concentration µM/ml ± S.E.M.	Net serosal accumulation/ gram dry gut weight ± S.E.M.	Serosal/ mucosal ratio ± S.E.M.
			D-GLUCOSE		
Posterior esophagus	(4)	1.41 ± 0.18	0.56 ± 0.04	0.48 ± 0.01	2.51 ± 1.08
Anterior intestine	(2)	0.76 ± 0.18	0.88 ± 0.01	- 4.32 ± 1.28	0.86 ± 0.10
Posterior intestine	(2)	0.49 ± 0.03	0.92 ± 0.01	- 2.49 ± 2.13	0.53 ± 0.01
			D-GALACTOSE		
Posterior esophagus	(4)	0.62 ± 0.06	0.77 ± 0.01	- 3.74 ± 0.04	0.80 ± 0.01
Anterior intestine	(4)	0.38 ± 0.02	0.94 ± 0.01	- 4.91 ± 0.70	0.40 ± 0.01
Posterior intestine	(4)	0.58 ± 0.07	0.97 ± 0.01	- 2.71 ± 0.03	0.60 ± 0.01

Everted segments were incubated aerobically for 3 hours. Initial serosal and mucosal concentrations were 1 µM/ml - indicates net loss of monosaccharide.

TABLE 2 (con't.) MAPPING DETERMINATIONS OF M. CRENULATA GUT I.

Region of the gut	Number of observations	Final serosal concentration μM/ml ± S.E.M.	Final mucosal concentration μM/ml ± S.E.M.	Net serosal accumulation/ gram dry gut weight ± S.E.M.	Serosal/ mucosal ratio ± S.E.M.
			3-METHYLGLUCOSE		
Posterior esophagus	(5)	0.92 ± 0.01	0.95 ± 0.09	- 0.94 ± 0.02	0.94 ± 0.01
Anterior intestine	(5)	0.79 ± 0.01	1.06 ± 0.01	- 5.36 ± 0.22	0.74 ± 0.01
Posterior intestine	(2)	0.74 ± 0.08	0.97 ± 0.01	-.3.02 ± 0.98	0.76 ± 0.03

Everted segments were incubated aerobically for 3 hours. Initial serosal and mucosal concentrations were 1 μM/ml - indicates net loss of monosaccharide.

were used as it was possible that a transport mechanism existed which had a high affinity for monoaccharides. Therefore, the presence of high concentrations of monosaccharides would essentially saturate the transport mechanism and active transport would not be observed. The very low concentrations of the various monosaccharides were different as each of these concentrations was the lowest available and represented the activity levels of the labeled monosaccharides. Positive S/M ratios in spite of decreased serosal and mucosal concentrations were obtained (Table 3) from the posterior esophagus in the presence of glucose. Fractional S/M ratios with decreased mucosal and serosal concentrations were again observed in the anterior and posterior intestine. Likewise fractional S/M ratios with decreased mucosal and serosal concentrations occurred with galactose in all regions of the gut. These data tend to confirm data obtained at the higher concentration showing that glucose undergoes active transport in the posterior esophagus, but not in the anterior or posterior intestine and that galactose is not actively transported by any region tested.

However, an initial concentration of 0.047 μM/ml 3-methylglucose on both sides of the posterior esophagus resulted in a significant ($P < 0.05$) positive S/M ratio (Table 3). An increase in serosal concentrations was also

TABLE 3 MAPPING DETERMINATIONS OF M. CRENULATA CUT II.

Region of the gut	Number of observations	Final serosal concentration µM/ml ± S.E.M.	Final mucosal concentration µM/ml ± S.E.M.	Net serosal accumulation/ gram dry gut weight ± S.E.M.	Serosal/ mucosal ratio ± S.E.M.
			D-GLUCOSE		
Posterior esophagus	(8)	0.0024 ± 0.0001	0.0022 ± 0.0001	- 0.0014 ± 0.0001	1.09 ± 0.06
Anterior intestine	(5)	0.0023 ± 0.0001	0.0026 ± 0.0001	- 0.0146 ± 0.0001	0.88 ± 0.01
Posterior intestine	(6)	0.0028 ± 0.0001	0.0038 ± 0.0001	- 0.0052 ± 0.0001	0.73 ± 0.01
			D-GALACTOSE		
Posterior esophagus	(4)	0.053 ± 0.001	0.079 ± 0.001	- 0.1327 ± 0.0200	0.67 ± 0.01
Anterior intestine	(4)	0.037 ± 0.001	0.076 ± 0.001	- 0.5071 ± 0.0035	0.49 ± 0.01
Posterior intestine	(3)	0.050 ± 0.001	0.065 ± 0.000	- 0.2572 ± 0.0002	0.76 ± 0.01

Everted segments were incubated aerobically for 3 hours.
- indicates net loss of monosaccharide
Initial concentration of D-glucose was 0.0045 µM/ml.
Initial concentration of D-galactose was 0.081 µM/ml.

TABLE 3 (con't) MAPPING DETERMINATIONS OF M. CRENULATA GUT II.

Region of the gut	Number of observations	Final serosal concentration µM/ml ± S.E.M.	Final mucosal concentration µM/ml ± S.E.M.	Net serosal accumulation/ gram dry gut weight ± S.E.M.	Serosal/ mucosal ration ± S.E.M.
			3-METHYLGLUCOSE		
Posterior esophagus	(4)	0.053 ± 0.001	0.047 ± 0.001	0.0010 ± 0.0006	1.12 ± 0.01
Anterior intestine	(6)	0.043 ± 0.001	0.048 ± 0.001	- 0.0037 ± 0.0001	0.90 ± 0.01
Posterior intestine	(5)	0.035 ± 0.001	0.041 ± 0.001	- 0.0274 ± 0.0031	0.87 ± 0.01
			D-FRUCTOSE		
Posterior esophagus	(4)	0.0015 ± 0.0001	0.0019 ± 0.0001	- 0.0031 ± 0.0001	0.79 ± 0.02
Anterior intestine	(4)	0.0012 ± 0.0001	0.0019 ± 0.0001	- 0.0043 ± 0.0001	0.63 ± 0.02
Posterior intestine	(4)	0.0017 ± 0.0001	0.0020 ± 0.0001	- 0.0002 ± 0.0000	0.85 ± 0.01

Everted segments were incubated aerobically for 3 hours.
- indicates net loss of monosaccharide
Initial concentration of 3-methylglucose was 0.047 µM/ml.
Initial concentration of D-fructose was 0.0021 µM/ml.

observed. Fractional S/M ratios and decreased mucosal and serosal concentrations were again observed in both the anterior and posterior intestine. In contrast to the date obtained using a 1 μM/ml concentration, these data suggest active transport of 3-methylglucose by the posterior esophagus and tend to confirm the absence of active transport of 3-methylglucose in the anterior and posterior intestine.

Table 4 presents the calculated per cent recovery of ^{14}C- labeled monosaccharides studied in the mapping determinations. The final mucosal concentrations only were used to calculate the per cent recovery since the amount of monosaccharide present in the serosal solution represents less than 1.0% of the total amount of monosaccharide present in each experiment using segments from the posterior esophagus and less than 0.05% of the total amount using either the anterior or posterior intestine. Per cent recoveries provide information as to the loss of monosaccharide due to metabolism and/or accumulation of the monosaccharide within the gut tissue. Since the size of the segments obtained varied greatly between the regions of the gut studied it is difficult to compare possible metabolism between the various regions of the gut. For example, the increased percent recoveries observed in the anterior and posterior intestine over the per cent recoveries seen in the posterior esophagus for the same monosaccharide are probably due to a much smaller segments used from the

TABLE 4 PERCENT RECOVERY OF CARBON-14 LABELED MONOSACCHARIDES USED IN THE IN VITRO TECHNIQUE

	D-glucose		D-galactose		3-methylglucose		D-fructose
	1 uM/ml	0.0045 uM/ml	1 uM/ml	0.081 uM/ml	1 uM/ml	0.047 uM/ml	0.0021 uM/ml
Posterior esophagus	56%	49%	86%	84%	95%	100%	100%
Anterior intestine	88%	58%	94%	93%	100%	100%	99%
Posterior intestine	92%	84%	97%	80%	100%	87%	99%

The values reported are the mean recoveries obtained from the final mucosal solutions.

anterior and posterior intestine rather than a different metabolic rate in these regions. However, as the segment sizes tested within each region were approximately constant, comparisons can be made between the various monosaccharides in each region. Thus, it appears that in all regions glucose is metabolized. Galactose also appears to be metabolized by all three regions of the gut. The metabolism of glucose and galactose is signficant as these data partially explain the reduced serosal and mucosal concentrations of the compounds and the fractional S.M ratios observed in Table 2 and 3.

The increased per cent recoveries observed with the 1 μM.ml concentrations of these metabolized monosaccharides is to be expected because there is a smaller concentration of labeled monosaccharide present in relation to the total concentration of monosaccharide at the 1 μM.ml concentration. Therefore, the increased per cent recoveries at the higher concentration do not necessarily indicate a difference in metabolic rates.

The recovery of essentially 100% of the initial concentration of 3-methylglucose indicates that 3-methylglucose is not metabolized by any region tested in the gut of the limpet and that very small amounts of this monosaccharide are retained by the gut tissue.

The mean dry gut weight ($\pm$ S.E.M) of the posterior esophagus was 0.087 $\pm$ 0.001. The mean dry gut weights of the

anterior and posterior intestine were 0.041 ± 0.001 and 0.019 ± 0.001 respectively.

Measurements of serosal sac volumes at the end of each incubation period showed that no measurable volume change had occurred in any of the experiments reported in Tables 2 and 3.

SERIES II Anaerobic inhibition studies

One of the characteristics of active transport is the requirement of a constant metabolic energy supply. Therefore, these studies were carried out in order to obtain further evidence of active transport. However, an increase in S/M ratios and serosal concentrations were observed during anaerobic conditions (Table 5). This was especially evident at the lower (0.0045 μM/ml) concentration and implies that the active transport of glucose by the posterior esophagus is not inhibited by anaerobic conditions.

SERIES III NaF inhibition studies

As it was probable that enough metabolic energy was being supplied from anaerobic monosaccharide metabolism to support active transport the effect of the glycolytic inhibitor (NaF) was studied. Table 6 shows that addition of NaF during the second incubation period reduced the serosal concentration to 38% of the value obtained at the end of the first incubation period. Upon removal of NaF, positive S/M ratios and increased serosal concentrations were re-established,

TABLE 5 EFFECT OF ANAEROBIA UPON D-GLUCOSE TRANSPORT IN M. CRENULATA GUT

Experimental condition	Number of observations	Final serosal concentration μM/ml ± S.E.M.	Final mucosal concentration μM/ml ± S.E.M.	Net serosal accumulation gram dry gut weight ± S.E.M.	Serosal/ mucosal ratio ± S.E.M.
			1μM/ml		
Air	(4)	1.02 ± 0.01	1.03 ± 0.13	0.48 ± 0.01	0.99 ± 0.01
N_2	(4)	1.13 ± 0.01	1.03 ± 0.18	1.25 ± 0.04	1.09 ± 0.01
Air	(4)	1.06 ± 0.01	1.01 ± 0.01	0.66 ± 0.01	1.05 ± 0.01
			0.0045μM/ml		
Air	(6)	0.0018 ± 0.0001	0.0033 ± 0.0001	- 0.02 ± 0.01	0.54 ± 0.05
N_2	(5)	0.0401 ± 0.0001	0.0031 ± 0.0001	0.21 ± 0.01	12.93 ± 2.30
Air	(6)	0.0099 ± 0.0001	0.0033 ± 0.0001	0.24 ± 0.01	3.00 ± 0.19

Everted posterior esophagus segments only were used. All incubation periods were 2 hours each.
- indicates net loss of monosaccharide.

TABLE 6 EFFECT OF NaF ON D-GLUCOSE TRANSPORT IN M. CRENULATA GUT

Experimental condition	Number of observations	Final serosal concentration μM/ml ± S.E.M.	Final mucosal concentration μM/ml ± S.E.M.	Net serosal accumulation/ gram dry gut weight ± S.E.M.	Serosal/ mucosal ratio ± S.E.M.
		0.0045uM/ml D-Glucose			
D-glucose	(10)	0.0042 ± 0.0001	0.0029 ± 0.0001	- 0.0025 ± 0.0005	1.45 ± 0.10
D-glucose and 7.5mM/ml NaF	(7)	0.0016 ± 0.0001	0.0030 ± 0.0001	- 0.0029 ± 0.0001	0.53 ± 0.05
D-glucose	(8)	0.0054 ± 0.0001	0.0032 ± 0.0001	0.0018 ± 0.0001	1.69 ± 0.02

Everted posterior esophagus segments were used. All incubation periods were 2 hours.
- indicates net loss of monosaccharide.

indicating that the effect of NaF on glucose active transport is reversible. These data imply that active transport of glucose in the posterior esophagus requires metabolic energy to maintain its selective absorption against an apparent concentration gradient and that a considerable amount is derived from anaerobic processes.

SERIES IV Transmural potential studies

Transmural potentials were measured across segments from the anterior and posterior intestine as a means of demonstrating that these segments were viable in the in vitro preparation used in this study. Furthermore, transmural potentials are indicative of ionic transport. For these reasons, transmural potentials across the three regions of the gut of the limpet were determined during mapping studies to obtain evidence for transport of ions by the limpet gut. All determinations were made in the presence of glucose in the incubation fluid. It is seen in Table 7 that transmural potentials were observed in all regions of the gut. The transmural potentials were significantly ($P < 0.05$) larger in the posterior esophagus than in the anterior or posterior intestine. All transmural potential were negative at the serosal and although small were significantly ($P < 0.05$) different from zero.

Since the initial data obtained from Series II indicated that anaerobic inhibition of active transport of glucose by

TABLE 7 TRANSMURAL POTENTIALS ACROSS THE M. CRENULATA GUT

Segment and number observations	0.5 hr.	1.0 hr.	2.0 hr.	3.0 hr.
Posterior esophagus (4)	- 0.47 ± 0.07	- 0.52 ± 0.06	- 0.40 ± 0.14	- 0.45 ± 0.001
Anterior intestine (4)	- 0.12 ± 0.01	- 0.15 ± 0.05	- 0.13 ± 0.02	- 0.12 ± 0.002
Posterior intestine (4)	- 0.14 ± 0.01	- 0.12 ± 0.002	- 0.10 ± 0.00	- 0.13 ± 0.02

Potential differences are expressed in mV ± standard error of mean. Potentials are given with mucosal compartment as reference of 0 mV. Zero time is taken at the beginning of incubation.

the posterior esophagus had not occurred it was of interest to determine whether or not anaerobic conditions were actually present. Therefore during all the pre-incubation and incubation periods in the anaerobic inhibition studies simultaneous measurements of transmural potentials were made as a means of demonstrating that anaerobic conditions did exist during the second incubation period. Table 8 shows transmural potentials determined across the posterior esophagus during the nitrogen and NaF induced anaerobic inhibition studies reported in Table 5. During anaerobic conditions there was a significant ($P < 0.05$) decrease in transmural potentials. The transmural potentials were observed to return to their original level upon re-establishment of aerobic conditions. This reversibility of transmural potentials indicates an active origin of these potentials and that anaerobic conditions had been established during Series II experiments.

Table 8 also shows transmural potentials determined across the posterior esophagus during the NaF inhibition studies reported in Table 6. Here again a significant ($P < 0.05$) decrease was observed during the period of inhibition followed by a return to the original level upon removal of NaF. These observations indicate that the measured transmural potentials are of active origin and that conditions of inhibition were established.

TABLE 8 EFFECT OF ANAEROBIA AND NcF ON TRANSMURAL POTENTIALS

Period and number observations	Glucose only		Period and number observations		Glucose and NcF
	1 uM/ml	0.0045 uM/ml			0.0045 uM/ml
Air (4)	- 0.53 ± 0.11	- 0.45 ± 0.01	Air (4)	D-glucose only	- 0.54 ± 0.02
N_2 (4)	- 0.25 ± 0.05	- 0.17 ± 0.08	Air (4)	D-glucose and NcF	- 0.15 ± 0.05
Air (4)	- 0.46 ± 0.03	- 0.45 ± 0.02	Air (4)	D-glucose only	- 0.47 ± 0.12

Posterior esophagus segments only were used. Potential differences are expressed in mV ± standard error of mean. Potentials are given with mucosal compartment as reference of $\overline{0}$ mV.

DISCUSSION

The development of S/M ratios > 1 which are due to an increased concentration of monosaccharide in the serosal compartment is evidence that active transport has occurred. Thus glucose and 3-methyl glucose are actively transported by the posterior esophagus. The development of S/M ratios < 1 is explained by the following. First, assume an equal rate of loss of monosaccharide due to metabolism and/or accumulation within the gut tissue from both compartments and essentially no movement of monosaccharide across the gut (no active transport). Then, there would be a larger decrease in the concentration of the monosaccharide in the serosal compartment per unit time because of the much smaller serosal compartment volume (the mucosal volume is approximately 100 times the serosal volume) as compared to the mucosal volume. Consequently, positive S/M ratios were also considered to be evidence for active transport even though there was no net accumulation of the monosaccharide further, one would assume that the fractional S/M ratios which are seen in Tables 2 and 3 were the result of metabolism and/or accumulation with very little movement of the monosaccharide across the gut and therefore are considered as evidence against the presence of an active transport mechanism. These fractional values make a positive S/M ratio so obtained much more significant. Even an S/M ratio of

approximately 1 would be indicative of active transport. Consequently, the data using 1 μM/ml and 0.047 μM/ml 3-methyl glucose in the posterior esophagus are not conflicting.

It is possible that glucose and 3-methylglucose are transported by the same transport mechanism since they are actively transported by the same region of the limpet gut. This has been reported to occur in the vertebrate intestine (Csaky, 1958) and in Cryptochiton stelleri (Lawrence and Lawrence, 1967, 1968). Glucose and 3-methylglucose are not actively transported in either the anterior or posterior intestine. This corresponds to the absence of glucose and 3-methylglucose active transport in the distal region of the intestine of Cryptochiton stelleri (Lawrence and Lawrence, 1967).

Startling, however, is the lack of evidence of transport of galactose in any region of the gut. Galactose is actively transported by all regions of the posterior intestine of Cryptochiton stelleri under both aerobic and anaerobic conditions (Lawrence and Lawrence, 1967) but presumably by a transport mechanism distinct from that for glucose. However, as these data indicate a lack of evidence for the presence of active transport of galactose in any region of the limpet gut tested it would appear that only one transport system is present. It is also of signficance that active transport of galactose by the vertebrate gut has been documented many times

(see reviews cited in Introduction section). However, the segments used in this study represent approximately 2/3 of the total gut therefore these data indicate that active transport of galactose appears to be absent only in those areas tested during the mapping determinations (refer to figure 1 for regions of the gut used in mapping determinations).

It is possible that the lack of evidence for monosaccharide transport by the anterior and posterior intestine is due to abnormal conditions within the segments from these regions. This is possible because these regions of the limpet gut are difficult to isolate from the visceral mass. However, because transmural potentials and amino acid transport (Lawrence and Wright, unpublished observations) can be obtained across segments from the anterior and posterior intestine it is more logical to assume that the lack of evidence for monosaccharide transport is due to the absence of an active transport mechanism for monosaccharide by these regions.

The probable existance of only one active transport system for monosaccharides in the gut of Megathura crenulata is of marked significance. Inferences which have been made as to the nature and specificity of active transport of monosaccharides by animal guts have been made on the assumption of a common pathway for all monosaccharides. However, Lawrence and Lawrence (1967, 1968) concluded that two distinct pathways

are present in the mollusc, Cryptochiton stelleri, thus indicating that this assumption is not valid for all phyla. Therefore, the presence of a distinct transport mechanism for galactose in the amphineuran Cryptochiton stelleri, yet no indication of galactose transport in the archegastropod Megathura crenulata, points out an interesting area of future research in terms of the nature of monosaccharide active transport itself and the comparative aspect of monosaccharide active transport within the phylum Mollusca.

The importance of the anaerobic studies was to establish whether or not anaerobic conditions had a specific and reversible effect upon active transport mechanisms due to removal of oxidative energy sources. This has been seen in several vertebrate guts (Darlington and Quastel, 1953; Wilson and Wiseman, 1954; Wilson and Vincent, 1955; Cordier and Chanel, 1961; Lluch and Ponz, 1962; and Musacchia et al., 1966). However, glucose and galactose active transport under anaerobic conditions has been observed in fetal and newborn rabbits (Wilson and Lin, 1960), in the bullhead catfish (Musacchia et al., 1964) and for galactose but not glucose in chiton (Lawrence and Lawrence, 1967). The movement of glucose in the posterior esophagus of the limpet gut persisted during anaerobic conditions and, in fact, appear to be stimulated by the presence of anaerobia. Simultaneous reduction of transmural potentials measured during anaerobic conditions

indicate that the observed movement of glucose actually occurred in the presence of anaerobic conditions which would reduce ion transport. The movement of glucose during anaerobic conditions indicates that the transport mechanism was able to function by utilizing ATP synthesized from anaerobic metabolism.

The dependence upon metabolic energy for active transport of glucose was shown, however, by the use of the glycolytic inhibitor, NaF. This indicates the requirement of metabolic energy for the active transport of glucose by the posterior esophagus. The fact that complete recovery was seen upon removal of the inhibitor indicates that the effect of NaF on the active transport mechanism is reversible and that no irreversible effects (e.g. tissue death, cellular damage, etc.) were suffered by the gut segments during inhibition. The remarkable tolerance of this tissue to two hours of inhibition is also signficant.

Fructose was not observed to undergo active transport in any region of the gut. This was also observed in Cryptochiton stelleri (Lawrence and Lawrence, 1967). Per cent recoveries of approximately 99% also indicate that fructose is not metabolized or converted to other compounds (e.g. glucose). Fructose has been reported to be metabolized and actively transported (Wilson and Vincent, 1955; Riklis and Quastel, 1958; and Lawrence, 1963). The apparent inability to convert

fructose to glucose by the mammalian gut has been associated with the absence of glucose-6-phosphatase (Kiyasu and Chaikoff, 1957). It is possible that this enzyme is lacking both in the limpet and chiton intestine.

Likewise, per cent recovery of the ^{14}C-labeled monosaccharides used in the mapping studies (Table 4) indicates that 3-methyl-glucose is not metabolized. This has also been observed in the vertebrate gut (Bihler and Adamic, 1967) and in Cryptochiton stelleri (Lawrence and Lawrence, 1967).

Both glucose and galactose appear to be metabolized by the gut Megathura crenulata. The rate of metabolism of glucose appears to be greater than the rate of metabolism of galactose, especially in the posterior esophagus.

Another similarity observed between the chiton and the limpet is the absence of net water movement from the mucosal compartment to the serosal compartment in any region of the intestine in the presence of glucose. However, both of these molluscs differ from the vertebrates in that the presence of glucose affects water movement by some regions of the vertebrate gut (Fisher and Parsons, 1953; Fullerton and Parsons, 1956; Smyth and Taylor, 1957; Parsons et al., 1958; Barry et al., 1961; Detheridge et al., 1966).

The presence of transmural potentials across the gut is assumed to indicate ion transport. The transmural potentials

observed in the vertebrate gut. In most vertebrate guts the serosal surface is positive with respect to the mucosal surface. This has been shown with respect to movement of HCl into the stomach (Bernstein et al., 1959) and movement of NaCl out of the ileum and colon (Curran, 1959-1960; Curran and Schwartz, 1959-60; and Schultz and Zalusky, 1964). In all regions of the limpet gut studied the serosal surface is negative with respect to the mucosal surface. Negative transmural potentials have been observed in Cecropia midgut and are attributed to a potassium pump (Haskell et al., 1965).

As the transmural potentials observed in the gut of Megathura crenulata are negative in all regions studied these transmural potentials are also dissimilar to the transmural potentials reported in the posterior intestine of Cryptochiton stelleri (Larence and Mailman, 1967). The anterior intestine of the chiton has a negative transmural potential while the posterior intestine (region of active transport of galactose) has positive transmural potentials. This difference between the two posterior intestines is interesting in the light of the lack of evidence for active transport of galactose by the limpet posterior intestine. Clarification as to the type of ion transport which caused this transmural potential was not made nor were concentration differences of sodium, potassium, or chloride determined.

In summary, glucose and 3-methylglucose are actively transported while galactose and fructose are not actively transported in the posterior esophagus of Megathura crenulata. There is no evidence for active transport of monosaccharides in either of the two other regions of the intestine studied. Negative transmural potentials are present in all regions of the gut studied with the largest values being obtained from the posterior esophagus.

The degree to which monosaccharides are actively transported indicates that the gut of the limpet is important in the absorption of monosaccharides and quite possibly organic nutrients in general.. Though previous investigators using gastropods (Hirsch, 1925; Peczenik, 1925; Graham, 1932; Millot, 1937a,b; Graham, 1938; Fretter, 1939; Graham, 1939; Howells, 1942; Carriker, 1946; Morton, 1956a,b; Martoja, 1961; van Weel, 1961; Fretter and Graham, 1962; Pugh, 1963; Sumner, 1965; Morse, 1966; and Ward, 1966) conclude that the digestive gland rather than the gut is the primary site of absorption, their data do not disagree with the findings of this study. This is because the data on which the previous findings were based did not deal with active transport of monosaccharides. Consequently, both the digestive gland and the gut of the limpet are important in the absorption of nutrients. The relative importance of each has yet to be determined.

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