ABSORPTION OF MONOSACCHARIDES, *IN VITRO*, FROM THE GUT OF THE ECHIUROID, *URECHUS CAUPO*

A thesis

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for

Senior Honors in Biology

By

Joan Wu

August, 1971

ACKNOWLEDGMENT

To the Head Magician, Dr. A. L. Lawrence,
who taught me the secret words;
To Mr. M. E. Hanisch, who taught me the tricks;
To Mr. D. M. Jessel, who gave me the magic
numbers;
To my father, who taught me to reach further
than I knew how ...

Thank you.

ABSORPTION OF MONOSACCHARIDES, *IN VITRO*, FROM THE GUT OF THE ECHIUROID, *URECHUS CAUPO*

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ABSTRACT

The purpose of these studies was to obtain evidence for the active transport of monosaccharides by the gut of the U. Caupo by determining if D-glucose, D-galactose, 3-O-methyl-D-glucose and/or D-mannitol were absorbed against a concentration difference, and if this ability was specific for one or more of these monosaccharides.

The following results were obtained. Positive evidence was shown for the active transport of D-glucose and was suggested for D-galactose with initial ambient concentrations of 1.00×10^{-9} moles/ml and 1.00×10^{-6} moles/ml. No evidence for the active transport of 3-O-methyl-D-glucose and D-mannitol was obtained. D-glucose at an initial ambient concentration of 1.00×10^{-9} moles/ml appears to be closer to the optimum transport concentration than D-glucose at 1.00×10^{-6} moles/ml. Anaerobic conditions did not inhibit active transport of D-glucose or D-galactose. This suggests the utilization of a different energy-producing mechanism, independent of oxidative processes. No evidence was found for the anabolism of 3-O-methyl-D-glucose, D-galactose, or D-mannitol, whereas significant amounts of D-glucose were anabolized. No net water movement was observed between the serosal and mucosal sides of gut preparations.

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INTRODUCTION

Active transport is the most important cellular mechanism for the absorption of monosaccharides by the intestine of animals. Although active transport studies of monosaccharide absorption have been done extensively in the vertebrates (Wilson and Vincent, 1955; Crane, 1960; Wilson and Landau, 1960; Lawrence, 1962); among the marine invertebrates, however, only the molluscs (Lawrence and Lawrence, 1967a,b) and echinoderms (D'Agostino and Farmanfarmaian, 1960; Lawrence *et al.*, 1966) have been studied. Thus, it would be pertinent to extend this information to other invertebrates.

Urechus caupo, a worm-like animal of the phylum Echiuroidea (Figure 1), was selected for these transport studies of monosaccharide absorption for the following reasons. First, transport studies have never been conducted with the gut of this very primitive animal. Secondly, the morphology of the gut seemed applicable to *in vitro* studies (Figure 2). In addition, the presence of a high blood sugar content (Lawrence *et al.*, 1971) suggests that the animal may have the ability to absorb large quantities of sugar and that this movement is possibly against a concentration gradient.

The purpose of this study was to obtain evidence for the active transport of monosaccharides by the gut of the *Urechus caupo* by determining if D-glucose, D-galactose, 3-O-

methyl-D-glucose and/or D-mannitol were absorbed against a concentration difference, and if this ability was specific for one or more of these monosaccharides.

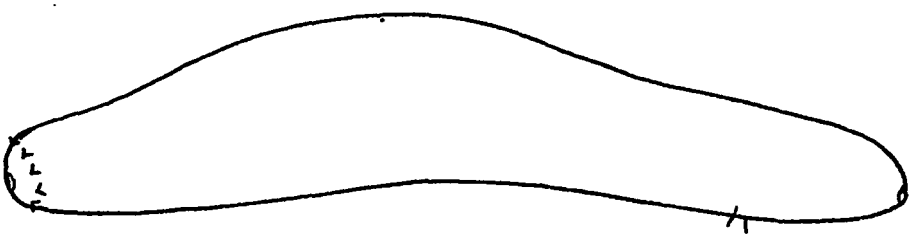

Figure 1: *Urechus caupo* (Posterior → Anterior)

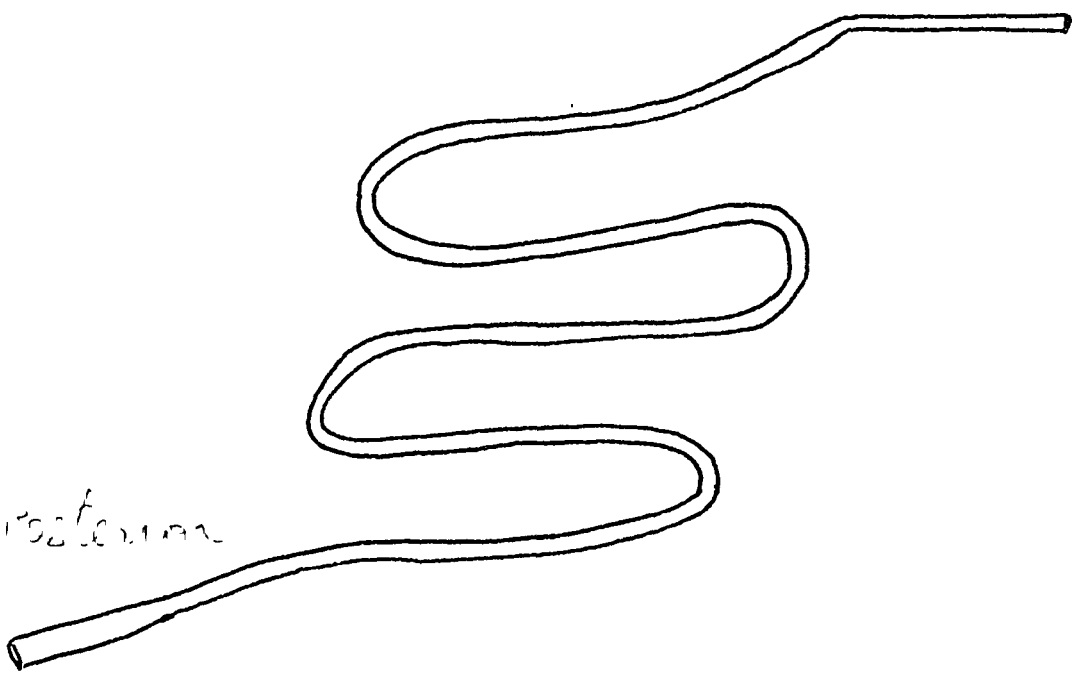

Figure 2: Diagram of *U. caupo* gut

METHODS AND MATERIAL

Animals

Adult animals; obtained from Pacific BioMarine Supply Company, Venice, California; were starved and kept under aeration in a cold room at 5°C for one week prior to experimentation. Animals were used over a period of twenty-eight days. One animal was used per experiment.

In each experiment, animals were sacrificed in the following manner. A longitudinal incision was made through the body wall, whereupon the intestines were removed and placed in Urechus Ringers Solution (Table 1). The length of the total gut was measured and approximately four-inch segments were used for each observation. Whenever possible, observations were prepared consecutively from the proximal to the distal end of the gut.

Chemicals

The following concentrations of three different test monosaccharides were used (Table 2). In addition, a concentration of D-mannitol equal to that of the test monosaccharide was also present in one of the experimental approaches used (exception was when 1.00×10^{-8} moles/ml of 3-O-methyl-D-glucose was used with 1.00×10^{-9} moles/ml of D-mannitol).

Radioactive dual labeling was also employed as an experimental device in these studies. The following radioactive

TABLE 1: COMPOSITION OF "URECHUS RINGERS"

Compound	g/L	mM
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	0.55

TABLE 2: TEST COMPOUNDS AND CONCENTRATIONS

Test compound	Concentration of test compound (moles/ml)	Concentration of D-mannitol (moles/ml)
D-Glucose	1.00×10^{-9}	1.00×10^{-9}
	1.00×10^{-6}	1.00×10^{-6}
D-Galactose	1.00×10^{-9}	1.00×10^{-9}
	1.00×10^{-6}	1.00×10^{-6}
3-0-Methyl-D-Glucose	1.00×10^{-8}	1.00×10^{-9}
	1.00×10^{-6}	1.00×10^{-6}

compounds were employed; D-glucose-U-^{14}C, D-galactose-1-^{14}C, 3-O-$^{14}CH_3$-D-glucose, and D-mannitol-U-^{3}H. In each case, the activity for ^{14}C was 5μc per liter and ^{3}H was 50μc per liter.

Experimental Design

The following is a description of the in vitro everted sac method used in this study (Crane and Wilson, 1958 - as revised by Lawrence, 1963).

a) Everting Segments

Gut segments, 8 and 12 inches long, were isolated and placed in a dish of Urechus Ringers. Each segment was inserted over a glass everting rod and the gut segment end at the tip of the rod ligated. Then the loose end was pulled back over the ligated end, thus exposing the mucosal surface of the isolated segment (Figure 3). These everted segments were then cut into four-inch segments.

b) Mounting Segments

A small glass weight was tied to one end of the everted segment (Figure 4). The free end was slipped over the tip of a glass cannula and ligated, thus enclosing the serosal compartment (Figure 5).

c) Incubation

The entire apparatus (rubber stopper, glass cannula, and ligated gut segment) was immersed in a wide-mouth test tube containing 32 ml of the test solution made from Urechus Ringers (Figure 6). This 32 ml will hereafter be

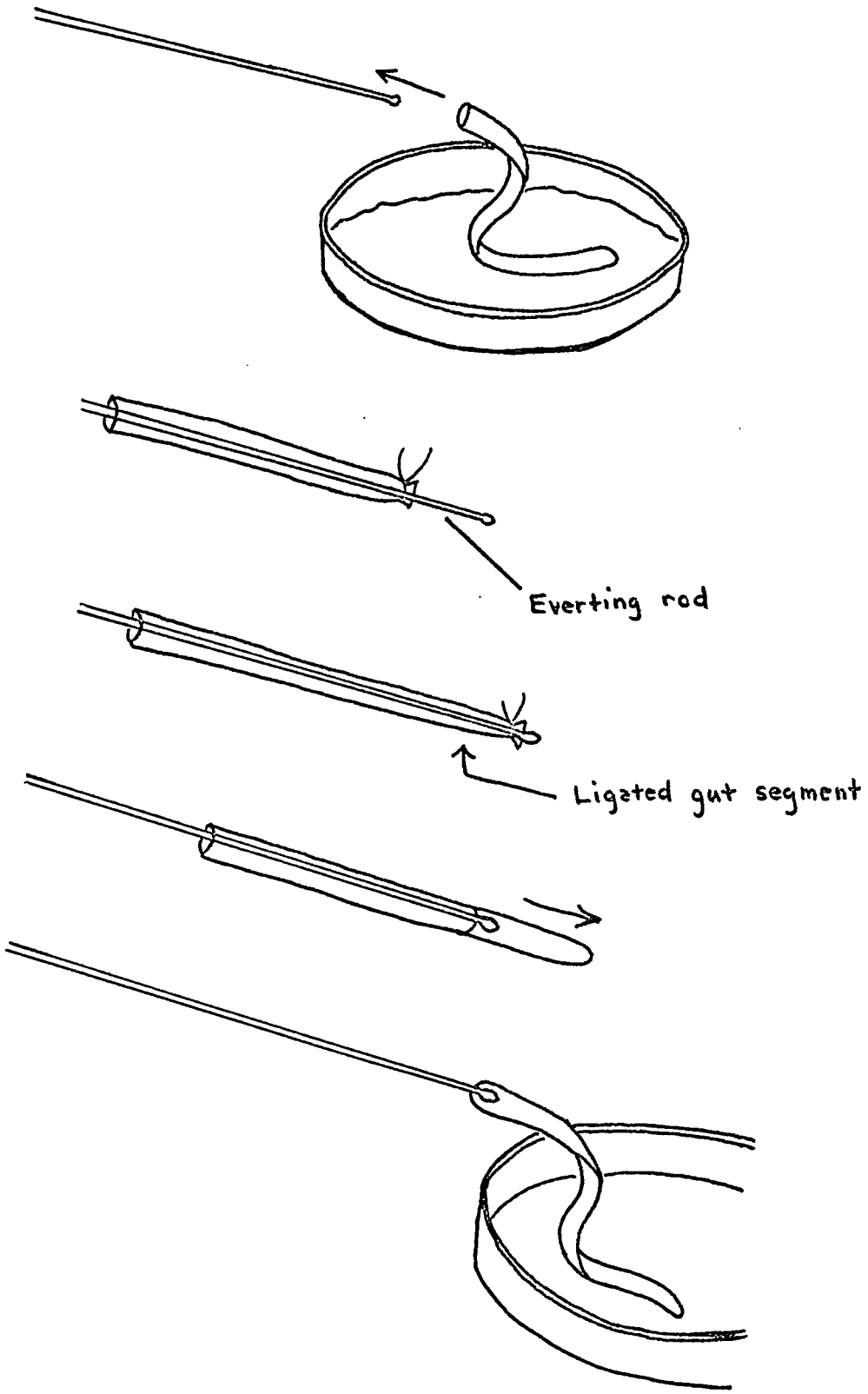

Figure 3: Everting gut segment

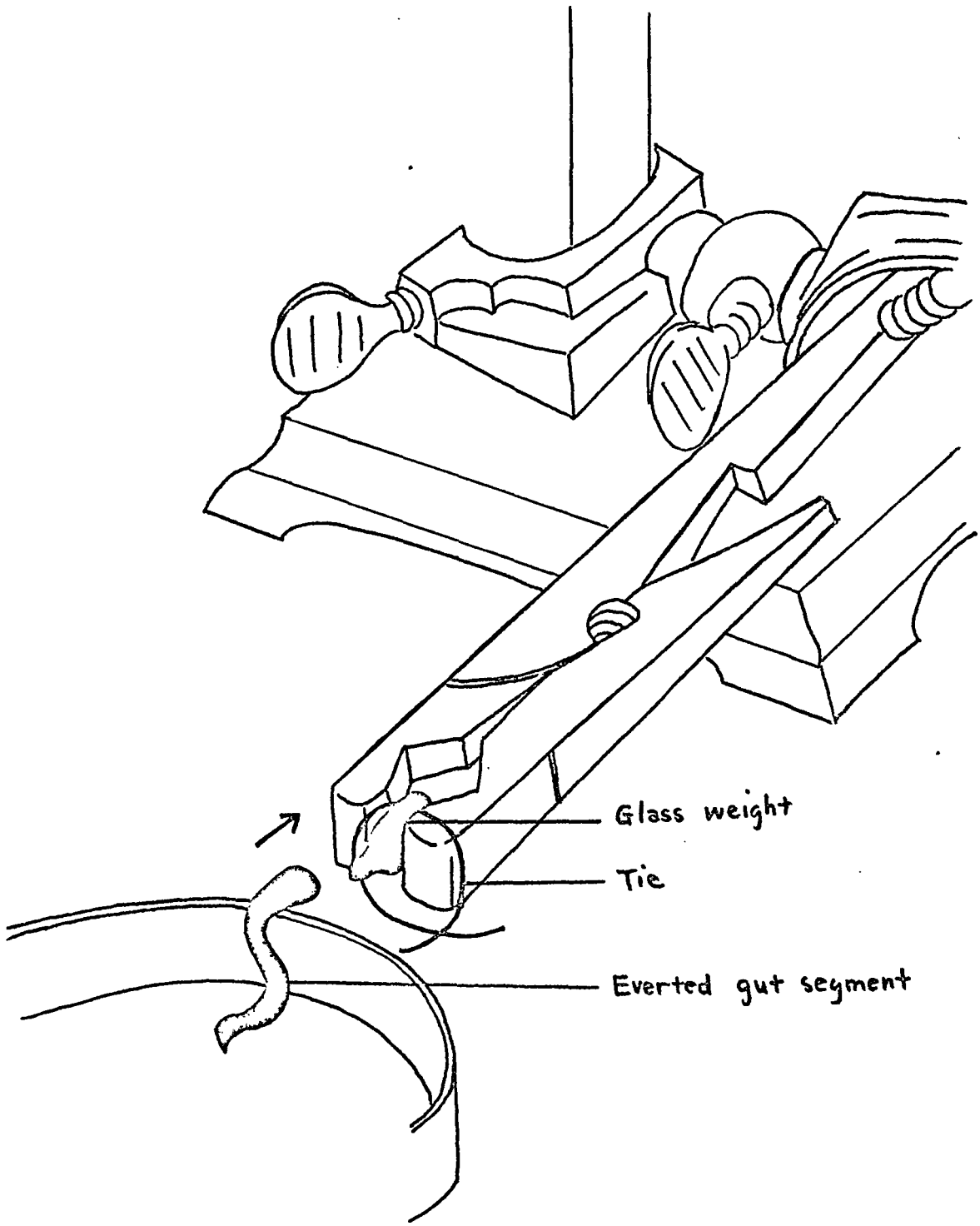

Figure 4: Attaching glass weight to everted gut segment

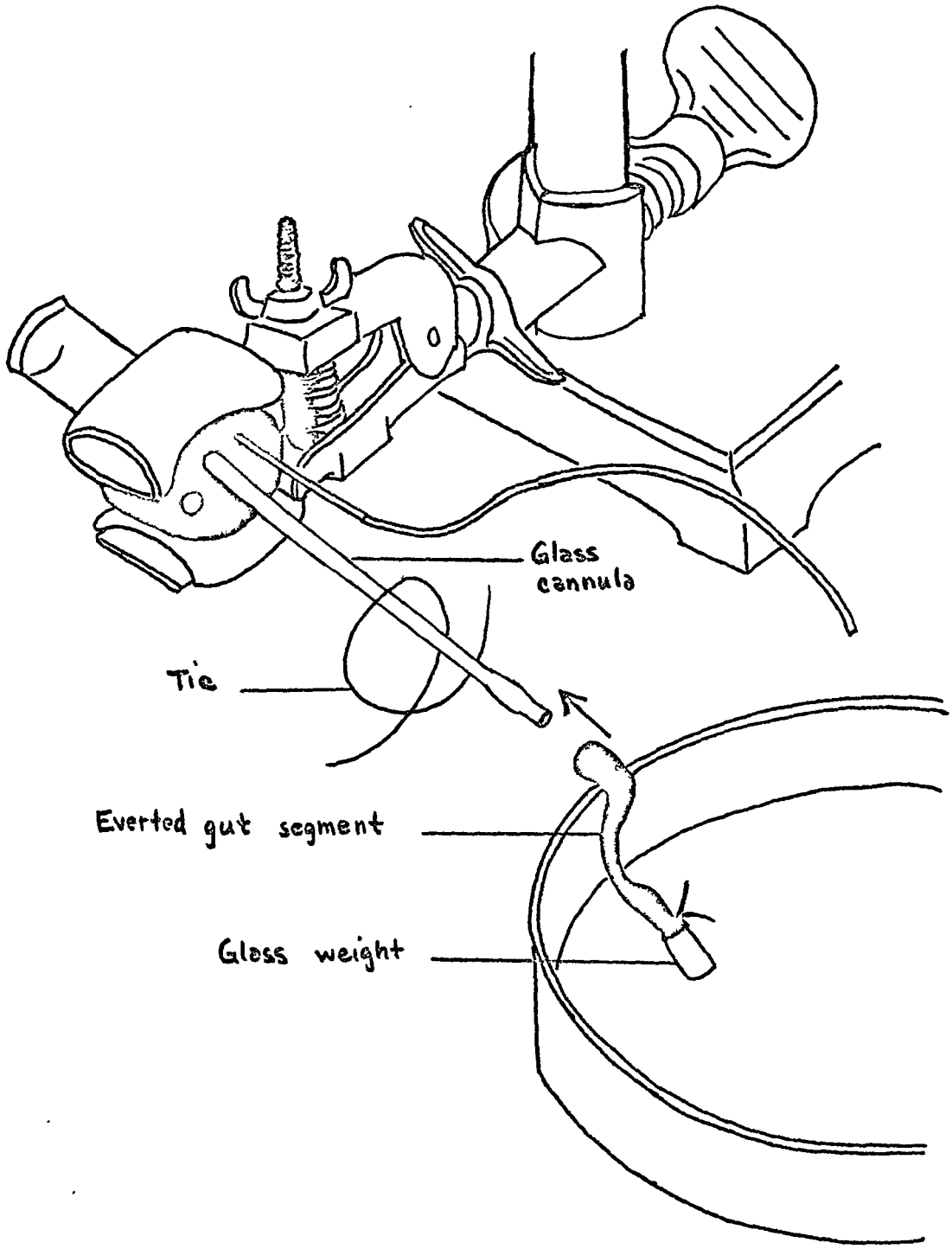

Figure 5: Attaching everted gut segment (with glass weight) to tip of glass cannula

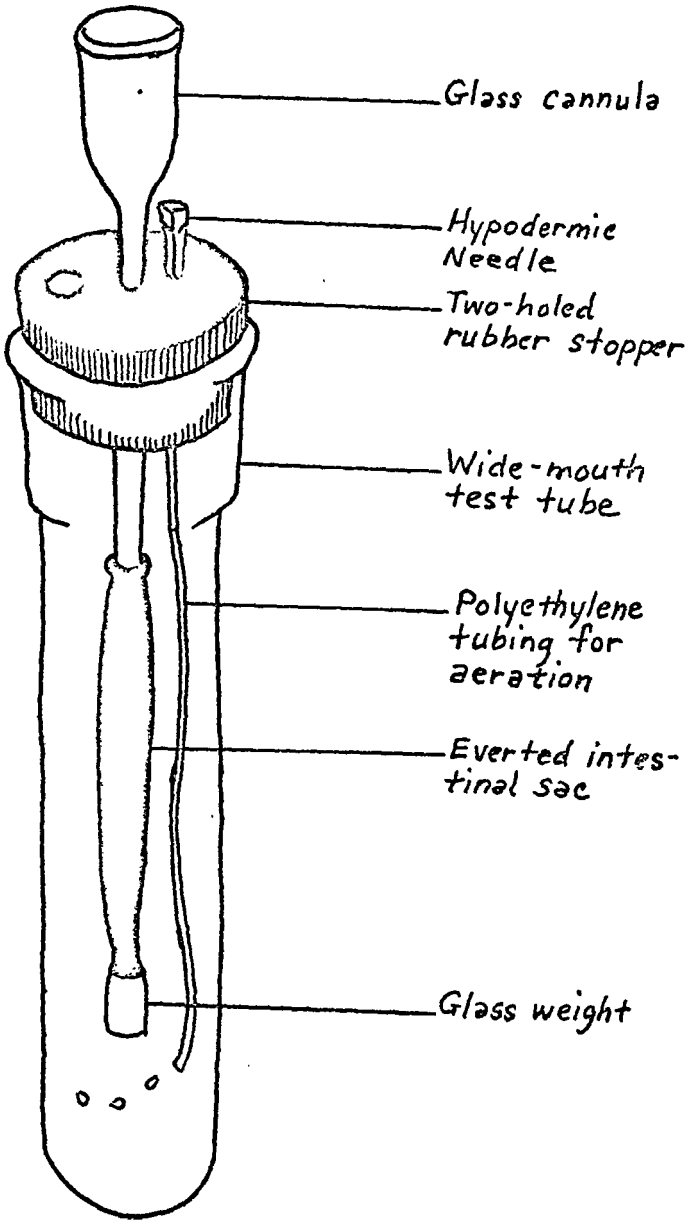

Figure 6: The experimental apparatus

referred to as the mucosal solution since it bathes the mucosal surface. An identical solution to the mucosal solution was immediately added to the serosal compartment. This solution will hereafter be referred to as the serosal solution since this solution bathes the serosal surface.

This technique was used in three types of experimental approaches. In Approach I, half of the gut preparations were immersed in a mucosal solution containing 1.00×10^{-9} moles/ml of D-glucose while the remaining samples were in a 1.00×10^{-6} moles/ml of D-glucose mucosal solution. The serosal compartments were filled with serosal solution containing concentrations of D-glucose identical to the mucosal solutions. These gut preparations were then incubated for three hours at 15°C with constant aeration of the mucosal solutions. In order to detect any net water movement, initial as well as final serosal volumes were measured. At the end of incubation, 200λ each of serosal and mucosal samples were obtained and placed in 15ml of Scintillation Cocktail I (Table 3). These samples were counted in a Beckman Liquid Scintillation Counter at 2% error.

Approach II was similar to Approach I except for the following changes. D-mannitol was present in an equal concentration as D-glucose. Also, a one-hour preincubation preceded a one-hour experimental period. Immediately after pre-

incubation, each segment was placed in tubes containing a test solution which was identical to the preincubation solution. Subsequently, the serosal fluid was removed with a long, slender pipett and was replaced with a fresh, identical solution.

Finally, in Approach III the same two different concentrations were employed in a comparison of aerobic versus anaerobic conditions for D-glucose and D-galactose. The only exception was 3-O-methyl-D-glucose which was 1.00×10^{-8} moles/ml, and D-mannitol was 1.00×10^{-9} moles/ml.

1. Aerobic Conditions

   This phase was equivalent to Approach II. Additionally, following sampling of serosal and mucosal fluids, each apparatus was placed into fresh test solutions, identical to the previous incubation fluid; a fresh, identical serosal fluid was also added.

2. Anaerobic Conditions

   This period was similar to aerobic conditions except mucosal solutions were bubbled with a gas mixture of 0.5% carbon dioxide and 95.5% nitrogen. Also preincubation was one-half hour instead of one hour.

3. Aerobic Conditions

   Experimental procedures were similar to anaerobic conditions except mucosal solutions were bubbled under atmospheric air.

TABLE 3: SCINTILLATION COCKTAIL I

Reagents	Proportions (ml)
Toluene (Reagent grade)	500
Liquifluor (New England Nuclear)	21
BBS-3 (Beckman)	42

TABLE 4: SCINTILLATION COCKTAIL II

Reagents	Proportions
Toluene (Reagent grade)	1000 ml
PPO (2,5-diphenyloxazole) (Beckman)	6 g

Tissue Accumulation

The subsequent procedures were employed in all of the experimental approaches except the first. At the end of each experiment, each gut segment was rinsed in Urechus Ringers, and these procedures were followed.

a) Ethanol Extraction

Approximately 100 mg of each gut segment was placed in five ml of 75% ethanol (789 ml of 95% ethanol and 211 ml of distilled water). Ethanol, a polar hydrophobic solvent, can extract free organic molecules from the tissue and dissolve it into solution. Thus, by sampling the ethanol-soluble portion and counting its radioactive label, an estimation of the amount of ^{14}C and ^{3}H accumulated by the tissue could be made.

b) Ethanol-insoluble Extraction

After subjection to ethanol extraction, each piece of gut was immersed in one ml of Soluene (Packard), a tissue solubilizer. The tissue dissolved after 48 hrs, and 15 ml of Scintillation Cocktail II (Table 4) was added. Five days later, samples were counted in a Beckman Liquid Scintillation Counter. Thus, an evaluation of how much test monosaccharide was incorporated into the tissue which could not be extracted by 75% ethanol could be made. This incorporated non-ethanol-soluble ^{14}C and ^{3}H was assumed to represent the amount of ^{14}C

and ^{3}H anabolized into large organic compounds.

No dry weight determinations were made, as these were done earlier. Therefore, all dry weight values were calculated as 22% of the total wet weight (based upon earlier percent dry weight determinations for U. caupo by Lawrence, unpublished observations).

Calculations

The following parameters were used in this study to determine the presence o absence of active transport in monosaccharide absorption:

a) Serosal-mucosal ratio (S/M) is the final serosal concentration versus the final mucosal concentration, given as follows:

$$\frac{S}{M} = \frac{\text{Final serosal concentration}}{\text{Final mucosal concentration}}$$

b) The final net serosal accumulation is the concentration of the actively transported compound per unit dry gut tissue weight, given as follows:

$$\text{Final net serosal accumulation} = \frac{\text{Final serosal concentration} - \text{Initial serosal concentration}}{\text{100 mg dry tissue weight}}$$

c) The ethanol-soluble tissue accumulation was calculated as follows:

$$\text{Ethanol-soluble tissue accumulation per ml of tissue water} = \frac{\text{Total amount of ethanol-extractable test substance in terms of radioactive label}}{\text{Total tissue volume}}$$

A S/M ratio > 1, positive net serosal accumulation, and ethanol-soluble tissue accumulation per ml of tissue water greater than initial ambient concentration indicate the presence of active transport of a given test substance. However, negative values do not disprove active transport, necessarily, since a test substance may be metabolized so quickly and/or in such a great quantity as it is transported that no immediate accumulation is evident (This will be analyzed further in the discussion).

The total net tissue accumulation was calculated as follows:

$$\text{Total net tissue accumulation per ml tissue water} = \frac{\text{Total amount of ethanol-extractable test substance in terms of radioactive label} + \text{Total amount of ethanol-nonextractable test substance in terms of radioactive label}}{\text{Total tissue volume}}$$

Thus, the difference between the total net tissue accumulation and the ethanol-soluble tissue accumulation values represents the ethanol-insoluble amount of the test substance which was assumed to be anabolized into larger organic compounds.

RESULTS

In absorption studies of D-glucose, 3-O-methyl-D-glucose, D-galactose, and D-mannitol, no net movement of water was observed between the mucosal and serosal sides of the gut preparations.

Measurements recorded for the length of the U. caupo gut averaged four times the animal's body length (24" versus 6").

Equal initial concentrations of D-mannitol and D-glucose, D-galactose, or 3-O-methyl-D-glucose constituted a test set. An exception was made when D-mannitol was at a concentration of 1.00×10^{-9} moles/ml and 3-O-methyl-D-glucose at 1.00×10^{-8} moles/ml. Each test set was subjected to two initial ambient concentrations, 1.00×10^{-9} moles/ml and 1.00×10^{-6} moles/ml. In all cases tested, D-mannitol values were always less than those of the other monosaccharide in the test set. Also in all cases tested, no evidence for the active transport of D-mannitol against a concentration difference was found, as observed by S/M ratios < 1, negative net serosal accumulation values, and ethanol-soluble tissue accumulation per ml tissue water less than initial ambient concentration (Tables 5-11).

No evidence for the active transport of D-galactose was obtained although S/M ratios and net serosal accumu-

lation values were positive, but not significantly ($P>0.05$), and ethanol-soluble tissue accumulation values were less than the initial ambient concentration (Tables 5 & 6). However, when compared with D-mannitol values for these respective parameters, D-galactose values were always found to be greater than those for D-mannitol, which is indicative of active transport for D-galactose (Tables 5 & 6). Under anaerobic conditions, D-galactose transport, if indeed present, was not inhibited (Tables 5 & 6).

No evidence for the active transport of 3-O-methyl-D-glucose was found as demonstrated by S/M ratios < 1, negative net serosal accumulations, and ethanol-soluble tissue accumulation per ml tissue water less than initial ambient concentrations (Tables 7 & 8). When these values were compared with the respective values for D-mannitol, equated at the same concentrations, they were found to be similar ($p> 0.05$). This also indicated that 3-O-methyl-D-glucose was not actively transported.

Evidence for the active transport of D-glucose using an initial ambient concentration of 1.00×10^{-6} moles/ml or 1.00×10^{-9} moles/ml were obtained, as indicated by S/M ratios > 1, positive net serosal accumulations, and ethanol-soluble tissue accumulations per ml tissue water greater than initial ambient concentrations (Tables 9 & 10). Furthermore, since D-mannitol values were significantly less than those

of D-glucose ($P < 0.05$), this was also indicative of active transport for D-glucose (Tables 9 - 12). It was also found that D-glucose accumulated at a faster rate at an initial ambient concentration of 1.00×10^{-9} moles/ml as compared with the initial concentration of 1.00×10^{-6} moles/ml (Tables 9-12). When data from aerobic test periods were compared with those obtained in the anaerobic test periods for both concentrations of D-glucose (Tables 9 & 10), no significant differences were found ($P > 0.05$).

After five hours of <u>in vitro</u> conditions, the mucosal surfaces of the gut preparations sloughed badly; although, surprisingly, the active transport mechanism showed no signs of deterioration, since S/M ratios either remained constant or continued to rise.

In comparing the degree of anabolism of all test sets at both initial ambient concentrations, it was found that only D-glucose was anabolized into ethanol-insoluble materials in all experiments using D-glucose (Tables 9 & 10), whereas neither D-galactose (Tables 5 & 6), 3-O-methyl-D-glucose (Tables 7 & 8) nor D-mannitol (Tables 5-11) were shown to be anabolized at either initial ambient concentration.

TABLE 5: THE EFFECTS OF ANAEROBIC CONDITIONS ON ABSORPTION OF D-GALACTOSE AND D-MANNITOL IN U. CAUPO GUT

Condition and test compound; number of observations	S/M ratio*	Final serosal concentration (moles/ml)*	Net serosal accumulation (moles/100 mg dry gut weight)*	Total net tissue accumulation (moles/ml tissue water)*	Ethanol-soluble accumulation (moles/ml tissue water)*
D-Galactose:					
Aerobic (3)	1.08 ±0.06	1.06×10^{-9} $\pm 7.00 \times 10^{-11}$	8.00×10^{-11} $\pm 8.00 \times 10^{-11}$	----	----
Anaerobic (2)	1.31 ±0.03	1.37×10^{-9} $\pm 8.00 \times 10^{-11}$	3.00×10^{-11} $\pm 9.00 \times 10^{-11}$	----	----
Aerobic (2)	1.47 ±0.09	1.45×10^{-9} $\pm 1.17 \times 10^{-10}$	4.10×10^{-10} $\pm 2.00 \times 10^{-10}$	9.60×10^{-10} $\pm 2.50 \times 10^{-10}$	8.10×10^{-10} $\pm 9.00 \times 10^{-11}$
D-Mannitol:					
Aerobic (3)	0.95 ±0.02	9.20×10^{-10} $\pm 2.00 \times 10^{-11}$	-9.00×10^{-11} $\pm 3.00 \times 10^{-11}$	---- ----	---- ----
Anaerobic (2)	0.95 ±0.04	9.50×10^{-10} $\pm 5.00 \times 10^{-11}$	-5.00×10^{-11} $\pm 4.00 \times 10^{-11}$	3.10×10^{-10} $\pm 5.00 \times 10^{-11}$	2.20×10^{-10} $\pm 5.00 \times 10^{-11}$
Aerobic (2)	1.00 ±0.03	9.70×10^{-10} $\pm 5.00 \times 10^{-11}$	-4.00×10^{-11} $\pm 4.00 \times 10^{-11}$	↓	↓

The initial serosal and mucosal solutions for all test periods contained 1.00×10^{-9} moles/ml each of D-galactose and D-mannitol. The duration for each test period was one hour. The experimental conditions for each test period were identical with the exception that a one-hour preincubation preceded the first test period; the second and third were preceded by a half-hour preincubation; the second test period was under anaerobic conditions, and the first and third under aerobic conditions. *All values were means ±S.E.M.'s.

TABLE 6: THE EFFECTS OF ANAEROBIC CONDITIONS ON ABSORPTION OF D-GALACTOSE AND D-MANNITOL IN U. CAUPO GUT

Condition, test compound, and number of observations	S/M ratio*	Final serosal concentration (moles/ml)*	Net serosal accumulation (moles/100 mg dry gut weight)*	Total net tissue accumulation (moles/ml tissue water)*	Ethanol-soluble accumulation (moles/ml tissue water)*
D-galactose:					
Aerobic (3)	1.04 ±0.11	$1.05 \times 10^{-6} \pm 1.33 \times 10^{-7}$	$9.37 \times 10^{-8} \pm 1.10 \times 10^{-7}$	----	----
Anaerobic (1)	1.12	1.11×10^{-6}	5.33×10^{-8}	----	----
Aerobic (2)	1.49 ±0.02	$1.48 \times 10^{-6} \pm 6.40 \times 10^{-8}$	$4.74 \times 10^{-7} \pm 2.64 \times 10^{-7}$	$5.87 \times 10^{-7} \pm 3.87 \times 10^{-7}$	$5.69 \times 10^{-7} \pm 4.06 \times 10^{-7}$
D-mannitol:					
Aerobic (3)	0.91 ±0.04	$9.01 \times 10^{-7} \pm 4.84 \times 10^{-8}$	$-7.93 \times 10^{-8} \pm 8.48 \times 10^{-9}$	----	----
Anaerobic (1)	0.92	9.14×10^{-7}	-4.14×10^{-8}	----	----
Aerobic (2)	1.01 ±0.01	$9.80 \times 10^{-7} \pm 4.25 \times 10^{-8}$	$-6.99 \times 10^{-9} \pm 1.85 \times 10^{-8}$	$1.83 \times 10^{-7} \pm 1.12 \times 10^{-7}$	$8.60 \times 10^{-8} \pm 3.83 \times 10^{-8}$

The initial serosal and mucosal solutions for all test periods contained 1.00×10^{-6} moles/ml each of D-galactose and D-mannitol. The duration for each test period was one hour. The experimental conditions for each test period were identical with the exception that a one-hour preincubation preceded the first test period; the second and third were preceded by a half-hour preincubation; the second test period was under anaerobic conditions, and the first and third under aerobic conditions. *All values were means ± S.E.M.'s with the exception that values for the second test period were based upon one observation.

TABLE 7: THE EFFECTS OF ANAEROBIC CONDITIONS ON ABSORPTION OF 3-0-METHYL-D-GLUCOSE AND D-MANNITOL IN U. CAUPO GUT

Condition, test compound, and number of observations	S/M ratio*	Final serosal concentration (moles/ml)*	Net serosal accumulation (moles/100 mg dry gut weight)*	Total net tissue accumulation (moles/ml tissue water)*	Ethanol-soluble accumulation (moles/ml tissue water)*
3-0-Methyl-D-Glucose:					
Aerobic (3)	0.81 ±0.04	$8.05 \times 10^{-9} \pm 3.00 \times 10^{-10}$	$-1.48 \times 10^{-9} \pm 4.00 \times 10^{-11}$	----	----
Anaerobic (3)	1.17 ±0.25	$9.25 \times 10^{-9} \pm 2.60 \times 10^{-10}$	$-5.20 \times 10^{-10} \pm 1.12 \times 10^{-10}$	----	----
Aerobic (3)	0.97 ±0.03	$9.60 \times 10^{-9} \pm 2.80 \times 10^{-10}$	$-2.70 \times 10^{-10} \pm 1.50 \times 10^{-10}$	$1.38 \times 10^{-9} \pm 2.30 \times 10^{-10}$	$1.17 \times 10^{-9} \pm 3.70 \times 10^{-10}$
D-Mannitol:					
Aerobic (3)	0.91 ±0.03	$9.00 \times 10^{-10} \pm 2.00 \times 10^{-11}$	$-8.00 \times 10^{-11} \pm 2.00 \times 10^{-11}$	----	----
Anaerobic (3)	1.24 ±0.28	$9.70 \times 10^{-10} \pm 1.00 \times 10^{-11}$	$-2.00 \times 10^{-11} \pm 1.00 \times 10^{-11}$	----	----
Aerobic (3)	0.99 ±0.02	$9.80 \times 10^{-10} \pm 2.00 \times 10^{-11}$	$-1.00 \times 10^{-11} \pm 2.00 \times 10^{-11}$	$1.20 \times 10^{-10} \pm 1.00 \times 10^{-11}$	$4.00 \times 10^{-11} \pm 1.00 \times 10^{-11}$

The initial serosal and mucosal solutions for all test periods contained 1.00×10^{-8} moles/ml of 3-0-methyl-D-glucose and 1.00×10^{-9} moles/ml of D-mannitol. The duration for each test period was one hour. The experimental conditions for each test period were identical with the exception that a one-hour preincubation preceded the first test period; the second and third were preceded by a half-hour preincubation; the second test period was under anaerobic conditions, and the first and third under aerobic conditions. *All values were means ± S.E.M.'s. There were no significant differences ($P > 0.05$) for any test periods of the same parameters for 3-0-methyl-D-glucose and D-mannitol.

TABLE 8: THE EFFECTS OF ANAEROBIC CONDITIONS ON ABSORPTION OF 3-0-METHYL-D-GLUCOSE AND D-MANNITOL IN U. CAUPO GUT

Condition, test compound, and number of observations	S/M ratio*	Final serosal concentration (moles/ml)*	Net serosal accumulation (moles/100 mg dry gut weight)*	Total net tissue accumulation (moles/ml tissue water)*	Ethanol-soluble accumulation (moles/ml tissue water)*
3-0-methyl-D-Glucose:					
Aerobic (3)	0.81 ±0.05	$7.84 \times 10^{-7} \pm 4.56 \times 10^{-8}$	$-1.29 \times 10^{-7} \pm 1.20 \times 10^{-8}$	----	----
Anaerobic (3)	0.94 ±0.01	$9.36 \times 10^{-7} \pm 1.62 \times 10^{-8}$	$-3.69 \times 10^{-8} \pm 4.83 \times 10^{-9}$	----	----
Aerobic (3)	0.97 ±0.02	$9.64 \times 10^{-7} \pm 2.20 \times 10^{-8}$	$-1.89 \times 10^{-8} \pm 5.74 \times 10^{-9}$	$8.71 \times 10^{-8} \pm 2.44 \times 10^{-8}$	$8.15 \times 10^{-8} \pm 2.02 \times 10^{-8}$
D-Mannitol:					
Aerobic (3)	0.89 ±0.04	$8.42 \times 10^{-7} \pm 4.91 \times 10^{-8}$	$-9.34 \times 10^{-8} \pm 1.42 \times 10^{-8}$	----	----
Anaerobic (3)	0.99 ±0.04	$9.68 \times 10^{-7} \pm 1.51 \times 10^{-8}$	$-1.68 \times 10^{-8} \pm 4.44 \times 10^{-9}$	----	----
Aerobic (3)	0.98 ±0.02	$9.87 \times 10^{-7} \pm 1.58 \times 10^{-8}$	$-6.78 \times 10^{-9} \pm 6.04 \times 10^{-9}$	$8.07 \times 10^{-8} \pm 1.20 \times 10^{-8}$	$2.69 \times 10^{-8} \pm 5.25 \times 10^{-9}$

The initial serosal and mucosal solutions for all test periods contained 1.00×10^{-6} moles/ml each of 3-0-methyl-D-glucose and D-mannitol. The duration for each test period was one hour. The experimental conditions for each test period were identical with the exception that a one-hour preincubation preceded the first test period; the second and third were preceded by a half-hour preincubation; the second test period was under anaerobic conditions, and the first and third under aerobic conditions. *All values were means ± S.E.M.'s. There were no significant differences ($P > 0.05$) for any test periods of the same parameters for 3-0-methyl-D-glucose or D-mannitol or between 3-0-methyl-D-glucose and D-mannitol.

TABLE 9: THE EFFECTS OF ANAEROBIC CONDITIONS ON ABSORPTION OF D-GLUCOSE AND D-MANNITOL IN U. CAUPO GUT

Condition, test compound, and number of observations	S/M ratio*	Final serosal concentration (moles/ml)*	Net serosal accumulation (moles/100 mg dry gut weight)*	Total net tissue accumulation (moles/ml tissue water)*	Ethanol-soluble accumulation (moles/ml tissue water)*
D-glucose:					
Aerobic (3)	16.28 ± 3.00	$11.35 \times 10^{-9} \pm 2.02 \times 10^{-9}$	$7.15 \times 10^{-9} \pm 1.33 \times 10^{-9}$	----	----
Anaerobic (3)	18.00 ± 0.80	$13.73 \times 10^{-9} \pm 9.80 \times 10^{-10}$	$8.80 \times 10^{-9} \pm 1.60 \times 10^{-9}$	----	----
Aerobic (3)	20.12 ± 7.21	$13.27 \times 10^{-9} \pm 5.04 \times 10^{-9}$	$7.40 \times 10^{-9} \pm 1.30 \times 10^{-9}$	$12.08 \times 10^{-9} \pm 4.40 \times 10^{-9}$	$8.98 \times 10^{-9} \pm 2.96 \times 10^{-9}$
D-mannitol:					
Aerobic (3)	0.80 ± 0.28	$8.10 \times 10^{-10} \pm 8.00 \times 10^{-11}$	$-1.15 \times 10^{-10} \pm 7.00 \times 10^{-11}$	----	----
Anaerobic (3)	1.27 ± 0.51	$1.27 \times 10^{-9} \pm 1.10 \times 10^{-10}$	$1.17 \times 10^{-10} \pm 2.00 \times 10^{-11}$	----	----
Aerobic (3)	1.39 ± 0.12	$1.35 \times 10^{-9} \pm 1.30 \times 10^{-10}$	$2.20 \times 10^{-10} \pm 2.00 \times 10^{-11}$	$6.90 \times 10^{-10} \pm 3.20 \times 10^{-10}$	$5.10 \times 10^{-10} \pm 2.60 \times 10^{-10}$

The initial serosal and mucosal solutions for all test periods contained 1.00×10^{-9} moles/ml each of D-glucose and D-mannitol. The duration for each test period was one hour. The experimental conditions for each test period were identical with the exception that a one-hour preincubation preceded the first test period; the second and third were preceded by a half-hour preincubation; the second test period was under anaerobic conditions, and the first and third under aerobic conditions. *All values were means ± S.E.M's. There were no significant differences ($P > 0.05$) between any test periods of the same parameters for D-glucose. All respective parameters for each test condition of D-glucose were significantly ($P < 0.05$) than the respective parameters for each test condition for D-mannitol.

TABLE 10: THE EFFECTS OF ANEROBIC CONDITIONS ON ABSORPTION OF D-GLUCOSE AND D-MANNITOL IN U. CAUPO GUT

Condition, test compound, and number of observations	S/M ratio*	Final serosal concentration (moles/ml)*	Net serosal accumulation (moles/100 mg dry gut weight)*	Total net tissue accumulation (moles/ml tissue water)*	Ethanol-soluble accumulation (moles/ml tissue water)*
D-glucose:					
Aerobic (3)	3.00 ±0.60	$3.08 \times 10^{-6} \pm 1.71 \times 10^{-6}$	$1.37 \times 10^{-6} \pm 2.02 \times 10^{-7}$	----	----
Anaerobic (3)	2.87 ±0.76	$3.18 \times 10^{-6} \pm 1.74 \times 10^{-6}$	$1.22 \times 10^{-6} \pm 3.09 \times 10^{-7}$	----	----
Aerobic (3)	2.35 ±0.65	$2.62 \times 10^{-6} \pm 8.28 \times 10^{-7}$	$8.87 \times 10^{-7} \pm 2.67 \times 10^{-7}$	$1.62 \times 10^{-6} \pm 3.07 \times 10^{-7}$	$1.14 \times 10^{-6} \pm 1.75 \times 10^{-7}$
D-mannitol:					
Aerobic (3)	0.81 ±0.10	$8.73 \times 10^{-7} \pm 8.86 \times 10^{-8}$	$-6.94 \times 10^{-8} \pm 4.33 \times 10^{-8}$	----	----
Anaerobic (3)	0.91 ±0.09	$1.04 \times 10^{-6} \pm 8.21 \times 10^{-8}$	$1.51 \times 10^{-8} \pm 3.63 \times 10^{-8}$	----	----
Aerobic (3)	0.91 ±0.13	$1.05 \times 10^{-6} \pm 1.76 \times 10^{-7}$	$6.51 \times 10^{-8} \pm 9.31 \times 10^{-8}$	$1.60 \times 10^{-7} \pm 2.84 \times 10^{-8}$	$9.90 \times 10^{-8} \pm 2.19 \times 10^{-8}$

The initial serosal and mucosal solutions for all test periods contained 1.00×10^{-6} moles/ml each of D-glucose and D-mannitol. The duration for each test period was one hour. The experimental conditions for each test period were identical with the exception that a one-hour preincubation preceded the first test period; the second and third were preceded by a half-hour preincubation; the second test period was under anaerobic conditions, and the first and third were under aerobic conditions. *All values were means ± S.E.M's. There no significant differences ($P > 0.05$) between any test periods of the same parameters for D-glucose. All respective parameters for each test condition of D-glucose were significantly greater ($P < 0.05$) than the respective parameters for each test condition for D-mannitol.

TABLE 11: THE EFFECTS OF CONCENTRATION ON ABSORPTION OF D-GLUCOSE AND D-MANNITOL IN U. CAUPO GUT

Test compound and initial concentration (moles/ml)	Number of Observations	S/M ratio*	Final serosal concentration (moles/ml)*	Total net tissue accumulation (moles/ml tissue water)*	Ethanol-soluble accumulation (moles/ml tissue water)*
D-Glucose:					
1.00×10^{-9}	3	15.60 ± 0.28	$1.21 \times 10^{-8} \pm 5.10 \times 10^{-11}$	$6.33 \times 10^{-9} \pm 5.64 \times 10^{-9}$	$4.09 \times 10^{-9} \pm 2.12 \times 10^{-9}$
1.00×10^{-6}	2	2.48 ± 0.27	$2.52 \times 10^{-6} \pm 3.87 \times 10^{-7}$	$9.64 \times 10^{-7} \pm 8.80 \times 10^{-8}$	$7.38 \times 10^{-7} \pm 2.04 \times 10^{-7}$
D-Mannitol:					
1.00×10^{-9}	3	1.05† ± 0.06	1.03×10^{-9}† $\pm 7.86 \times 10^{-11}$	3.35×10^{-10}† $\pm 1.44 \times 10^{-10}$	1.32×10^{-10}† $\pm 6.21 \times 10^{-11}$
1.00×10^{-6}	2	1.02† ± 0.01	9.84×10^{-7}† $\pm 5.00 \times 10^{-9}$	2.33×10^{-7}† $\pm 8.07 \times 10^{-9}$	6.60×10^{-8}† $\pm 1.52 \times 10^{-8}$

A one-hour preincubation preceded a one-hour test period. *All values were means ± S.E.M's. These mannitol values were significantly less ($P > 0.05$) than their respective D-glucose values. †All respective parameters for each test condition of D-glucose were significantly greater ($P < 0.05$) than the respective parameters for each test condition for D-mannitol.

TABLE 12: THE EFFECTS OF CONCENTRATION ON ACTIVE TRANSPORT OF D-GLUCOSE IN U. CAUPO GUT

Initial ambient concentration (moles/ml)	Number of observations	S/M ratio*	Final serosal concentration (moles/ml)*
1.00×10^{-9}	3	18.21 ± 8.00	9.76×10^{-9} $\pm 4.35 \times 10^{-9}$
1.00×10^{-6}	2	4.50 ± 2.42	4.25×10^{-6} $\pm 2.42 \times 10^{-6}$

The duration of the test period was three hours. *All values were means ± S.E.M's.

DISCUSSION

The parameters used to determine the presence of active transport were S/M ratios > 1, positive net serosal accumulation values, and ethanol-soluble tissue accumulations greater than the initial ambient concentration. Of course, these criteria are not absolute and do suffer limitations since they are designed to measure only terminal evidence of active transport. It must be pointed out that metabolic factors may affect these parameters and cause misinterpretation of data in terms of whether active transport occurred or not. Thus, for example, if a metabolizable sugar demonstrates negative evidence for active transport as measured By these parameters, this does not disprove the presence of an active transport mechanism.

In general, three aspects of metabolism, i.e., a simple chemical conversion of the test compound (e.g., glucose → lactic acid), anabolism of the test compound into larger organic compounds, and catabolism of the test compound into carbon dioxide and water, may affect these transport parameters and alter them in such a way that erroneous conclusions of transport would be made. For example, as a monosaccharide is absorbed by the gut tissue, it may be slightly chemically altered into a different organic compound and thus, is present as a different compound at the serosal side,

though still detectable by its radioactive label. Thus, despite the fact that a sugar demonstrated active transport in terms of final serosal and ethanol-soluble tissue accumulations according to the parameters discussed earlier, it cannot be absolutely concluded from these studies whether the sugar was actively transported unmodified or chemically altered into a non-diffusable compound which would account for the serosal and ethanol-soluble tissue accumulations. Chromatographic analysis, such as paper chromatography, could be employed to identify the components in the ethanol-soluble fraction and the final serosal volume which contain the radioactive label and thus determine whether the test compound was transported unaltered or chemically converted into a non-diffusable compound, resulting in serosal as well as ethanol-soluble tissue accumulations. In another aspect of metabolism, a monosaccharide may be anabolized into larger organic compounds as it is actively transported. The concentration of the monosaccharide anabolized is determined by the difference between the total net tissue accumulation value and the ethanol-soluble tissue accumulation value. Therefore, it is conceivable that a compound which is significantly anabolized would not demonstrate positive evidence for active transport based upon the parameters discussed earlier even though the substance is actively transported. For example, if the

rate of anabolism exceeds the rate of transport, then fractional S/M ratios, negative net serosal accumulations, and ethanol-soluble tissue accumulations less than the initial ambient concentration will be evidenced. Thirdly, a monosaccharide may be metabolized completely into carbon dioxide and water as it is actively transported. If a compound is catabolized extensively as it is transported, the rate of transport versus the rate of catabolism must be considered. As long as the rate of transport exceeds the rate of catabolism, S/M ratios > 1, positive net serosal accumulations, and ethanol-soluble tissue accumulations greater than the initial ambient concentration may still be obtained. However, when a compound is catabolized at a faster rate than it is transported, again as in the previous case when the rate of anabolism exceeded the rate of transport, then fractional S/M ratios, negative net serosal accumulations, and ethanol-soluble tissue accumulations less than the initial ambient concentration will be evidenced.

All three events could occur to some degree in the gut tissue for all monosaccharides tested. However, it is the interplay and extensive occurrence of the latter two events which may cause the failure to detect the active transport of a compound.

Since the parameters for the determination of active transport are not perfect, the inclusion of D-mannitol in

the experimental approaches further substantiated the case for active transport of a given monosaccharide. No evidence for the active absorption of D-mannitol by the U. caupo gut was found. This finding coincided with numerous known studies in which no evidence was found for the active transport of D-mannitol by any animal gut tissue to date studied (Cori, 1925; Davidson and Garry, 1940; Wilson and Vincent, 1955; Lawrence and Lawrence, 1967a). Consequently, movement of D-mannitol could be assumed to be due to diffusion. Thus, D-mannitol served as an added control for D-glucose, D-galactose, and 3-O-methyl-D-glucose, since the diffusion coefficient for mannitol equals the diffusion coefficient of D-glucose and D-galactose and is only slightly higher than that of 3-O-methyl-D-glucose. Thus, any significant movement of a monosaccharide greater than that of D-mannitol suggests processes other than diffusion. Further, this physiological process is due to active transport, which has been shown with gut tissues of other animals (Cori, 1925; Davidson and Garry, 1940; Wilson and Vincent, 1955; Lawrence and Lawrence, 1967a).

Of the four monosaccharides tested, D-galactose, 3-O-methyl-D-glucose, and D-mannitol did not show positive evidence for active transport in the U. caupo gut, as evaluated by S/M , net serosal and ethanol-soluble tissue accumulation parameters. Only evidence for the active trans-

port of D-glucose was suggested by these transport parameters. A faster rate of accumulation was obtained using the initial ambient concentration of 1.00×10^{-9} moles/ml as compared to 1.00×10^{-6} moles/ml. This indicated that the lower test concentration was closer to the optimum for transport; however, no kinetics studies have been done to determine whether this concentration is the most optimum for sugar transport by the U. caupo gut. The rate of absorption of D-galactose into and across the gut was always greater than that for D-mannitol, as indicated by S/M ratios, net serosal accumulations, and ethanol-soluble tissue accumulations higher than D-mannitol values (Two of fifteen values were significant at 5% probability). This evidence suggests that D-galactose is possibly actively transported but at a very slow rate. Additional observations would be required in order to definitely determine whether D-galactose is actively transported.

D-glucose was not inhibited by anaerobic conditions, and if D-galactose were indeed transported, it was also not inhibited. This leads to the speculation that perhaps this active transport mechanism was not dependent upon metabolic energy derived through oxidative processes. On the other hand, it is just as probable to ask whether anaerobic conditions were established. It is felt that the former explanation is the more probable, since these same techniques

have been used with other animal tissues in this laboratory in which inhibition of active transport resulted. Moreover, anaerobic conditions do not always inhibit active transport in all animal tissues, as illustrated by D-galactose transport in the distal gut region of C. stelleri (Lawrence and Lawrence, 1967a), some fish gut tissues (Wilson and Lin, 1960), and some embryonic tissues (Musacchia et al., 1964). In addition, the U. cauco is an intertidal animal living in a U-shaped burrow beneath the substratum, and during periods of low tides, it suffers from oxygen deprivation due to lack of fresh sea water. Thus, it would not be surprising that this animal has the ability to develop a large oxygen debt, since anaerobic conditions are not uncommon to its mode of existence. The use of a glycolytic inhibitor would substantiate this speculation.

When all test sets at both initial ambient concentrations were compared, no evidence was obtained for the anabolism of D-galactose, 3-O-methyl-D-glucose, and D-mannitol in terms of no significant net accumulation of these sugars into the ethanol-insoluble fractions. It was felt that the small insignificant amounts of 3-O-methyl-D-glucose, D-galactose, and D-mannitol accumulated into the ethanol-insoluble fractions were probably due to inefficiency of the ethanol extraction methods to remove all of

the test compound (ethanol extraction was 90-95% efficient). Further, no evidence for the anabolism of D-galactose (Lawrence, 1962), 3-O-methyl-D-glucose (Campbell and Young, 1952; Csaky and Glenn, 1957; Lawrence et al., 1966), or D-mannitol (Cori, 1925; Davidson and Garry, 1940; Wilson and Vincent, 1955; Lawrence and Lawrence, 1967a) has ever been demonstrated in other known studies. Significant amounts of D-glucose, in all experiments conducted with D-glucose, were anabolized into larger organic compounds, as indicated by total net tissue accumulations significantly greater than ethanol-soluble tissue accumulation values (i.e., amount anabolized was assumed to be 24-34% of total net tissue accumulation). The anabolism of D-glucose has been cited in other known studies (Lawrence et al., 1966).

In conclusion, the purpose of these studies was to obtain evidence for the active transport of monosaccharides by the gut of the U. caupo. Established parameters were used to determine if D-glucose , D-galactose, D-mannitol, and 3-O-methyl-D-glucose were actively transported. No evidence for the active transport of D-mannitol was shown. This coincided with all prior studies in which D-mannitol was not known to be actively transported in any animal gut tissue (Cori,1925; Davidson and Garry,1940; Wilson and Vincent,1955; Lawrence and Lawrence, 1967a). Data also suggests that 3-O-methyl-D-glucose did not demonstrate active absorption by

the U. caupo gut. This is surprising since 3-O-methyl-D-glucose is actively transported in mammalian (Wilson and Vincent, 1955; Wilson and Landau, 1960), bird (Westenbrink, 1936), amphibian (Csaky and Fernald, 1960), fish (Cordier and Worbe, 1955) tissues; and in the proximal gut region of the marine mollusc, C. stelleri (Lawrence and Lawrence, 1967a). If D-galactose is indeed actively transported by the U. caupo gut, then this information would agree with prior positive evidence for active transport of this monosaccharide in mammalian (Wilson and Vincent, 1955; Wilson and Landau, 1960), bird (Westenbrink, 1936), amphibian (Lawrence, 1962), and fish (Cordier and Worbe,1955) tissues, and in the gut of the mollusc, C. stelleri (Lawrence and Lawrence, 1967a). The conclusion that D-glucose was actively transported (assuming that most of the monosaccharide was not chemically altered to another form) correlates with other known evidence of its active transport in mammalian (Wilson and Vincent, 1955; Wilson and Landau, 1960), bird (Westenbrink, 1936), amphibian (Lawrence, 1962), fish (Cordier and Worbe, 1955), and molluscan (Lawrence and Lawrence, 1967a) tissues. Thus, the presence of active transport for some monosaccharides has been established in another group of invertebrates.

SUMMARY

The purpose of these studies was to obtain evidence for the active transport of monosaccharides by the gut of the U. caupo by determining if D-glucose, D-galactose, 3-O-methyl-D-glucose and/or D-mannitol were absorbed against a concentration difference, and if this ability was specific for one or more of these monosaccharides.

The following results were obtained:

a) Positive evidence was shown for the active transport of D-glucose and was suggested for D-galactose with initial ambient concentrations of 1.00×10^{-9} moles/ml and 1.00×10^{-6} moles/ml.

b) No evidence for the active transport of 3-O-methyl-D-glucose and D-mannitol was obtained.

c) D-glucose at an initial ambient concentration of 1.00×10^{-9} moles/ml appears to be closer to the optimum transport concentration than D-glucose at 1.00×10^{-6} moles/ml.

d) Anaerobic conditions did not inhibit active transport of D-glucose or D-galactose. This suggests the utilization of a different energy-producing mechanism, independent of oxidative processes.

e) No evidence was found for the anabolism of 3-O-methyl-D-glucose, D-galactose, or D-mannitol, whereas significant amounts of D-glucose were anabolized.

f) No net water movement was observed between the serosal and mucosal sides of gut preparations.

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