TRANSPORT MECHANISMS IN THE ANTERIOR INTESTINE OF *Cryptochiton stelleri*

A Dissertation

Presented to

The Faculty of the Department of Biophysics

University of Houston

In Partial Fulfillment

Of The Requirements For The Degree

DOCTOR OF PHILOSOPHY

By

Louis Evans Schneider, Jr.

May, 1971

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ABSTRACT

The *in vitro* everted sac method of Crane and Wilson as modified by Lawrence was employed in the study of the basic transport mechanisms for salt, water, and organic nutrients, specifically glucose and 3-O-methyl glucose, in the anterior intestine of the marine invertebrate, *Crytochiton stelleri*. The parameters measured were the sodium flux, sugar flux, water flux, transmural potential, and pH. The experimental conditions imposed upon the tissue included ion replacement, in which one or more ions was replaced in the bathing medium, and the use of inhibitors, particularly anaerobiosis and 1×10^{-6}M ouabain.

The results of these experiments seem to indicate that sodium ion is actively transported by the anterior chiton gut, and that this transport is ouabain sensitive and is inhibited by anaerobic conditions. The movement of potassium, chloride, and water is most likely passive. The active transport of glucose and 3-O-methyl glucose is dependent upon the presence of sodium. The negative potential across the anterior gut appears to be the result of the active transport of hydrogen ion from the serosal to the mucosal compartment. This transport is ouabain insensitive but is inhibited by anaerobic conditions. A model for the transport mechanisms in the anterior chiton gut encompassing these observations is proposed.

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INTRODUCTION

The intestinal mucosa represents an epithelial membrane that brings about a net transfer of materials across an intact cell layer. This transfer can involve simple diffusion, diffusion through pores, pinocytosis, carrier mediated transport, or any combination of these transport phenomena.

Carrier mediated transport involves a hypothetical carrier molecule and is characterized by a high Q_{10}, stereospecificity, saturable kinetics, competitive inhibition, and inhibition by enzyme poisons. Carrier mediated transport can be further divided into facilitated diffusion, exchange diffusion, and active transport.

Active transport is unique in that it (1) involves a movement against an apparent electrochemical gradient, (2) requires metabolic energy, and (3) does not chemically change the material being transported. It is through the process of active transport that many animals obtain their nutritional requirements and maintain their fluid and electrolyte balance.

Our knowledge of the active transport of organic nutrients and ions is limited primarily to the vertebrate intestine, where it has been found that the two are interrelated. In order to approach the problem of transport in the invertebrate gut, an area in which very little work has been done, the current concepts of transport in the vertebrate intestine should be thoroughly understood. Therefore, a review of the transport mechanisms for ions, monosaccharides, and water

in the vertebrate intestine will be given.

The study of the intestinal absorption of ions, particularly sodium, potassium, and chloride, in the vertebrate gut was significantly advanced by the work of Visscher in 1944. Extensive work has been done in the area since that time, as reviewed by Wilson (157), Schultz and Curran (133), Wiseman (159), and Fordtran and Inglefinger (94). That sodium is actively transported in all regions of the vertebrate intestine has been universally accepted. This active sodium transport is from the mucosal or luminal side to the serosal or plasma side in all regions of the intestinal tract. However, present information suggests the existence of a gradient of sodium transport properties along the intestine; and, therefore, each of the regions of the intestine can be characterized as to its net sodium movement. The unidirectional sodium fluxes are the greatest in the duodenum and jejunum, the influx being approximately equal to the efflux (20, 34, 35, 122, 154, 159). The result is a very small, if any, net absorption of sodium. In the ileum, the sodium fluxes into and out of the lumen are less than those in the jejunum (20, 34, 159). However, the flux from lumen to plasma exceeds the flux from plasma to lumen, and, hence, there is a larger net absorption of sodium. The largest net sodium absorption occurs in the colon, where the flux of sodium from lumen to plasma greatly exceeds the flux in

the opposite direction. In the human, the colonic epithelium absorbs about 90% of the sodium available in the lumen (159).

These variations in sodium behavior suggest different functions for the various intestinal regions. Thus, it is thought that the mucosa of the duodenum and jejunum fulfills the function of equilibrating its contents with the blood and maintaining an isotonic sodium concentration in the lumen by nearly equal and large movements of sodium and water in both directions, while that of the ileum, and particularly the colon, is fixed to serve in the net absorption of sodium (35, 67, 122). The importance of these functional differences will be realized when the active transport of monosaccharides is discussed.

At present, all evidence indicates that the active "pumps" responsible for the net sodium movement from the mucosa to the serosa are located at the basal end or lateral surface of the mucosal cell, since the net sodium flux is inhibited by ouabain only at the serosal surface (57, 58, 109, 137) and most of the ATPase activity of the cell has been found in the serosal membrane (127).

The situation for the transport of the chloride ion is not well understood and by no means clear, since experimental results vary, depending upon whether the technique employed is in vitro or in vivo. However, the behavior of chloride ion seems to be similar to that of sodium in that there

exists a gradient of transport mechanisms down the intestinal tract. In the duodenum and jejunum, there are, as with sodium, large and equal fluxes of chloride in both directions across the mucosal epithelium (159). The transport in the small intestine appears to be primarily active. Some workers, however, have presented evidence for the entirely passive movement of chloride (36, 37). The ileum seems to be the major site of absorption of chloride from the lumen (68, 122). The transport of chloride in the colon appears to occur passively (67).

The relation between sodium chloride transport and the transmural potential difference, which results from the active transport of ions by the intestinal epithelium, should be commented on briefly. In most in vitro intestinal preparations bathed on both sides with identical solutions, the orientation of the electrical potential can be attributed to the active transport of sodium ion from the mucosa to the serosa, the serosa being positive relative to the mucosa. When active chloride transport accompanies that of sodium, the resulting potential is the difference between the active chloride and sodium flows, and in some instances, for example in the jejunum, the potentials are close to zero or reversed, that is, serosal side relatively negative. This occurs frequently in in vivo studies (68).

Potassium ion moves with equal ease in both directions

across the gut wall. The efficiency of absorption of potassium is dependent to a considerable extent on the sodium content of the diet, the potassium ion appearing to be available as a means to regulating the cation concentration in the lumen of the small intestine (21). All movement of potassium appears to be passive (157, 159).

Hydrogen ion, another important cation, is actively secreted into the lumen of the vertebrate stomach. Recent work has also shown the presence of a gradient of hydrogen ion secretion down the small intestine, the duodenum having the lowest pH and the ileum the highest. Some workers have suggested the presence of a hydrogen-sodium exchange mechanism in the small intestine (122, 141, 149).

The active transport mechanism for monosaccharides has also been well characterized in the vertebrate gut. Excellent reviews on the subject have been given by Wilson (157), Wiseman (159), Crane (38), Benson and Rampone (18), and Smyth (144). There are at present 14 sugars known to be actively transported by the vertebrate gut. These sugars are characterized by (1) a pyranose ring, (2) a methyl or substituted methyl group at carbon number five, and (3) a hydroxyl group on carbon number two. Since it has been found that sodium is a specific requirement for the active transport of hexoses, there is evidence for the presence of a sodium activated hexose pump in the brush border of the mucosal

epithelial cell, at least in the jejunum where the greatest active absorption of organic nutrients occurs (7, 25, 90). The greatly enhanced transmural potential in the presence of actively transported hexoses indicates a sodium involvement in the carrier process (38). The sodium equilibration function of the duodenum, that is, the net flux of sodium into the lumen whenever its contents are hypotonic relative to the blood, seems to provide for the most efficient and maximal absorption of organic nutrients, since sodium is necessary for their transport.

The mobile carrier hypothesis of Crane (39, 42) is currently the most accepted explanation of active hexose transport. In this hypothesis, transport requires the formation of a complex of the hexose, sodium ion, and the carrier, a lipo-protein with specific binding sites for the hexose and sodium which has the ability to move these substances back and forth across the membrane barrier. The sodium ion is necessary for the carrier to have an increased affinity for the sugar. The net movement of sugars into the cell is determined by the sodium gradient between the medium and the cell interior. That is, the apparent "uphill" accumulation of sugar is attributed to a "downhill" sodium gradient maintained by the operation of an outwardly directed, energy-dependent sodium pump, present at a different locus in the membrane. The transport process is freely reversible and

is competitively inhibited by phlorizin (1, 2, 4, 5, 13, 111, 113, 116). All of the actively transported sugars in vertebrates seem to share a common carrier (3, 43, 158). Once inside the cell, the hexoses move along their concentration gradient and are extruded at the basal membrane.

The movement of water in the vertebrate gut seems to be entirely passive, responding to an osmotic gradient created by the active transport of sodium. In Curran's "Double Membrane Theory" (66) the transport of water is explained in terms of two membranes arranged in series, the first of which is permeable to water only, and a second which is permeable to water and salt. Sodium ion is actively transported across the first membrane and water follows along its osmotic gradient. Both water and sodium move through the second membrane along a hydrostatic pressure gradient. Diamond (73) has applied Curran's theory to the intestinal epithelial cell in his "standing gradient osmotic flow" theory. In it, sodium enters the cell at the brush border either by leakage or associated with a sugar pump and is actively pumped out of the cell and into the spaces between adjacent epithelial cells. Water is freely diffusable through the membrane and moves along its osmotic gradient. This represents Curran's first membrane. The sodium and water then move down the open channel along their concentration gradients into the serosal compartment. The open channel corresponds to the central compartment

proposed by Curran. Diamond's theory has been well substantiated by electron microscopy.

It is apparent, then, that considerable information is available concerning the transport mechanisms for salt, water, and organic nutrients in the vertebrate gut. There is an active sodium-requiring hexose pump at the mucosal border responsible for the net accumulation of at least fourteen different hexoses in the cell. The sodium is extruded at the sides of the cell or at the basal membrane, resulting in the net movement of sodium and the production of a transmural potential. Water follows its osmotic gradient.

Absorption in the nonvertebrate gut, however, is by no means clear. Research in the area has been scant and for the most part ignored completely. Those studies which have involved the active transport of monosaccharides in the invertebrate gut have been restricted to two classes of Echinodermata, one class of Arthropods, and two classes of Mollusca.

Lawrence and Lawrence (104) studied active monosaccharide absorption in the molluscan gut of the amphineuron, Cryptochiton stelleri. The tissue of this particular animal was chosen for study because (1) the primitive nature of the chiton might allow the determination of transport character-

istics which are masked in more advanced forms; (2) the *in vitro* technique which was employed is not too different from the *in vivo* situation due to the open-closed nature of the chiton circulatory system; (3) the thin muscle layers of the chiton gut allow diffusion to occur more freely and cause a reduction of back diffusion; (4) the chiton gut is viable for approximately twelve hours after its removal from the animal and ranges in length from two to three feet; (5) the anterior and posterior segments of the gut are clearly discernible from one another both in appearance and in function; and (6) these animals are so far removed from the chordate branch of evolution that any information obtained would be of value from the aspect of comparative and membrane physiology.

It was established in these studies that the active transport of organic nutrients does indeed occur in the gut of *Cryptochiton stelleri* and that this transport is apparently associated with the movement of ions, as indicated by the presence of a potential difference across the gut tissue (106). A pecularity of the potential across the chiton gut, however, is the opposite polarity in the anterior gut as compared to the posterior gut. In the vertebrate gut using *in vitro* preparations, the serosal surface is positive with respect to the mucosal. The negative serosal polarity in the anterior intestine of the chiton seems to indicate a transport mechanism

different from the posterior intestine and from the vertebrate gut as well.

Also of interest is the difference in the types of nutrients absorbed by the two gut regions. D-glucose and 3-O-methyl-D-glucose are actively transported in the anterior gut, as are the purines and pyrimidines thymine, guanine, and uracil (91). In the posterior gut, D-galactose (104), those amino acids which have been tested (129), and the purines and pyrimidines thymine, guanine, and hypoxanthine (91) are actively transported. The previously mentioned negative serosal potential in the anterior intestine has been shown to be dependent upon the presence of a metabolizable sugar (Puddy, unpublished).

It was the purpose of this investigation, then, to characterized the basic transport mechanisms for salt and water in the anterior intestine of Cryptochiton stelleri and to determine what interrelationships, if any, exist among the transport systems for salts, water, and sugars.

METHODS AND MATERIALS

Animals

Adult chitons (Cryptochiton stelleri) of both sexes weighing at least 600 grams were obtained commercially from the Pacific Ocean near Monterey, California. The animals were maintained in the laboratory at 15°C in aerated sea water for at least one week before they were used.

In vitro method

The procedure used for the in vitro study of sugar transport was a modification of the method described by Crane and Wilson (53).

The apparatus consisted of a glass tube with an enlarged top and a two holed rubber stopper with a glass cannula (Figure 1) . A hypodermic needle was forced through the stopper at a slight angle and a length of polyethylene tubing was attached to the needle.

The anterior and posterior intestine and the associated digestive gland were removed from the animal and placed in chiton Ringer solution, the composition of which is shown in Table 1. The anterior intestine was then isolated, everted over a glass rod, and rinsed with cold Ringer solution. Two to three segments, each approximately three-five cm in length, were used from each anterior intestine (Figure 2). One end of the everted segment was tied to the glass cannula and the other to a glass weight. The cannula with the everted seg-

ment was placed in the glass chamber which contained 32 ml of chiton Ringer solution of known composition. The cannula was adjusted so that the whole segment was immersed. The glass tube containing the intestinal preparation was placed in a constant temperature water bath at 15°C. Atmospheric air or other gases was passed through the solution, entering by way of the hypodermic needle and leaving by way of the hole in the stopper. After a preliminary incubation period of 30 minutes, a known volume (0.6-1.5 ml) of a second chiton Ringer solution, whose composition was identical to the solution contained by the glass tube except for the presence of radioactive label, was pipetted into the gut sac. The solution contained in the everted segment and the cannula will be referred to as the serosal solution, and that contained by the glass tube as the mucosal solution. Both solutions contained 1uM/ml glucose or 3-O-methyl glucose.

Initial samples of the mucosal and serosal solutions were obtained for the determination of the various parameters. At the end of a three hour period, referred to as the first control period, the serosal solution was removed and its volume recorded. Final mucosal and serosal samples were collected. The sac and cannula were transferred to a second tube containing fresh mucosal solution. A second serosal solution, again whose composition was identical to the mucosal solution except for the presence of radioactive lable, was

FIGURE 1 APPARATUS USED IN THE IN VITRO METHOD FOR THE STUDY OF INTESTINAL ABSORPTION IN THE CHITON

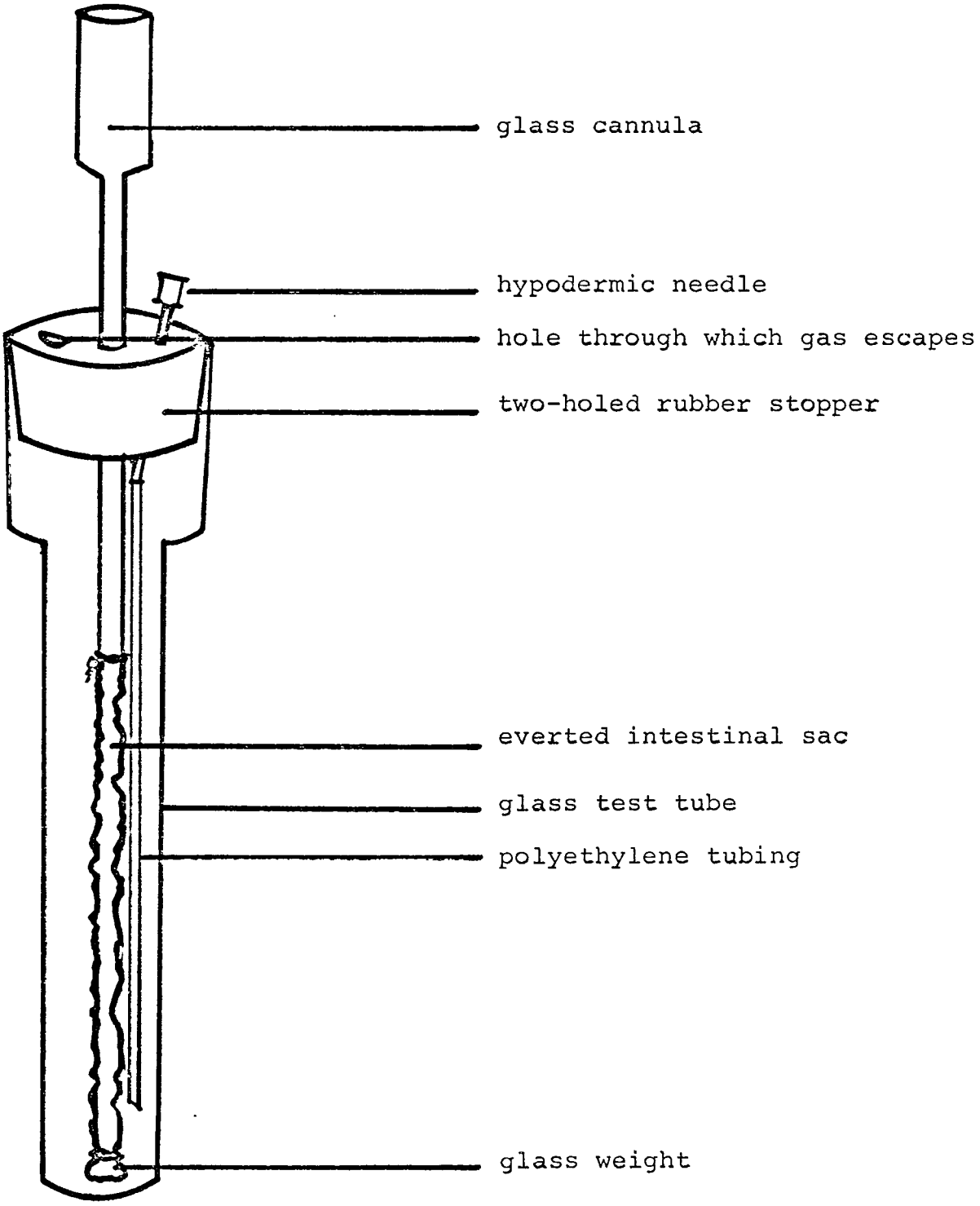
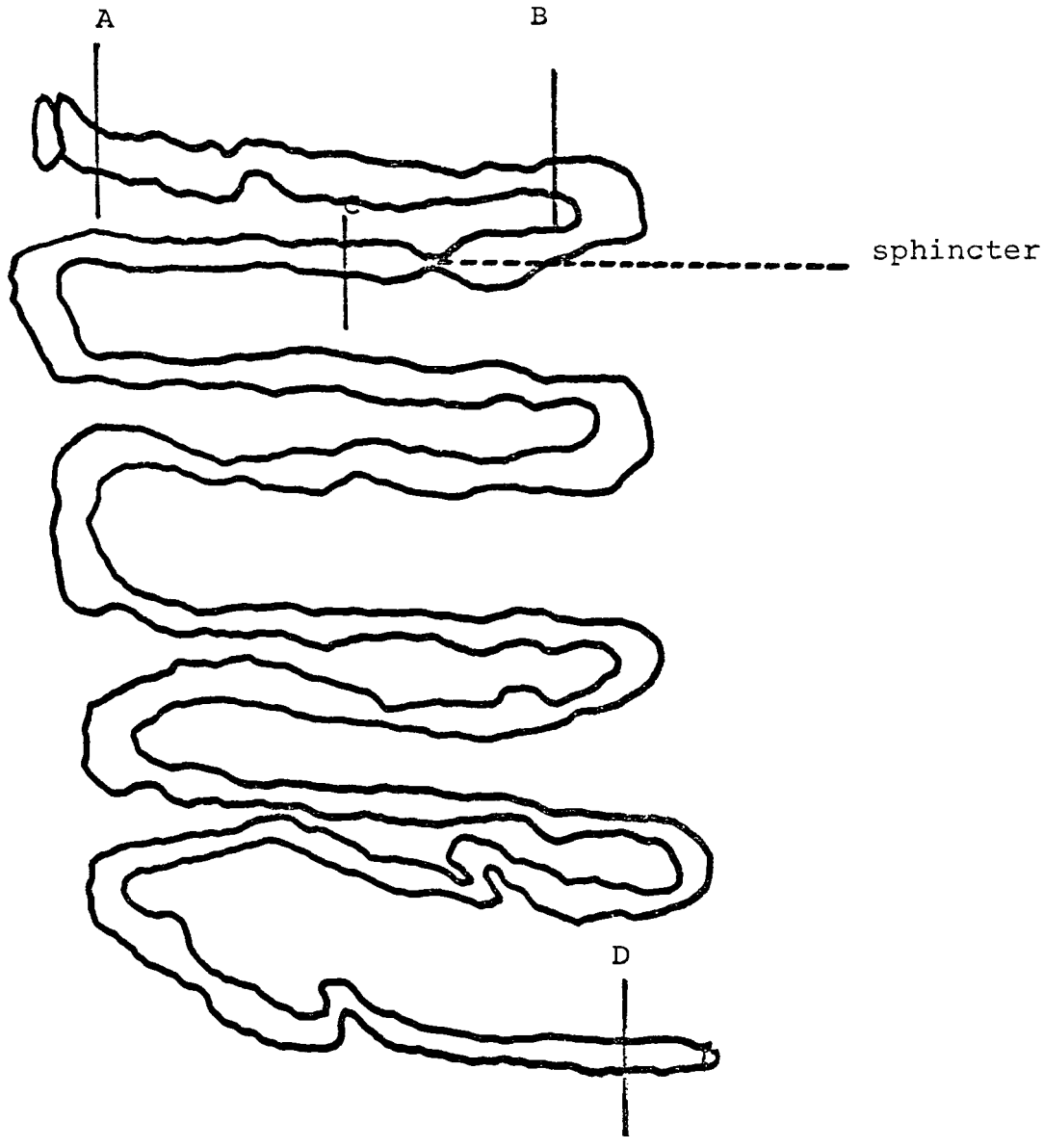


FIGURE 2 ISOLATED C. stelleri GUT



Regions of the gut are as follows:

A-B: anterior

C-D: posterior

TABLE 1

COMPOSITION OF RINGER SOLUTIONS USED IN INVERTEBRATE TRANSPORT STUDIES

COMPOUND	STANDARD RINGER	SODIUM FREE	CHLORIDE FREE	POTASSIUM FREE
NaCl	0.462M	x	x	0.462M
KCl	0.0121M	0.0121M	0.0121M	x
$NaHCO_3$	0.00238M	0.00238M	0.00238M	0.00238M
Na_2SO_4	x	x	0.231M	x
Sucrose	x	x	0.231M	x
Choline chloride	x	0.462M	x	x
$CaCl_2.2H_2O$	0.01135M	0.01135M	0.01135M	0.01135M
$MgCl_2.6H_2O$	0.00983M	0.00983M	0.00983M	0.00983M
$MgSO_4.7H_2O$	0.00243M	0.00243M	0.00243M	0.00243M
Glucose	luM/ml	luM/ml	luM/ml	luM/ml

added to the gut sac. These solutions were, however, different from those used in the first control period in all experiments except those with anaerobic conditions, in which the solutions in all periods were the same. The second period, which will be referred to as the experimental period, was terminated in the same manner as the first. Because of the viability of the chiton gut, a third period of three hours, referred to as the second control period, was performed so that the results obtained from the experimental period could not be attributed to time effects. The second control period was identical to the first.

Analytical Methods, Radioactivity Determination, and Transmural Potential Measurement

After the initial and final mucosal and serosal solutions had been collected, duplicate aliquots of ten λ were dissolved in five ml of doubly deionized water for the determination of sodium and potassium concentrations by flame photometry (Eppendorf) and chloride by the Cotlove chloridometer (Buchler). Duplicate aliquots of approximately 15 λ were used to determine the pH of the samples in an ultramicro pH meter (Instrumentation Laboratories). The amount of total carbohydrate was determined by the phenol method of Dubois et al. (75).

The potential difference across the gut wall was measured hourly during the course of each control and experimental period using a high impedence electrometer (Keithly) with

calomel reference electrodes and 3% agar-invertebrate Ringer salt bridges.

The radioactivity of 50 λ aliquots of the initial and final samples containing uniformly labeled ^{14}C D-glucose or 3-O-$^{14}CH_3$-D-glucose, $^{3}H_2O$, and ^{22}Na was measured in a liquid scintillation counter (Beckman) with automatic quench correction using the ^{3}H, $^{14}C/^{3}H$, and $^{32}P/^{14}C$ (^{22}Na) isosets for the three channel windows. Counting was done to 2% standard error. A toluene cocktail containing 7g/l PPO, 100ml/l Biosolve, and 30 ml/l water was employed.

Calculations

The equal unidirectional fluxes of ^{22}Na and $^{3}H_2O$ were determined by the method of Berger (22). The equation used was:

$$\text{Flux in} = \text{Flux out} = \frac{-\ln\frac{(^{\Theta}A-^{\Theta}B)}{(^{\Theta}AO-^{\Theta}BO)}}{\frac{t_1-t_0}{Na_1} + \frac{t_2-t_1}{Na_2} + \cdots \frac{t_n-t_{n-1}}{Na_n}}$$

where $^{\Theta}A$ is the specific activity of the serosal compartment at time = t_n, $^{\Theta}B$ is the specific activity of the mucosal compartment at time = t_n, $^{\Theta}AO$ is the intial specific activity of the serosal side, $^{\Theta}BO$ is the initial specific activity of the mucosal side, Na is the (initial volume-sample volumes) times the ion concentration, and $\underline{t}$ is the time. The sodium concentration did not change significantly in either compartment during the course of the experiments.

The net flux of sugar was determined by the initial sugar concentration times the initial volume minus the final sugar concentration times the final volume divided by the time. The S/M ratios were calculated by dividing the terminal serosal sugar concentration by the terminal mucosal sugar concentration for each control and experimental period.

The means of the parameters obtained from the first control period were compared with those of the experimental period by the paired T test for the determination of significance. The means of parameters obtained from the first and second control periods were also compared by the paired T test to show that there was no significant variation between these two periods.

Experimental Design

There were several experimental approaches employed in this investigation, each experiment involving two control periods, one before and one after the experimental period. One experimental approach involved ion replacement studies, in which one or more ions was replaced in the mucosal and serosal solutions during the experimental period. For example, a series of experiments was performed using experimental solutions of various sodium concentrations, that is, sodium free, quarter sodium, half sodium, and three-quarter sodium Ringer solutions. Other experimental solutions included isosmotic sucrose, potassium free, and chloride free

Ringer solutions. Another experimental approach involved ion replacement experiments in which each serosal solution was labeled with ^{14}C glucose or 3-O-$^{14}CH_3$ glucose, $^{3}H_2O$, and ^{22}Na. The mucosal solutions were not labeled. The third experimental approach used in this investigation involved the use of inhibitors, specifically anaerobic conditions and ouabain. In these studies, the experimental period was identical to the first control period except that 1.0×10^{-6} M ouabain was present in the mucosal compartment only, in the serosal compartment only, or in both compartments simultaneously. Anaerobic conditions were established during the experimental period by bubbling nitrogen through the mucosal solution.

RESULTS

The results of the initial ion replacement experiments on the transmural potential can be seen in Tables 2 through 8. In all of the partial sodium experiments, that is, sodium free (Table 2), one-quarter sodium (Table 3), one-half sodium (Table 4), and three-quarter sodium (Table 5) chiton Ringer solutions, the potentials significantly increased ($P < .05$) in magnitude four to five fold during the experimental period. A two fold increase in the magnitude of the potential ($P < .05$) is observed when the experimental solution consisted of a potassium free Ringer solution (Table 6). A very large increase (thirty-eight fold) in potential occured with isosmotic sucrose (Table 7). The potential significantly decreased to 50% of the control valve with a chloride free Ringer solution (Table 8).

In addition to potential measurements, ion replacement studies using a label allowed the determination of the fluxes of sodium, water, and sugar. Since there was no significant net accumulation of sodium or water in either the serosal or mucosal compartments, the unidirectional fluxes of sodium and water were equal within the experimental error. The sugar flux was considered a net flux from the mucosal to the serosal compartment because less than 5% of the serosal ^{14}C entered the mucosal compartment and there was no change

TABLE 2

EFFECT OF A SODIUM FREE RINGER SOLUTION ON ELECTRICAL POTENTIALS ACROSS THE ANTERIOR INTESTINE OF *C. stelleri*

TIME OF OBSERVATION		NUMBER OF OBSERVATIONS	mV	± SEM
HOUR	1	4	-2.55	0.53
	2	4	-2.06	0.44
	3	4	-1.47	0.38
	4	4	-1.36	0.25
	5	4	-1.29	0.21
	5 + 1 min.	4	-5.50*	0.58
	5 + 2 min.	4	-4.57	0.78
	5 + 3 min.	4	-3.77	0.68
	5 + 30 min.	4	-1.80	0.41
	6	4	-2.85	0.41

ELECTRICAL POTENTIAL MEASUREMENTS WERE MADE FOR SIX CONSECUTIVE HOURS AFTER THE GUT SEGMENT WAS MOUNTED. VALUES REPRESENT MEANS (mV) ± (SEM) FOR THE INDICATED NUMBER OF OBSERVATIONS.

* INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE PRECEEDING VALUE AT THE 5 PERCENT LEVEL.

TABLE 3

EFFECT OF A 0.116M NaCl RINGER SOLUTION (ONE-FOURTH SODIUM) ON ELECTRICAL POTENTIALS ACROSS THE ANTERIOR INTESTINE OF *C. stelleri*

TIME OF OBSERVATION	NUMBER OF OBSERVATIONS	mV	± SEM
HOUR 1	4	-1.27	0.08
2	4	-0.99	0.12
3	4	-0.86	0.14
4	4	-0.79	0.14
5	4	-0.82	0.15
5 + 1 min.	4	-3.17*	0.30
5 + 2 min.	4	-2.85	0.18
5 + 3 min.	4	-2.70	0.18
5 + 30 min.	4	-2.16	0.15
6	4	-1.92	0.24

ELECTRICAL POTENTIAL MEASUREMENTS WERE MADE FOR SIX CONSECUTIVE HOURS AFTER THE GUT SEGMENT WAS MOUNTED. VALUES REPRESENT MEANS (mV) ± (SEM) FOR THE INDICATED NUMBER OF OBSERVATIONS.

* INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE PRECEEDING VALUE AT THE 5 PERCENT LEVEL.

TABLE 4

EFFECT OF A 0.232M NaCl RINGER SOLUTION (ONE-HALF SODIUM) ON ELECTRICAL POTENTIALS ACROSS THE ANTERIOR INTESTINE OF *C. stelleri*

TIME OF OBSERVATION	NUMBER OF OBSERVATIONS	mV	± SEM
HOUR 1	4	-1.46	0.15
2	4	-0.97	0.11
3	4	-0.93	0.16
4	4	-1.12	0.09
5	4	-1.25	0.14
5 + 1 min.	4	-6.16*	0.86
5 + 2 min.	4	-5.92	0.77
5 + 3 min.	4	-5.77	0.68
5 + 30 min.	4	-3.52	0.51
6	4	-2.47	0.21

ELECTRICAL POTENTIAL MEASUREMENTS WERE MADE FOR SIX CONSECUTIVE HOURS AFTER THE GUT SEGMENT WAS MOUNTED. VALUES REPRESENT MEANS (mV) ± (SEM) FOR THE INDICATED NUMBER OF OBSERVATIONS.

* INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE PRECEEDING VALUE AT THE 5 PERCENT LEVEL.

TABLE 5

EFFECT OF A 0.348M NaCl RINGER SOLUTION (THREE-FOURTH SODIUM) ON ELECTRICAL POTENTIALS ACROSS THE ANTERIOR INTESTINE OF C. stelleri

TIME OF OBSERVATION	NUMBER OF OBSERVATIONS	mV	± SEM
HOUR 1	4	-1.69	0.46
2	4	-1.23	0.17
3	4	-1.31	0.13
4	4	-1.27	0.32
5	4	-1.25	0.31
5 + 1 min.	4	-4.36*	0.39
5 + 2 min.	4	-4.21	0.37
5 + 3 min.	4	-3.86	0.33
5 + 30 min.	4	-3.27	0.26
6	4	-2.38	0.13

ELECTRICAL POTENTIAL MEASUREMENTS WERE MADE FOR SIX CONSECUTIVE HOURS AFTER THE GUT SEGMENT WAS MOUNTED. VALUES REPRESENT MEANS (mV) ± (SEM) FOR THE INDICATED NUMBER OF OBSERVATIONS.

* INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE PRECEEDING VALUE AT THE 5 PERCENT LEVEL.

TABLE 6

EFFECT OF A POTASSIUM FREE RINGER SOLUTION ON ELECTRICAL POTENTIALS ACROSS THE ANTERIOR INTESTINE *C. stelleri*

TIME OF OBSERVATION	NUMBER OF OBSERVATIONS	mV	± SEM
HOUR 1	4	-1.70	0.30
2	4	-1.47	0.26
3	4	-1.56	0.22
4	4	-1.52	0.17
5	4	-1.31	0.17
5 + 1 min.	4	-2.20*	0.14
5 + 2 min.	4	-2.20	0.14
5 + 3 min.	4	-2.12	0.20
5 + 30 min.	4	-0.87	0.21
6	4	-1.40	0.25

ELECTRICAL POTENTIAL MEASUREMENTS WERE MADE FOR SIX CONSECUTIVE HOURS AFTER THE GUT SEGMENT WAS MOUNTED. VALUES REPRESENT MEANS (mV) ±(SEM) FOR THE INDICATED NUMBER OF OBSERVATIONS.

* INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE PRECEEDING VALUE AT THE 5 PERCENT LEVEL.

TABLE 7

EFFECT OF AN ISOSMOTIC SUCROSE SOLUTION ON ELECTRICAL POTENTIALS ACROSS THE ANTERIOR INTESTINE OF *C. stelleri*

TIME OF OBSERVATION	NUMBER OF OBSERVATIONS	mV	± SEM
HOUR 1	4	-0.81	0.09
2	4	-0.63	0.07
3	4	-0.66	0.06
4	4	-0.83	0.07
5	4	-0.78	0.15
5 + 1 min.	4	-29.7*	6.6
5 + 2 min.	4	-31.5	5.4
5 + 3 min.	4	-27.0	4.3
5 + 30 min.	4	-27.7	1.1
6	4	-28.0	1.47

ELECTRICAL POTENTIAL MEASUREMENTS WERE MADE FOR SIX CONSECUTIVE HOURS AFTER THE GUT SEGMENT WAS MOUNTED. VALUES REPRESENT MEANS (mV) ± (SEM) FOR THE INDICATED NUMBER OF OBSERVATIONS.

* INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE PRECEEDING VALUE AT THE 5 PERCENT LEVEL.

TABLE 8

EFFECT OF A CHLORIDE FREE RINGER SOLUTION ON ELECTRICAL POTENTIALS ACROSS THE ANTERIOR INTESTINE OF C. stelleri

TIME OF OBSERVATION	NUMBER OF OBSERVATIONS	mV	± SEM
HOUR 1	4	-3.20	1.00
2	4	-2.68	0.77
3	4	-2.52	0.50
4	4	-2.48	0.55
5	4	-2.18	0.46
5 + 1 min.	4	-1.35*	0.58
5 + 2 min.	4	-1.37	0.36
5 + 3 min.	4	-1.72	0.31
5 + 30 min.	4	-1.15	0.18
6	4	-0.72	0.22

ELECTRICAL POTENTIAL MEASUREMENTS WERE MADE FOR SIX CONSECUTIVE HOURS AFTER THE GUT SEGMENT WAS MOUNTED. VALUES REPRESENT MEANS (mV) ± (SEM) FOR THE INDICATED NUMBER OF OBSERVATIONS.

* INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE PRECEEDING VALUE AT THE 5 PERCENT LEVEL.

in total carbohydrate concentration in the mucosal compartment during the course of the experiments. However, this does not eliminate the fact that a tissue accumulation of the ^{14}C label occurred.

The results of these studies using either glucose or 3-O-methyl glucose were qualitatively similar although quantitatively different and can be seen in Figures 3 through 22. The parameters presented are the sodium flux, sugar flux, pH, transmural potential, and the S/M ratio.

The pH reported is for the serosal compartment only, since there was no apparent pH change during the course of the experiments in the mucosal compartment. The potential represents the transmural potential between the mucosal and serosal compartments, the mucosal side being considered as the reference at OmV.

Figure 3 illustrates the results of experiments performed with a sodium free chiton Ringer solution. During the experimental period, the data indicate that the sodium flux decreased to 1/15 of the control value ($P < .05$) and the sugar flux changed significantly from a net accumulation to a net disappearance ($P < .05$). This change from an accumulation of the sugar (positive net flux) to a disappearance of the sugar (negative net flux) is probably due to blockage of the sugar flux into the serosal compartment

by the experimental condition inflicted upon the tissue, in this case, a sodium free Ringer solution, and an uptake of the sugar by the gut tissue, resulting in a net sugar disappearance. The pH increased by 0.29 pH units ($P < .05$), i.e. became more alkaline, and the potential increased two fold in absolute magnitude ($P < .05$), i.e. became more electronegative. Figures 4 through 9 illustrate the results of the same experiment performed with one-quarter sodium, one-half sodium, and three-quarter sodium Ringer solutions, respectively, using either glucose or 3-O-methyl glucose in the medium. With one-quarter sodium Ringer solution, the sodium flux decreased to 1/3 of the control value ($P < .05$) and the sugar flux changed from a net accumulation to a net disappearance ($P < .05$), but the pH increased by 0.37 pH units ($P < .05$) and the potential increased by 60% in absolute magnitude ($P < .05$) using glucose as the sugar (Figure 4). With 3-O-methyl glucose in the medium (Figure 5), the sodium flux decreased to 1/2 of the control ($P < .05$) and the sugar flux changed from net accumulation to net disappearance ($P < .05$), while the pH increased by 0.28 pH units ($P < .05$) and the potential increased three fold in magnitude ($P < .05$). A one-half sodium Ringer solution resulted in a decrease in the sodium flux to 50% of the control ($P < .05$), a change from net accumulation to net disappearance in the sugar flux ($P < .05$), a 0.25 pH unit increase in the pH ($P < .05$), and a three fold

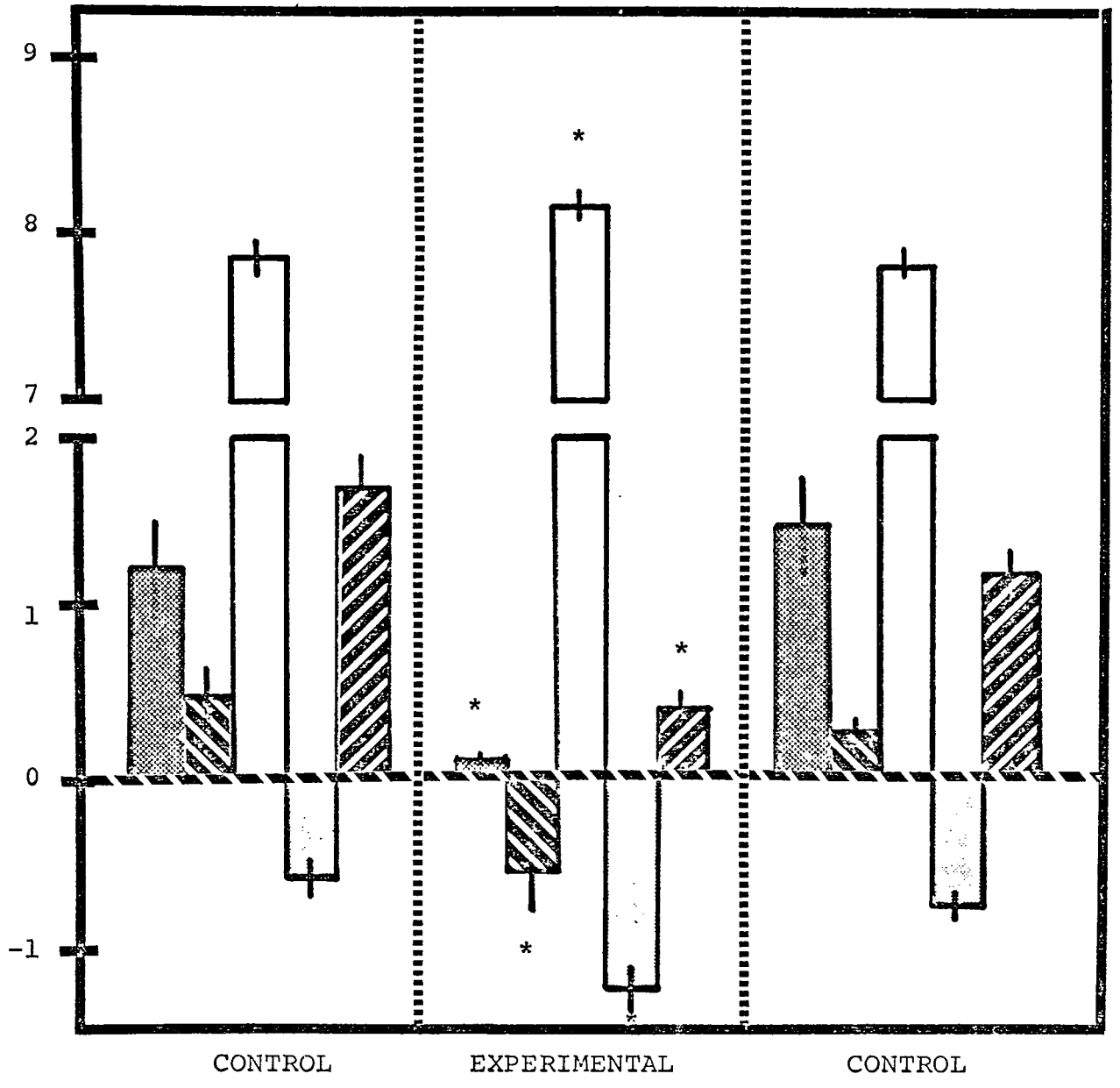


FIGURE 3 THE EFFECT OF A SODIUM FREE RINGER SOLUTION CONTAINING 1uM/ML GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR FOUR OBSERVATIONS. * INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

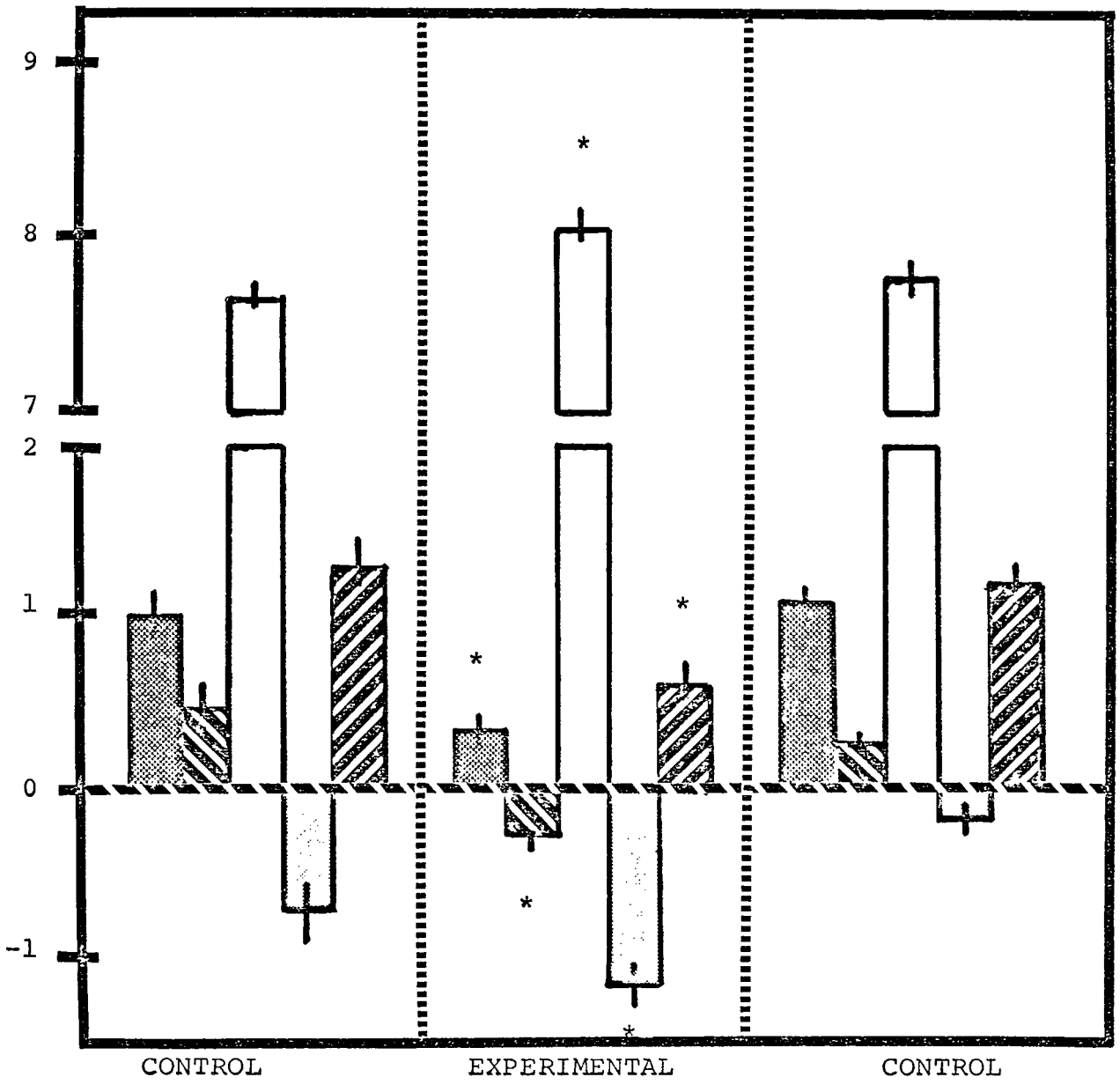


FIGURE 4 THE EFFECT OF A 0.116M NaCl RINGER SOLUTION (ONE-QUARTER SODIUM) CONTAINING 1uM/ML GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR FOUR OBSERVATIONS. * INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

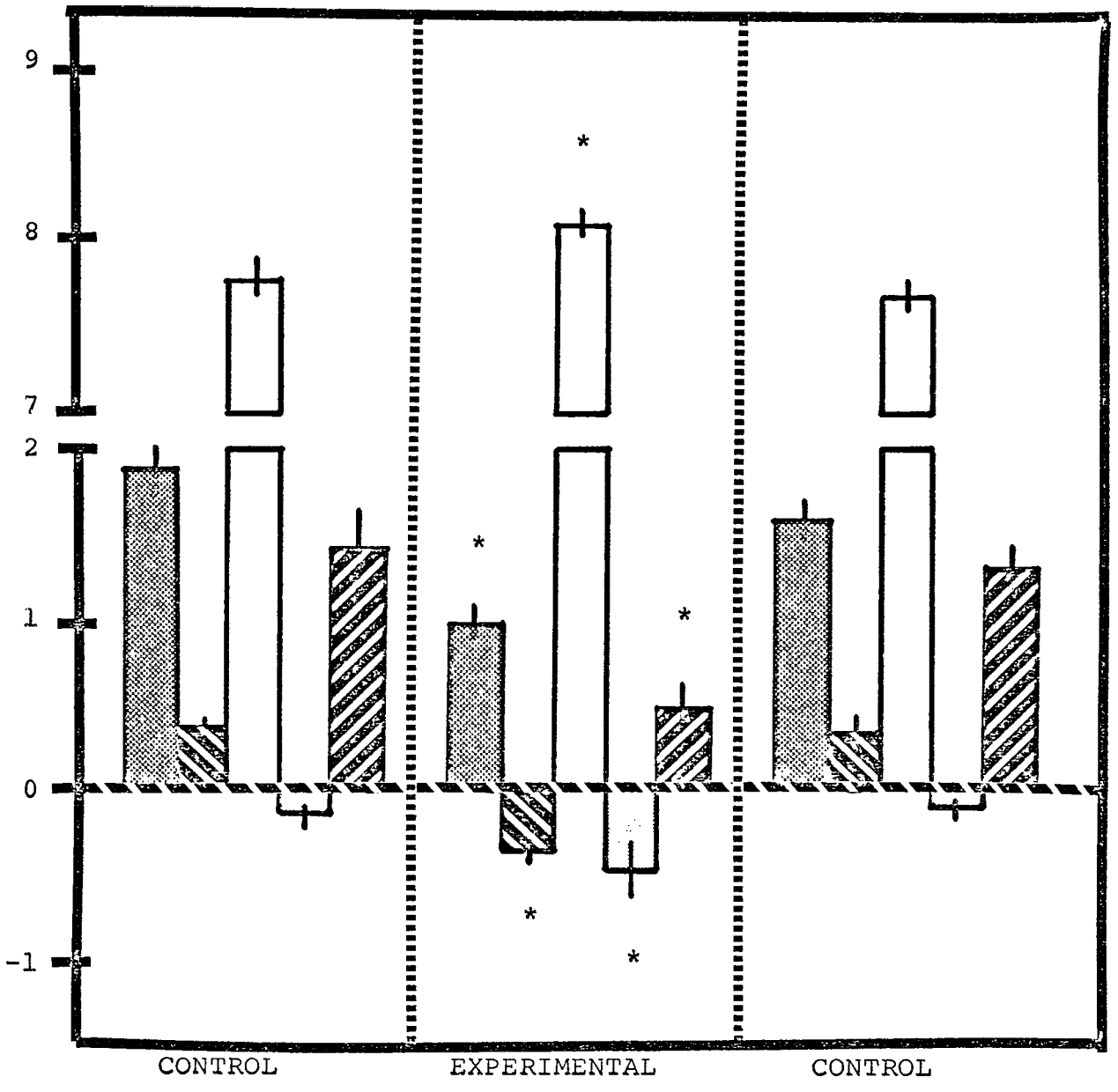


FIGURE 5 THE EFFECT OF A 0.116 M NaCl RINGER SOLUTION (ONE-QUARTER SODIUM) CONTAINING 1uM/ML 3-O-METHYL GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR THREE OBSERVATIONS. * INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

increase in the absolute magnitude of the potential ($P < .05$) using glucose (Figure 6), while the same experiment performed with 3-O-methyl glucose (Figure 7) also resulted in a decrease in the sodium flux to 50% of the control ($P < .05$), a change in the sugar flux from net accumulation to net disappearance ($P < .05$), a 0.41 pH unit increase in pH ($P < .05$), and a six fold increase in the potential ($P < .05$). With a three-quarter sodium Ringer solution, the sodium flux decreased 25% ($P > .05$), the sugar flux changed from net accumulation to net disappearance ($P > .05$), the pH increased by 0.19 pH units ($P > .05$), and the potential increased three fold ($P < .05$) with glucose (Figure 8); and with 3-O-methyl glucose (Figure 9), the sodium flux decreased to 1/2 of the control ($P < .05$), the sugar flux changed from net accumulation to net disappearance ($P < .05$), the pH increased by 0.31 pH units ($P < .05$), and the potential increased three fold in magnitude ($P < .05$).

As can be seen, all of the results using a partial sodium Ringer solution were similar; that is, the sodium and sugar fluxes significantly decreased while the pH and potential increased significantly during the experimental period. Figure 10 indicates the relationship between the sodium flux and sodium concentration obtained from data from the partial sodium experiments using either glucose or 3-O-methyl glucose. The relationship between sodium concentration and sugar flux, from data also obtained in the partial sodium experiments,

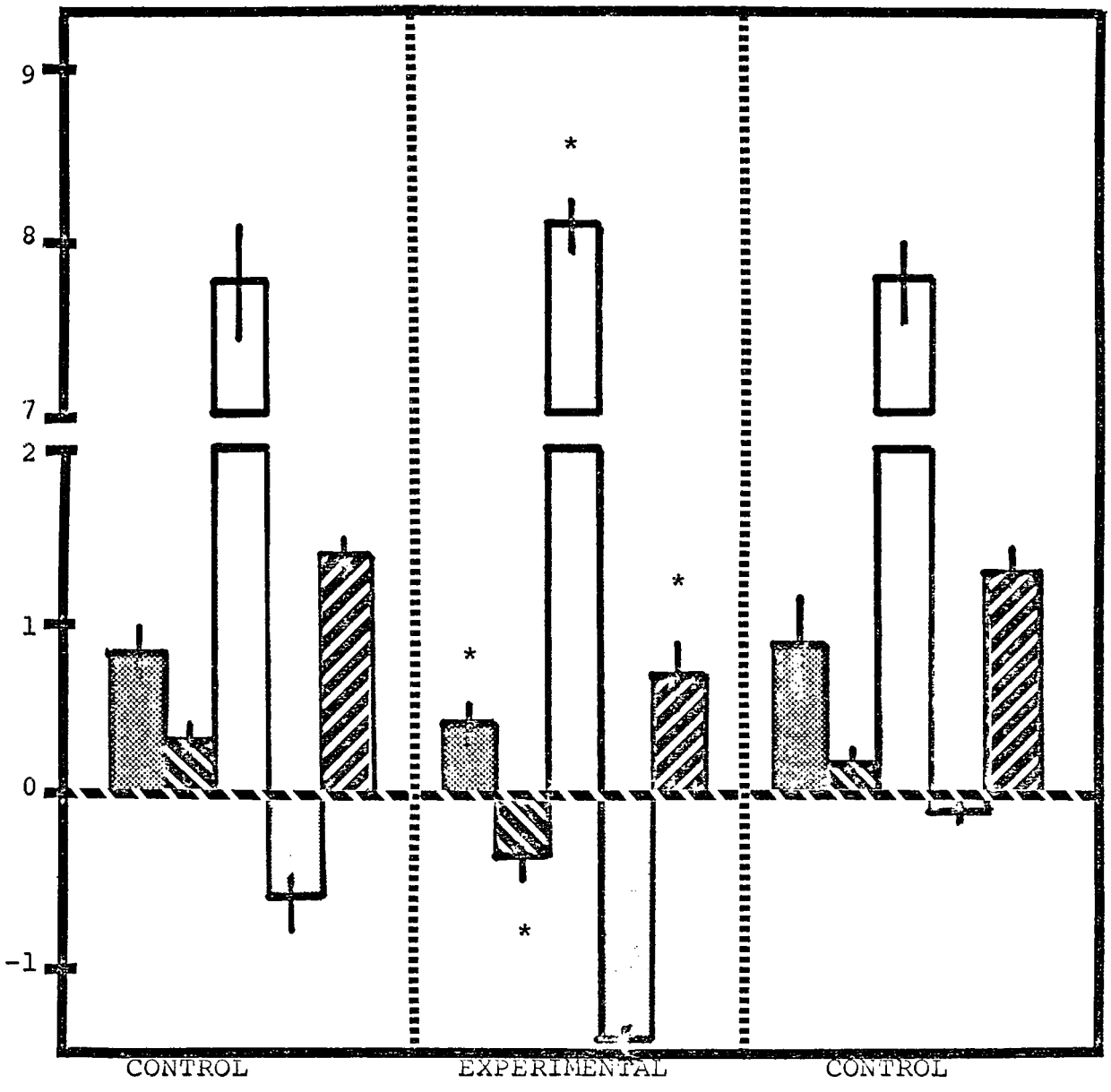


FIGURE 6 THE EFFECT OF A 0.232 M NaCl RINGER SOLUTION (ONE-HALF SODIUM) CONTAINING 1uM/ML GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR FOUR OBSERVATIONS. *INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

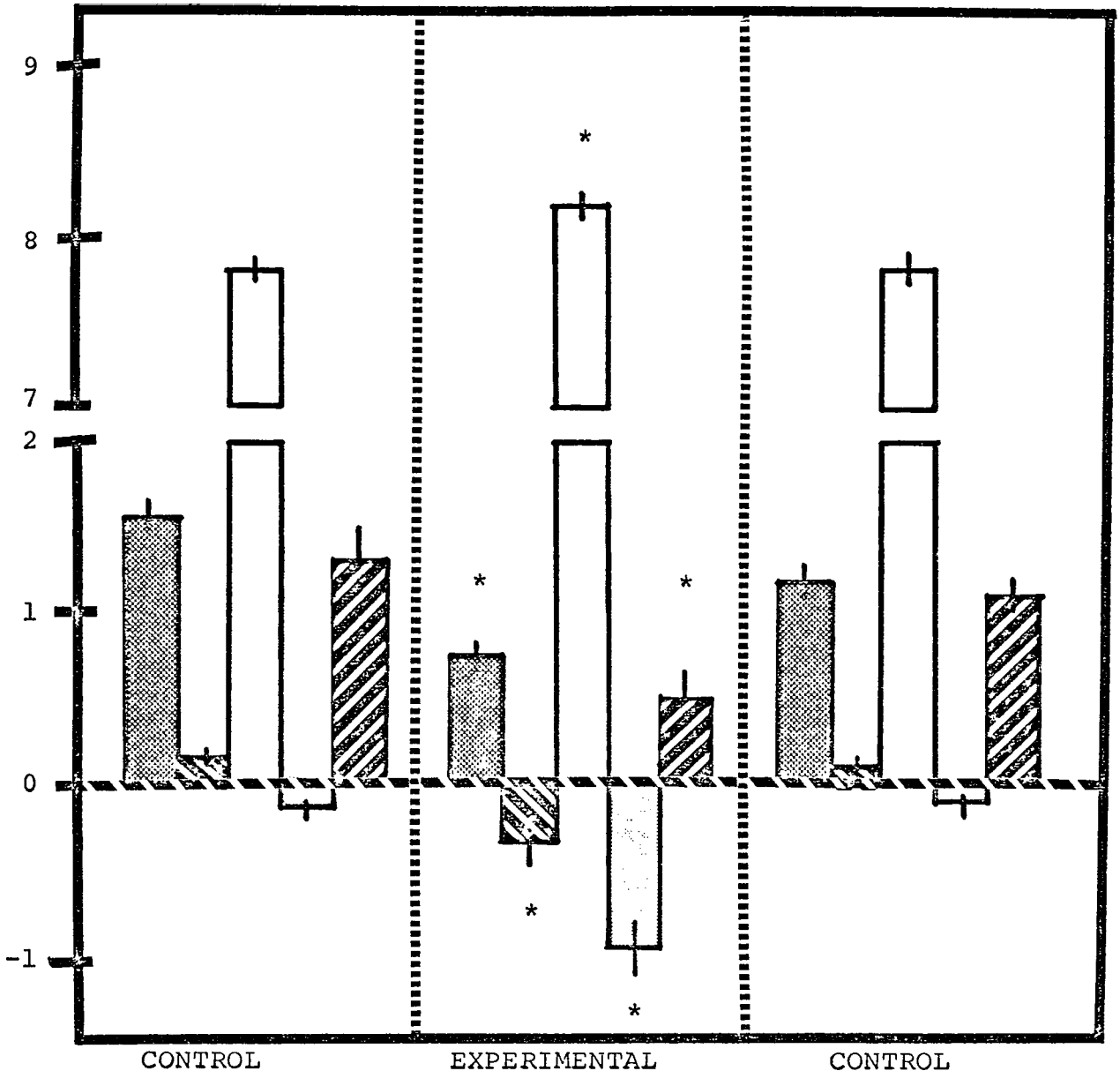


FIGURE 7 THE EFFECT OF A 0.232 M NaCl RINGER SOLUTION (ONE-HALF SODIUM) CONTAINING 1uM/ML 3-O-METHYL GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR THREE OBSERVATIONS. *INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

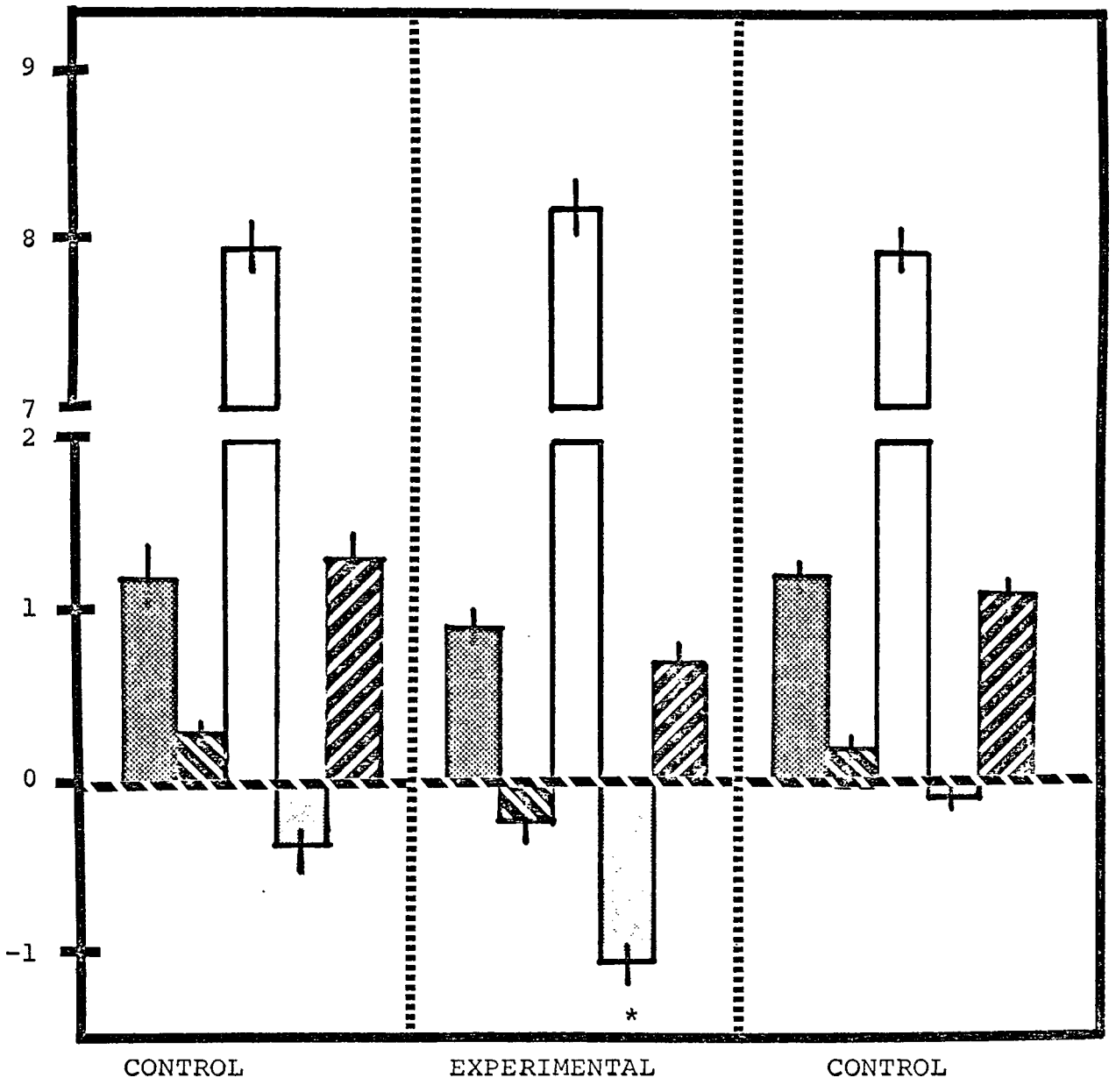


FIGURE 8 THE EFFECT OF A 0.348M NaCl RINGER SOLUTION (THREE-QUARTER SODIUM) CONTAINING 1uM/ML GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR TWO OBSERVATIONS. *INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

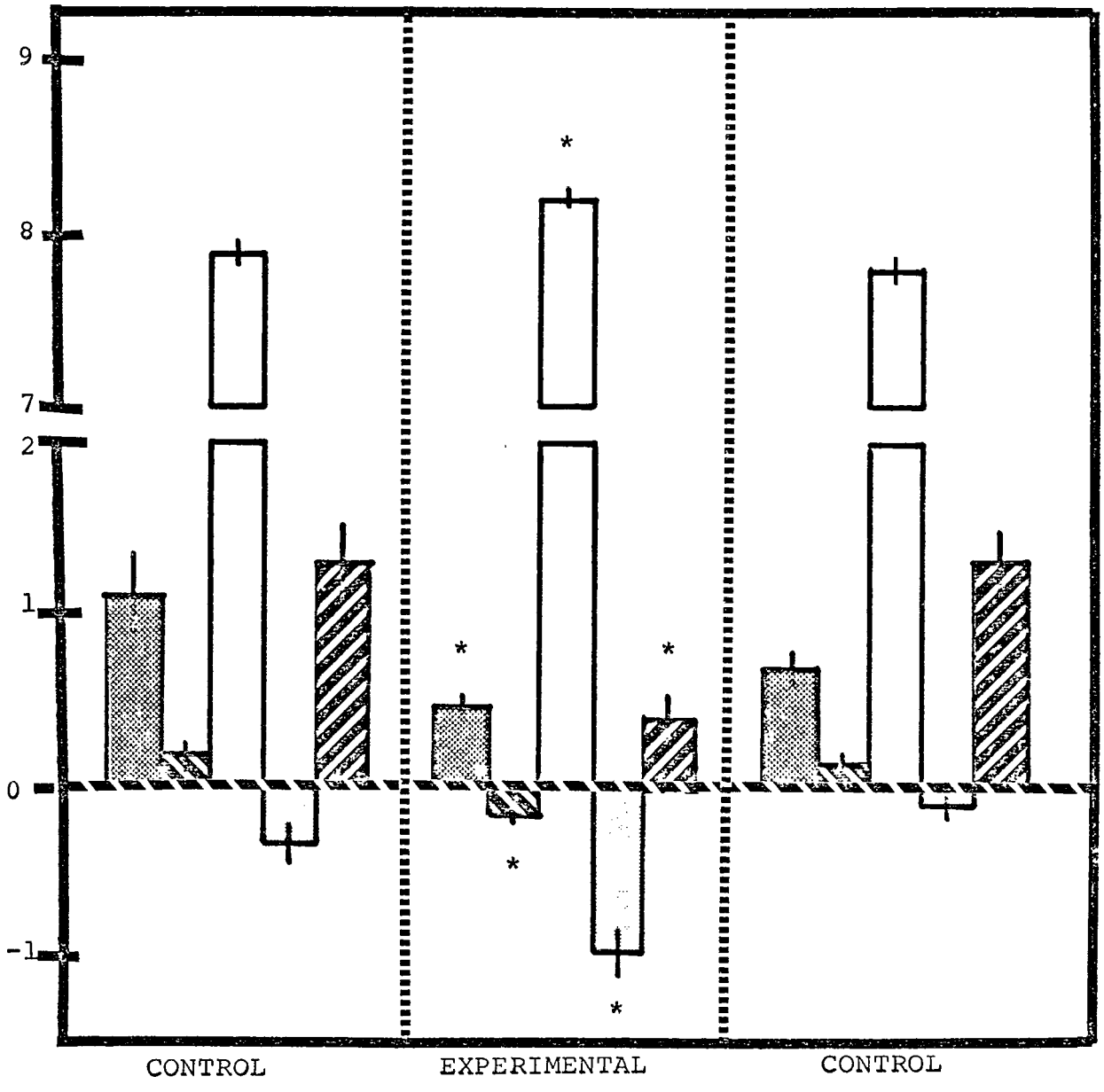


FIGURE 9 THE EFFECT OF A 0.348M NaCl RINGER SOLUTION (THREE-QUARTER SODIUM) CONTAINING 1uM/ML 3-O-METHYL GLUCOSE ON THE SODIUM FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR THREE OBSERVATIONS. *INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

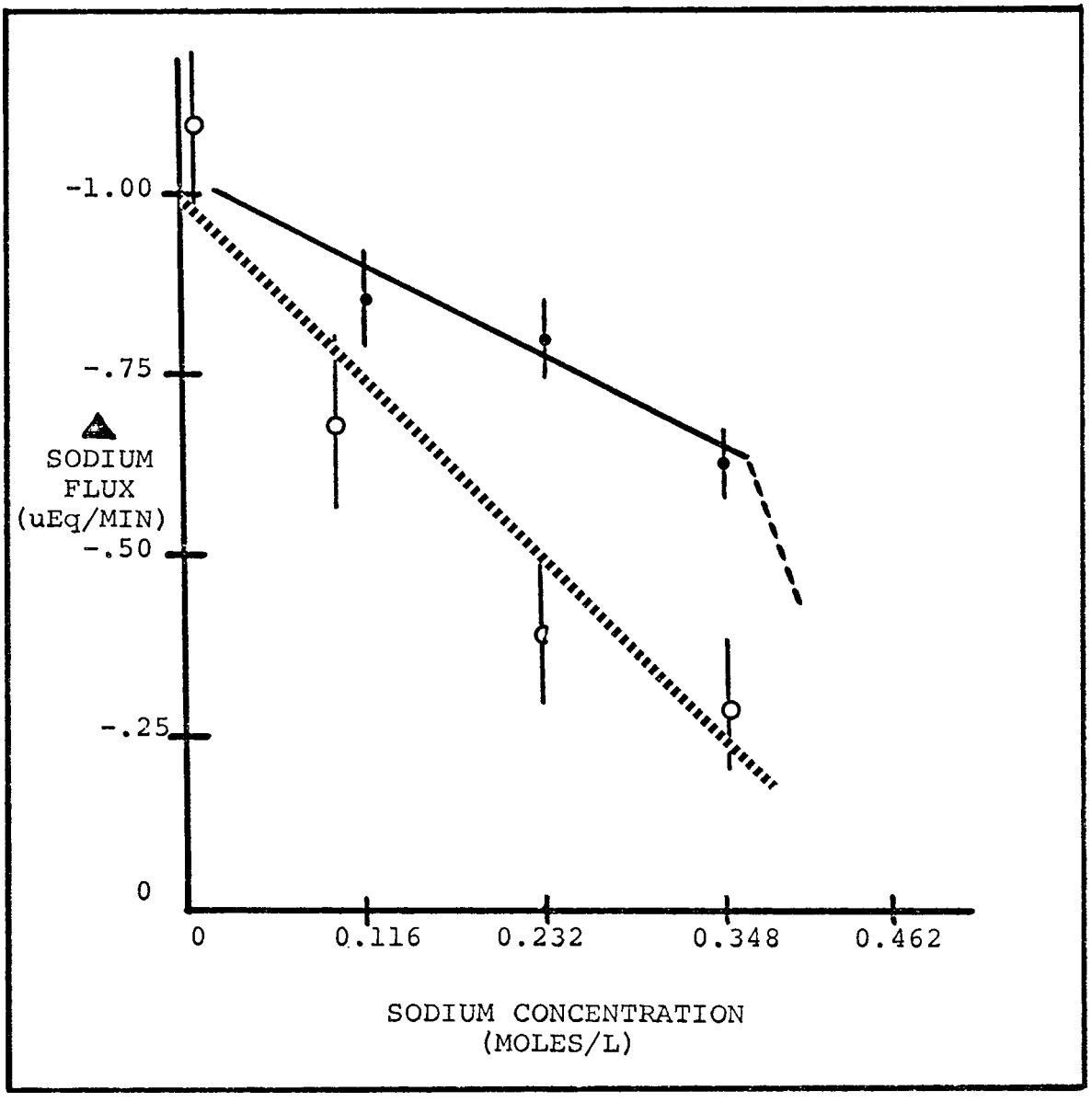


FIGURE 10 THE RELATIONSHIP BETWEEN SODIUM FLUX AND SODIUM CONCENTRATION USING 1uM/ML OF EITHER GLUCOSE OR 3-0-METHYL GLUCOSE. EACH POINT REPRESENTS THE MEAN OF FOUR EXPERIMENTS. ▲ SODIUM FLUX REPRESENTS THE MEAN OF THE DIFFERENCE BETWEEN THE SODIUM FLUX IN THE CONTROL PERIOD AND THE SODIUM FLUX IN THE EXPERIMENTAL PERIOD. THE NEGATIVE VALUE REPRESENTS A DECREASE IN THE FLUX DURING THE EXPERIMENTAL PERIOD. ---- REPRESENTS RESULTS USING GLUCOSE AND ——— REPRESENTS RESULTS USING 3-0-METHYL GLUCOSE. THE CURVES WERE DRAWN BY EYE. VERTICAL LINES REPRESENT THE SEM VALUES.

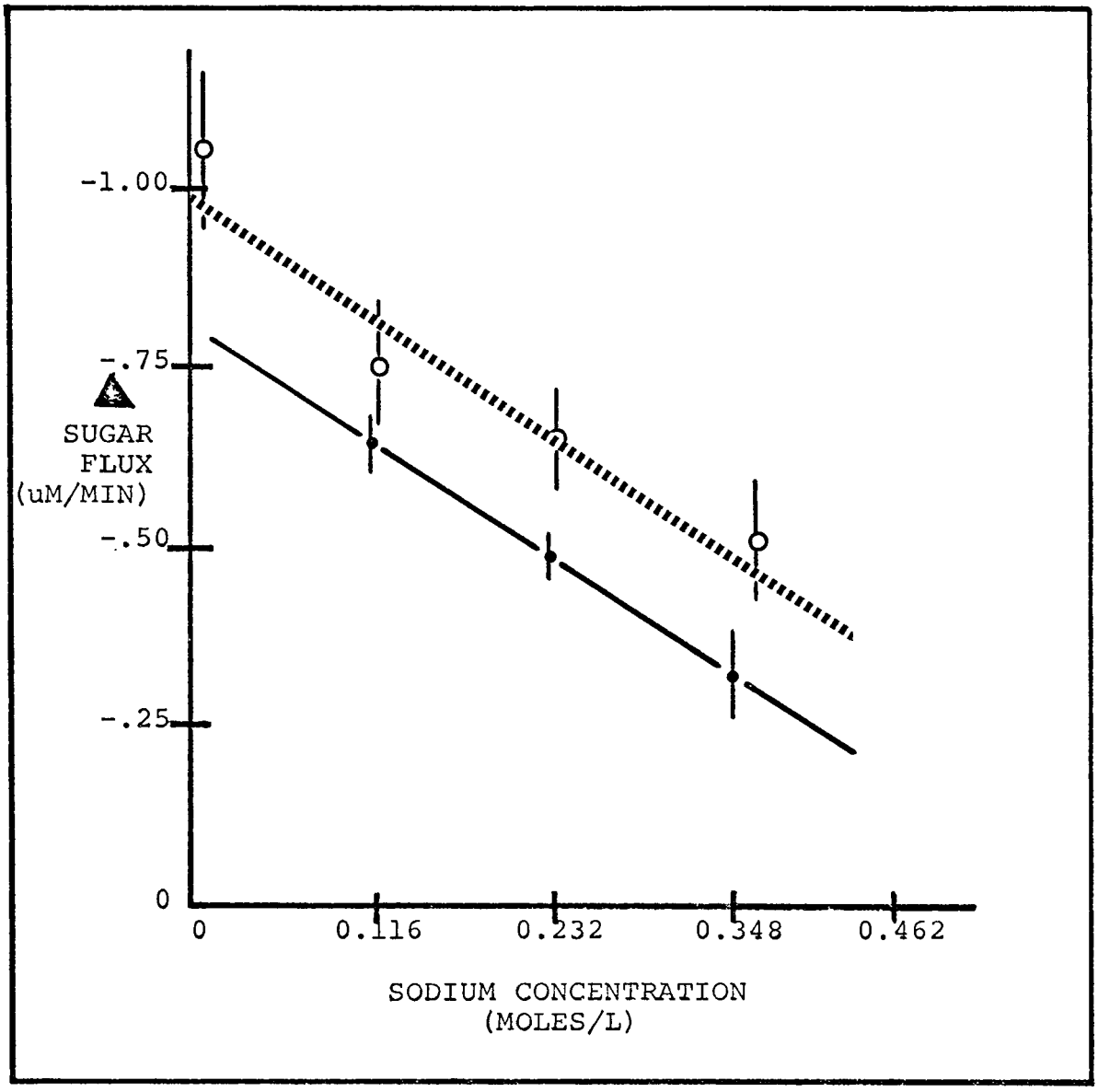


FIGURE 11 THE RELATIONSHIP BETWEEN SUGAR FLUX AND SODIUM CONCENTRATION USING 1uM/ML OF EITHER GLUCOSE OR 3-O-METHYL GLUCOSE. EACH POINT REPRESENTS THE MEAN OF FOUR EXPERIMENTS. ▲ SUGAR FLUX REPRESENTS THE MEAN OF THE DIFFERENCE BETWEEN THE SUGAR FLUX IN THE CONTROL PERIOD AND THE SUGAR FLUX IN THE EXPERIMENTAL PERIOD. THE NEGATIVE VALUE REPRESENTS A DECREASE IN THE FLUX DURING THE EXPERIMENTAL PERIOD. ----- REPRESENTS RESULTS USING GLUCOSE AND ——— REPRESENTS RESULTS WITH 3-O-METHYL GLUCOSE. THE CURVES WERE DRAWN BY EYE. VERTICAL LINES REPRESENT THE SEM VALUES.

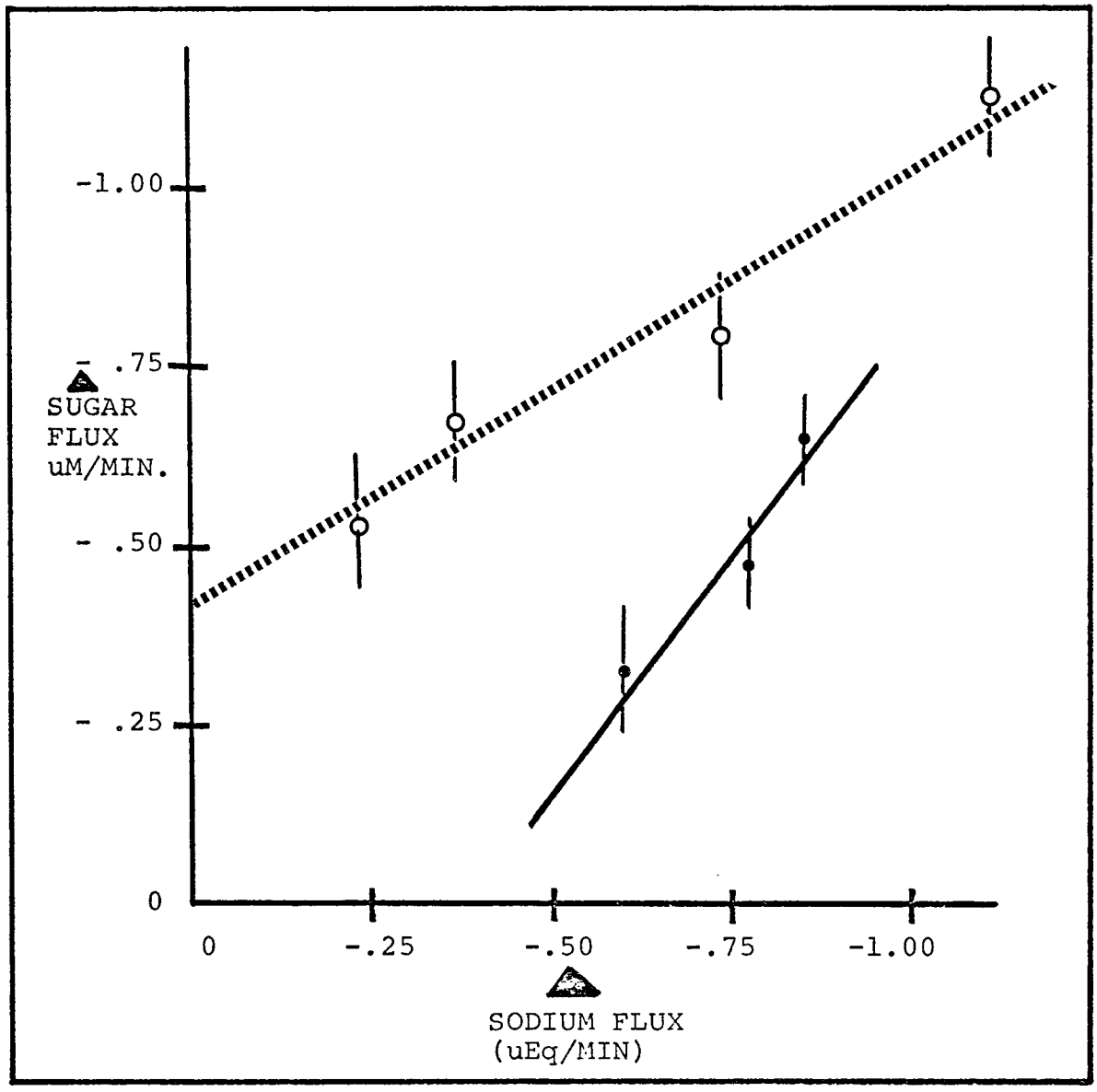


FIGURE 12 THE RELATIONSHIP BETWEEN SUGAR FLUX AND SODIUM FLUX USING 1uM/ML OF EITHER GLUCOSE OR 3-O-METHYL GLUCOSE. EACH POINT REPRESENTS THE MEAN OF FOUR EXPERIMENTS. ▲ SUGAR FLUX AND ▲ SODIUM FLUX REPRESENT THE MEANS OF THE DIFFERENCES BETWEEN THE RESPECTIVE FLUXES IN THE CONTROL PERIODS AND IN THE EXPERIMENTAL PERIODS. THE NEGATIVE VALUE REPRESENTS A DECREASE IN THE FLUX DURING THE EXPERIMENTAL PERIOD. ---- REPRESENTS RESULTS USING GLUCOSE AND ——— REPRESENTS RESULTS USING 3-O-METHYL GLUCOSE. THE CURVES WERE DRAWN BY EYE. VERTICAL LINES REPRESENT THE SEM VALUES.

can be seen in Figure 11. Figure 12 represents a combination of Figures 10 and 11 and indicates a linear relationship between the sodium and sugar fluxes.

The results of a potassium free Ringer solution can be seen in Figures 13 and 14. Using glucose as the sugar (Figure 13), the sodium flux decreased to one third of the control ($P > .05$), the sugar flux decreased to 1/3 of the control value ($P > .05$), the pH increased 0.14 pH units ($P > .05$), and the potential increased two fold in absolute magnitude ($P < .05$). With 3-O-methyl glucose (Figure 14), the sodium flux decreased 35% ($P < .05$), the sugar flux changed from net accumulation to net disappearance ($P < .05$), the pH increased 0.30 pH units ($P < .05$), and the potential increased two fold ($P < .05$). Here again, then, the sodium and sugar fluxes both decreased, while the pH and potential increased.

Figures 15 and 16 illustrate the effects of anaerobic conditions upon the various parameters during the experimental period. Again, except for magnitude effects, the results were equivalent with glucose and 3-O-methyl glucose. In these experiments, the sodium flux decreased to 1/3 of the control value ($P < .05$), the sugar flux changed from net accumulation to net disappearance ($P < 05$), the pH decreased 0.12 pH units ($P < .05$), and the potential decreased 40% in magnitude ($P < .05$) using glucose as the sugar (Figure 15), while the

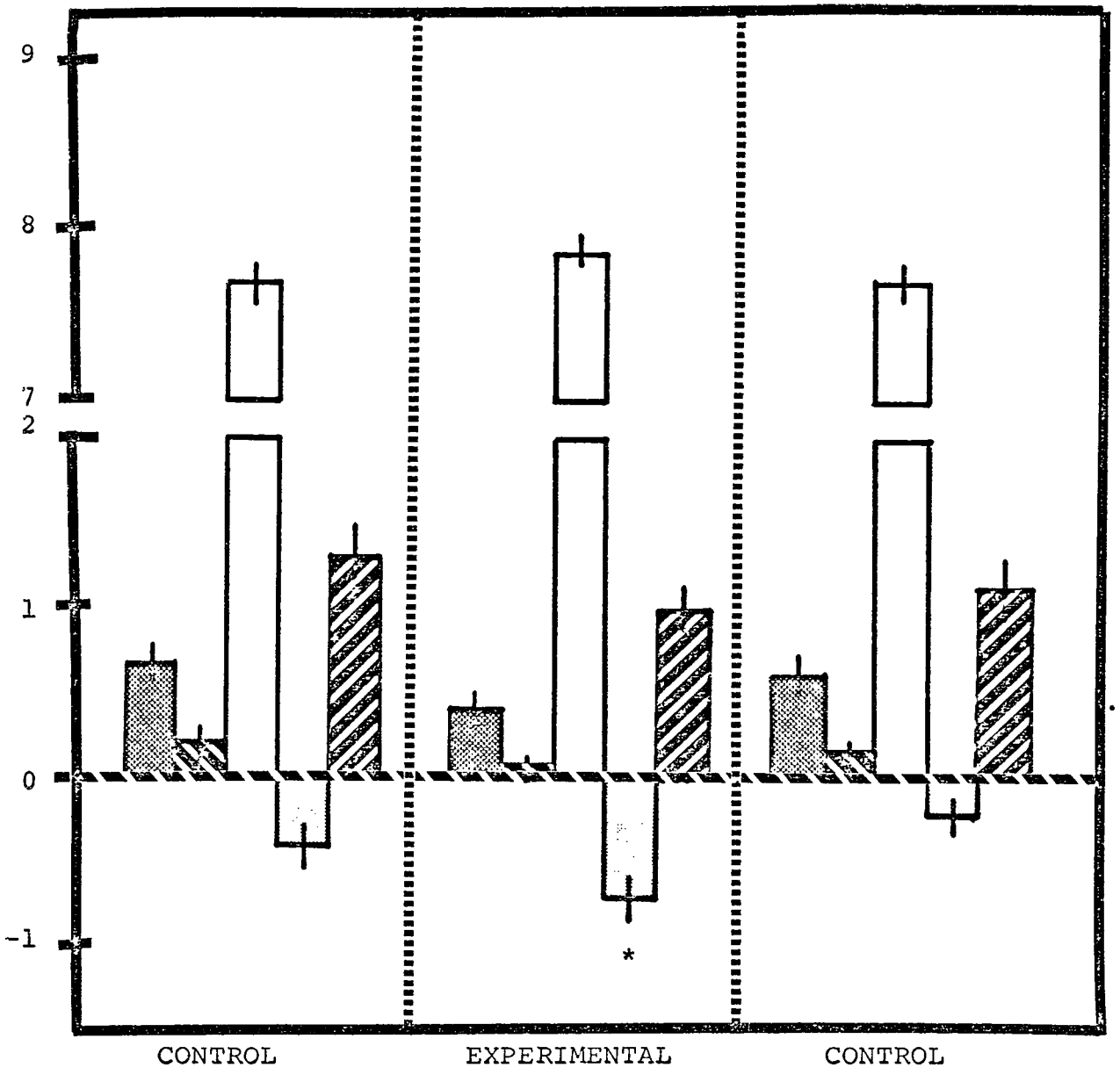


FIGURE 13 THE EFFECT OF A POTASSIUM FREE RINGER SOLUTION CONTAINING 1uM/ML GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR TWO OBSERVATIONS. * INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

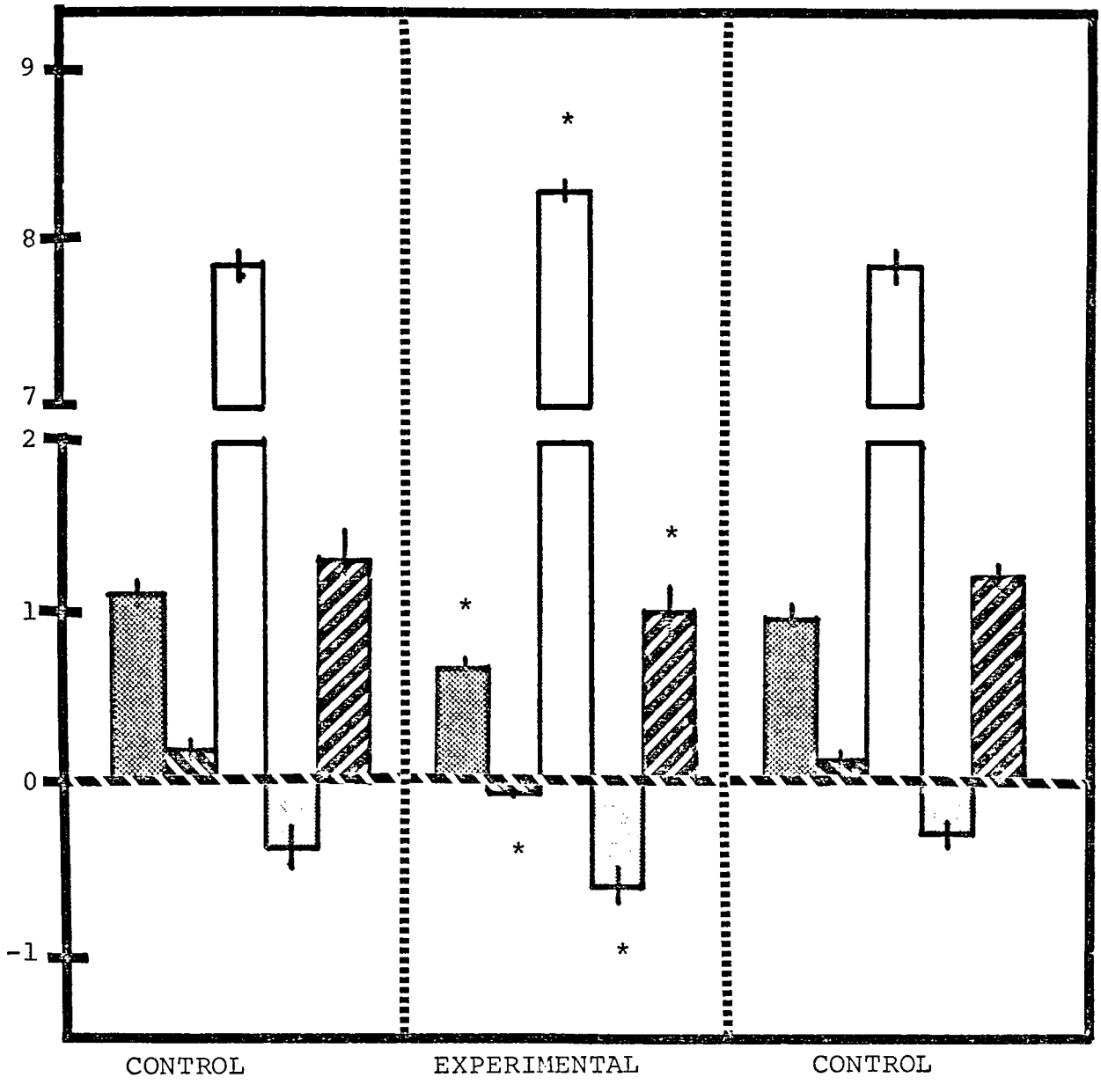


FIGURE 14 THE EFFECT OF A POTASSIUM FREE RINGER SOLUTION CONTAINING 1uM/ML 3-O-METHYL GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR THREE OBSERVATIONS. * INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

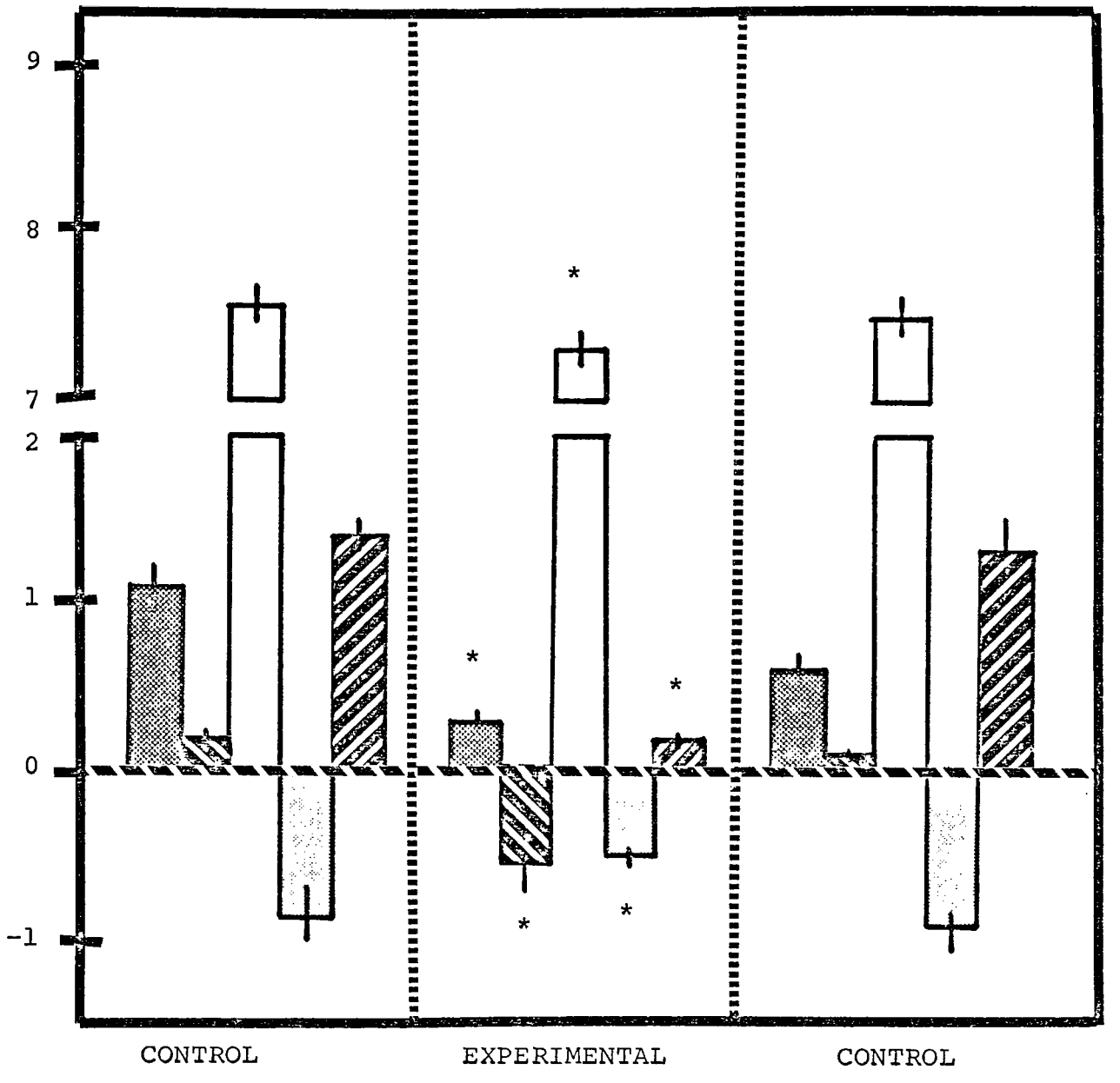


FIGURE 15 THE EFFECT OF ANAEROBIC CONDITIONS USING 1uM/ML GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR FOUR OBSERVATIONS. * INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

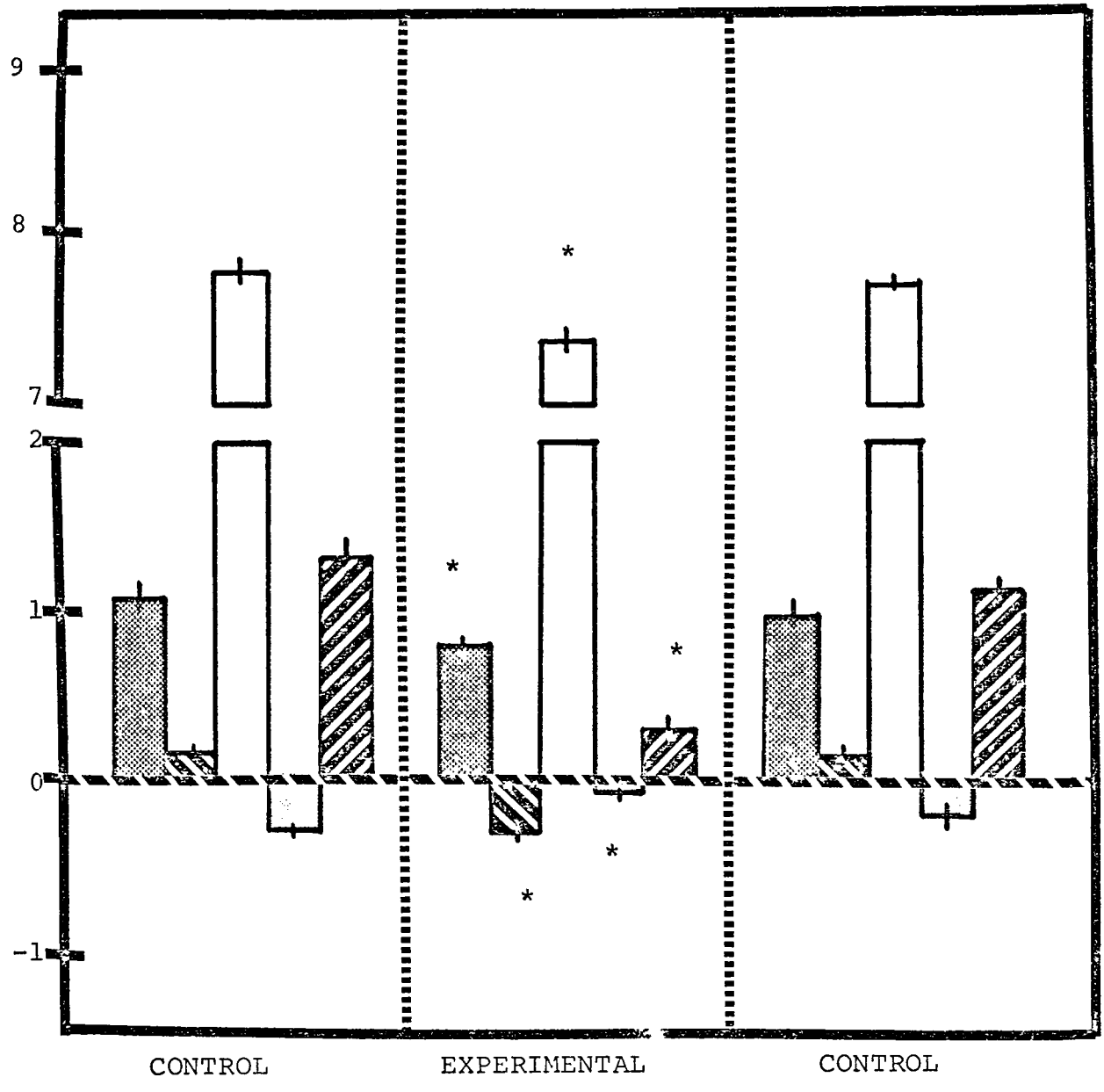


FIGURE 16 THE EFFECT OF ANAEROBIC CONDITIONS USING 1uM/ML 3-O-METHYL GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR ON THE MEAN FOR SIX OBSERVATIONS. *INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

sodium flux decreased 22% ($P < .05$), the sugar flux again changed from net accumulation to net disappearance ($P < .05$), the pH decreased 0.33 pH units ($P < .05$), and the potential decreased in magnitude to 1/4 of the control ($P < .05$) using 3-O-methyl glucose (Figure 16). Here, then, in addition to a decrease in the sodium and sugar fluxes, the pH decreased, i.e. became more acidic, and the potential decreased, i.e. became less negative.

The results of experiments using 1×10^{-6}M ouabain on the mucosal side only, on the serosal side only, and in both compartments simultaneously with either glucose or 3-O-methyl glucose are seen in Figures 17 through 22. The results of all three experimental conditions were the same. Table 9 summarizes the experimental changes observed using ouabain in the experimental solutions and illustrates the differences between results obtained with ouabain present on the mucosal side only, on the serosal side only, and on both sides simultaneously. Although the results of experiments using glucose as the sugar are not significant due to the low number of observations, a definite pattern is apparent. The magnitude of the effect of ouabain was greatest when ouabain was present on the serosal surface only. The next largest effect upon the parameters occurred with ouabain present in both the mucosal and serosal compartments simultaneously, the results probably being due to the inhibitory effects of ouabain upon the serosal

surface only. It follows, then, that the least effect of ouabain upon the magnitude of the experimental parameters occurred with ouabain present on the mucosal surface only. If the results of experiments with ouabain on the serosal side only are grouped with results of experiments with ouabain on both the mucosal and serosal surfaces simultaneously, the differences are significant to the 5% level. If a similar grouping is made with the results of experiments with ouabain present on the mucosal surface only, the differences remain insignificant. Table 9 also illustrates that the major effect of ouabain was on the sodium and sugar fluxes, with a lesser and indirect effect upon the potential and pH. Although the addition of ouabain to the serosal compartment caused a very marked initial increase in the magnitude of the potential across the gut tissue, the potential returned to its control value within an hour, and, hence, the effect of ouabain on the potential was insignificant ($P > .05$) when considered over the entire three hour experimental period.

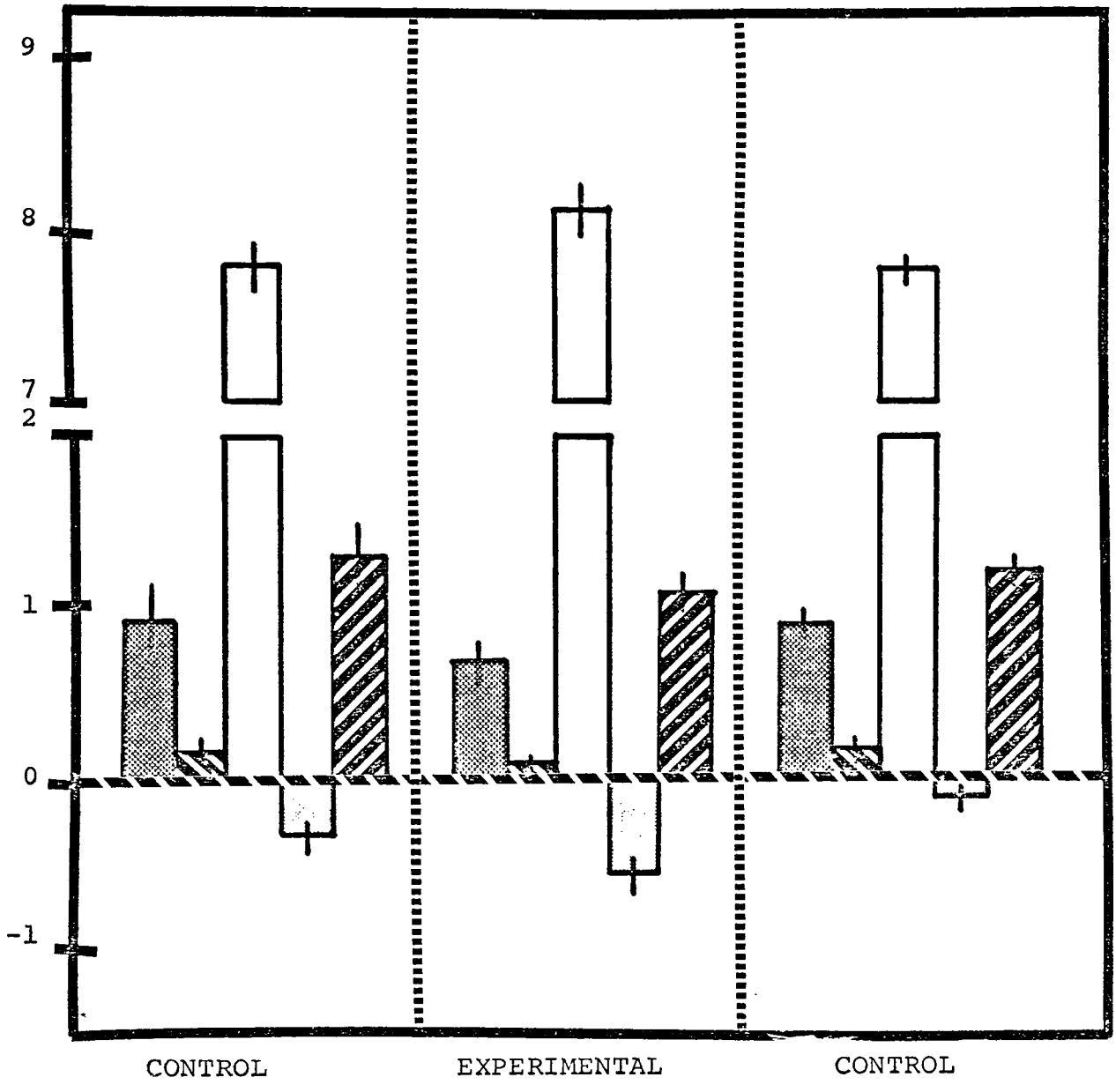


FIGURE 17 THE EFFECT OF 1.0 X 10^{-6}M OUABAIN IN THE MUCOSAL COMPARTMENT ONLY USING 1uM/ML GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MILLIEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR TWO OBSERVATIONS. *INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

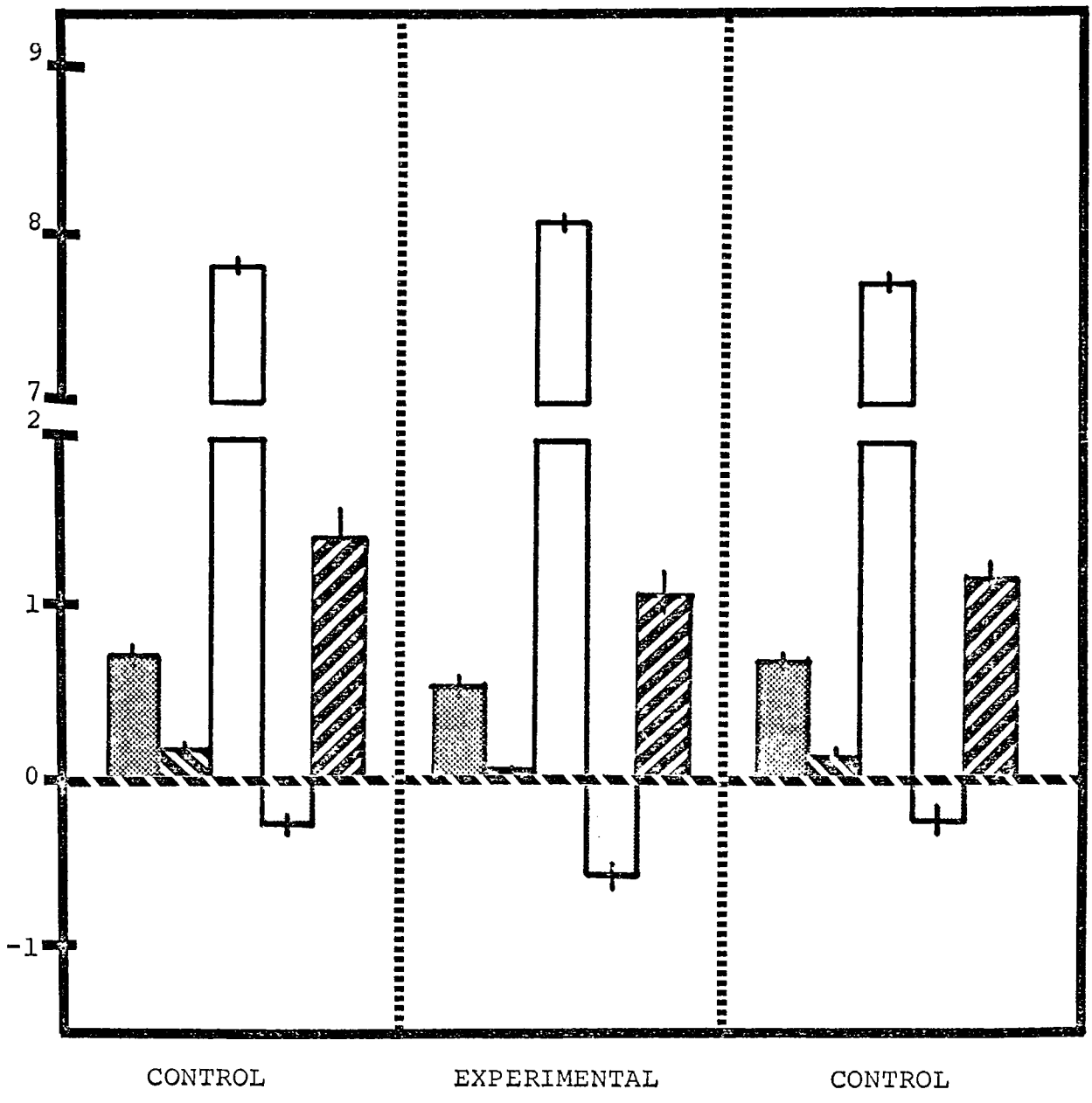


FIGURE 18 THE EFFECT OF 1.0 X 10^{-6}M OUABAIN IN THE MUCOSAL COMPARTMENT ONLY USING 1uM/ML 3-O-METHYL GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR SIX OBSERVATIONS. *INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

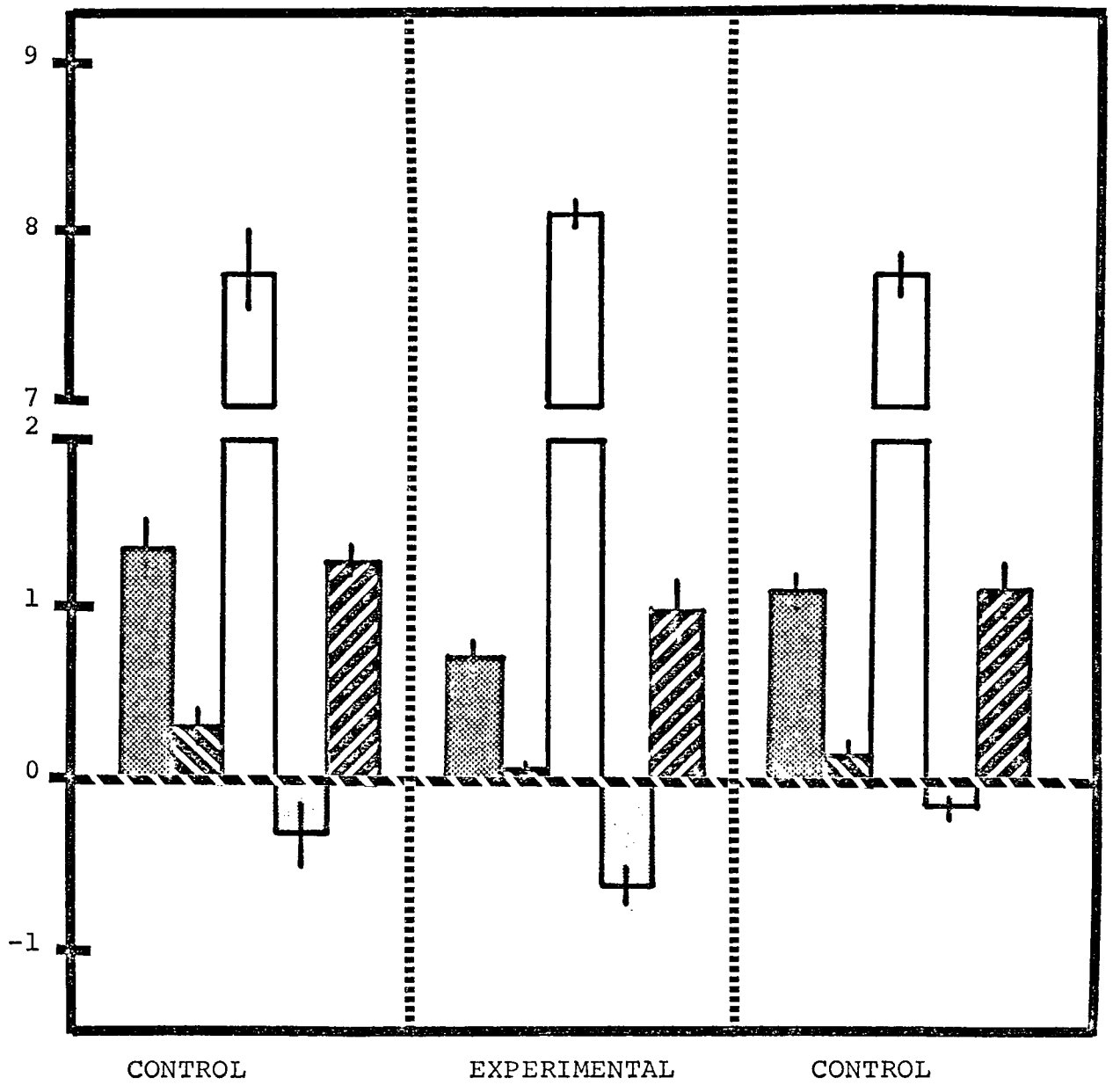


FIGURE 19 THE EFFECT OF 1.0 X 10^{-6}M OUABAIN IN THE SEROSAL COMPARTMENT ONLY USING luM/ML GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR TWO OBSERVATIONS. * INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

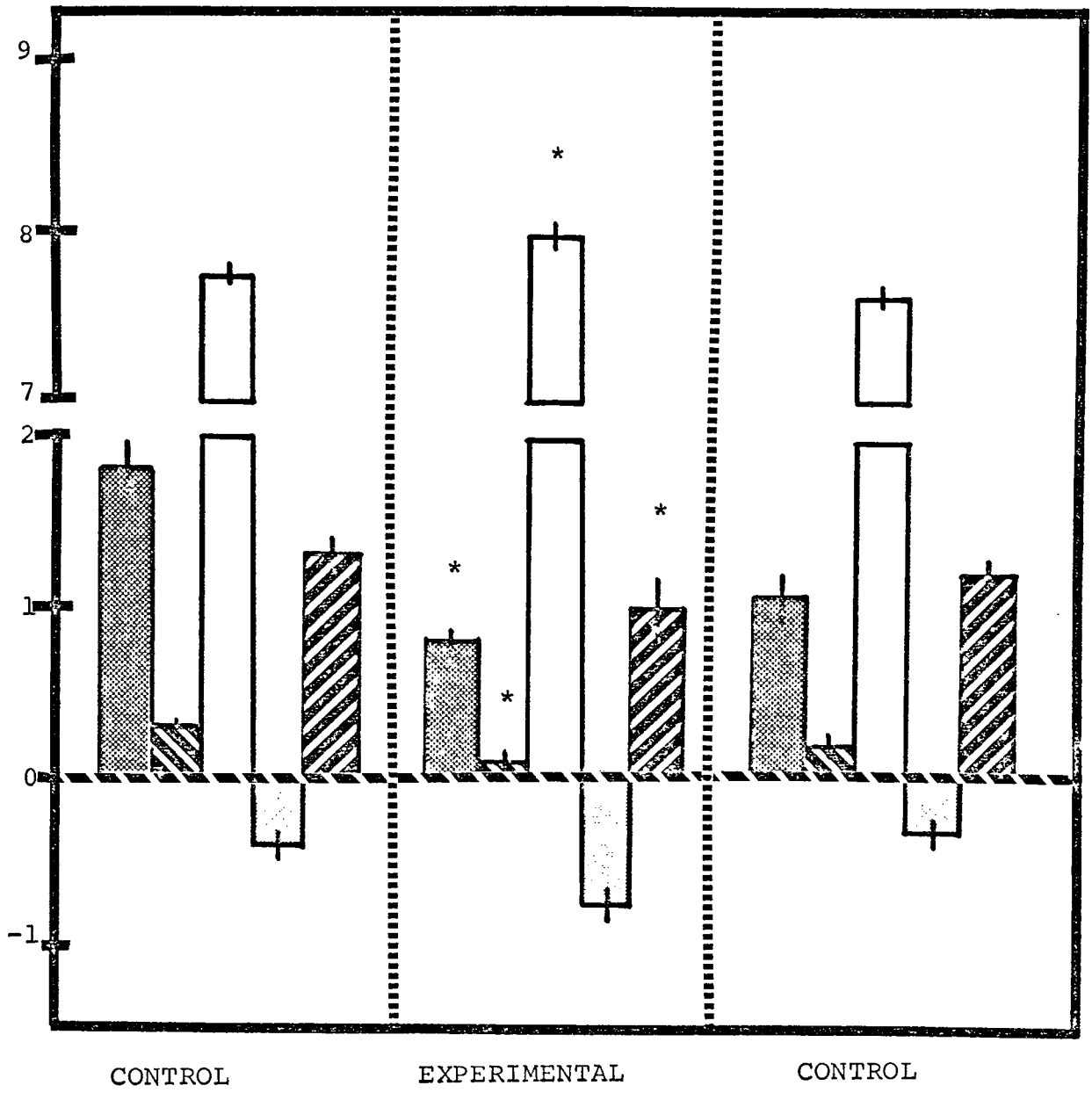


FIGURE 20 THE EFFECT OF 1.0 X 10^{-6}M OUABAIN IN THE SEROSAL COMPARTMENT ONLY USING 3-O-METHYL GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTES.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR THREE OBSERVATIONS. * INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

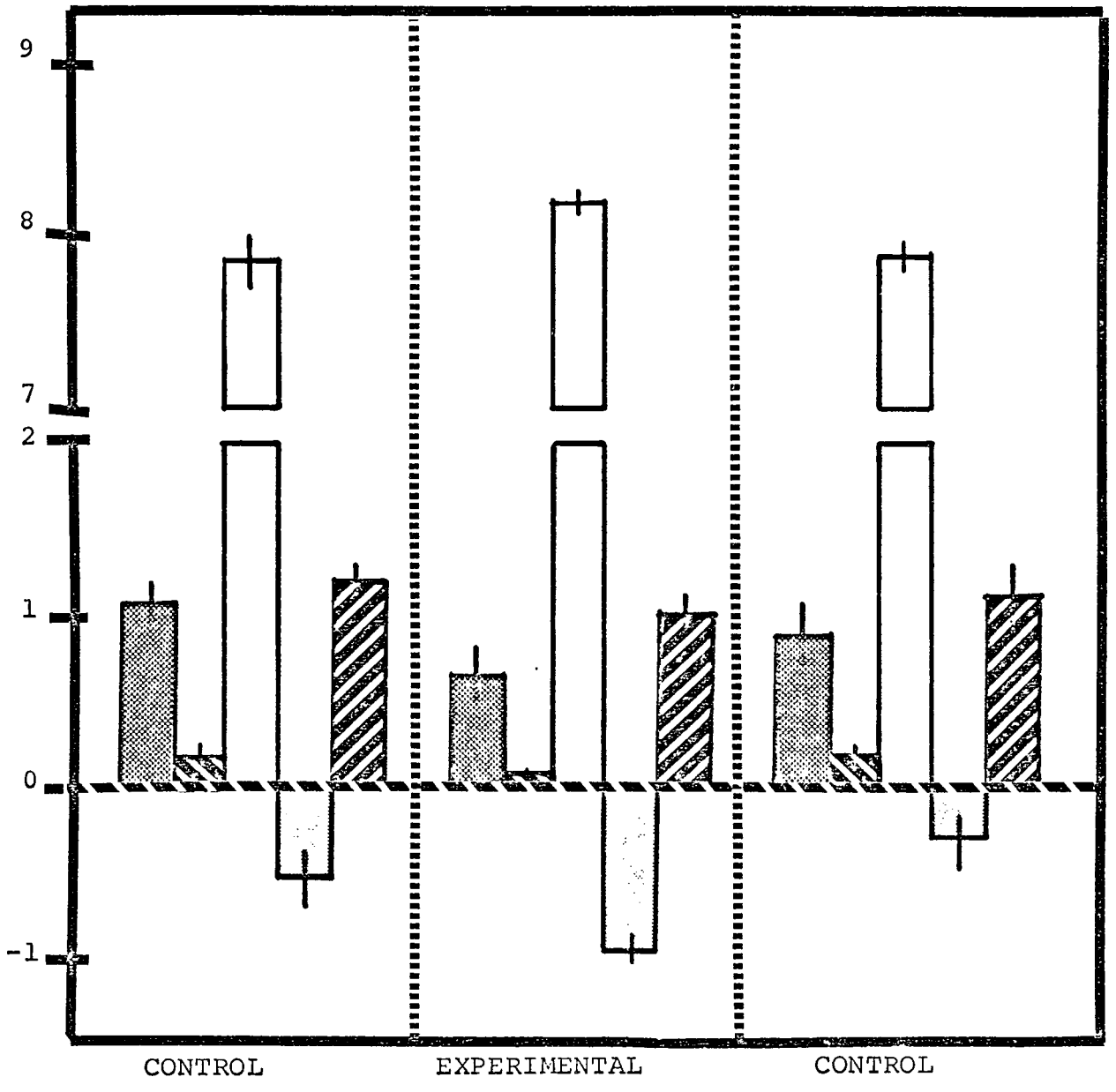


FIGURE 21 THE EFFECT OF 1.0 X 10^{-6}M OUABAIN IN THE MUCOSAL AND SEROSAL COMPARTMENTS USING 1uM/ML GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR TWO OBSERVATIONS. * INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

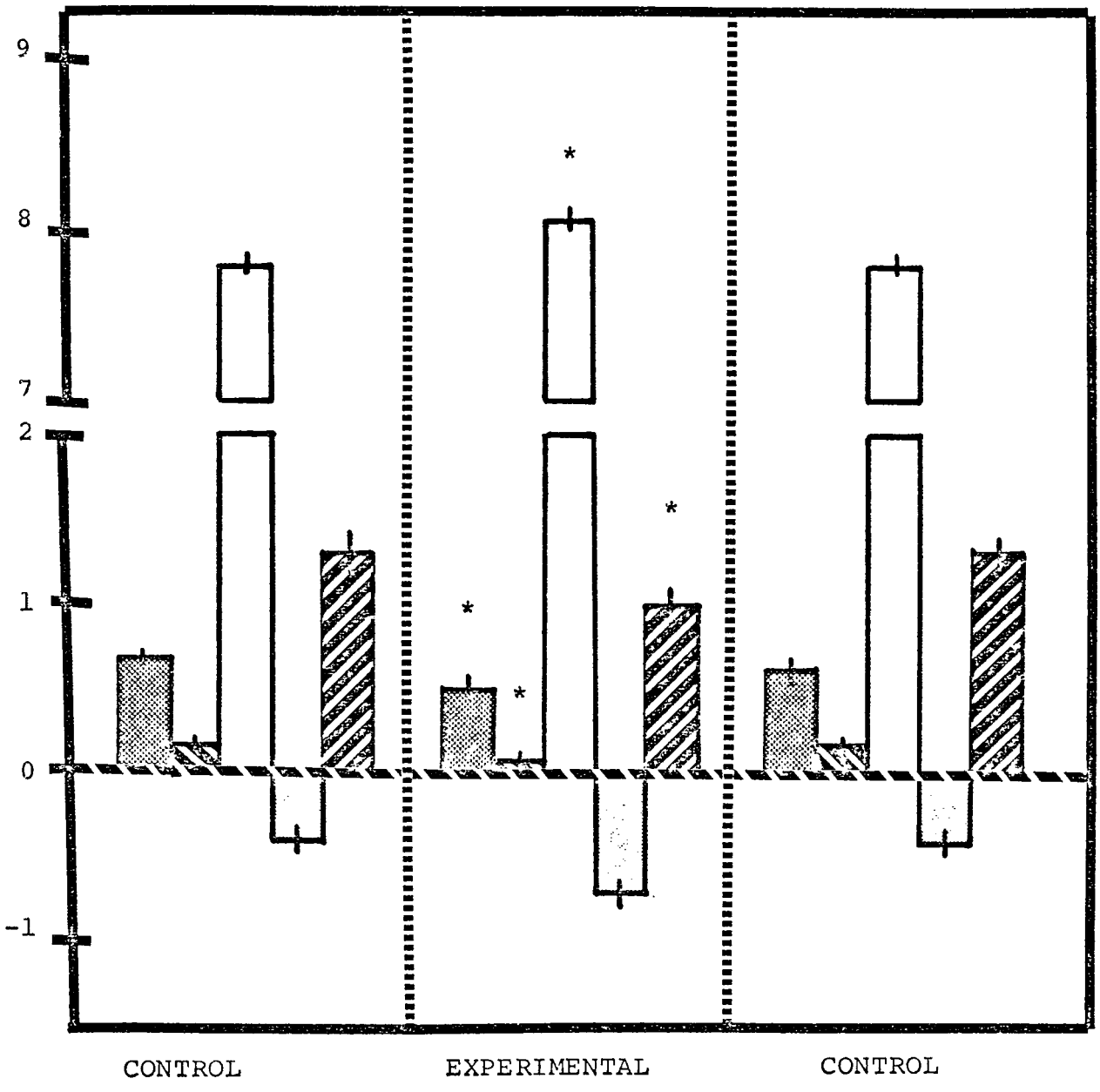


FIGURE 22 THE EFFECTS OF 1.0 X 10^{-6}M OUABAIN IN THE MUCOSAL AND SEROSAL COMPARTMENTS USING 1uM/ML 3-O-METHYL GLUCOSE ON THE SODIUM FLUX, SUGAR FLUX, pH, TRANSMURAL POTENTIAL, AND FINAL S/M RATIO OF SUGAR CONCENTRATION.

INDICATES SODIUM FLUX IN MICROEQUIVALENTS/MINUTE.
INDICATES SUGAR FLUX IN MICROMOLES/MINUTE.
INDICATES pH IN STANDARD pH UNITS.
INDICATES TRANSMURAL POTENTIAL IN mVOLTS.
INDICATES S/M RATIO.

LINES INDICATE THE STANDARD ERROR OF THE MEAN FOR THREE OBSERVATIONS. * INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL VALUE AT THE 5% LEVEL.

TABLE 9

THE MAGNITUDE OF EFFECTS OF 1.0 X 10^{-6}M OUABAIN IN THE MUCOSAL COMPARTMENT ONLY, IN THE SEROSAL COMPARTMENT ONLY, AND IN BOTH COMPARTMENTS SIMULTANEOUSLY ON THE SODIUM FLUX, SUGAR FLUX, pH, AND TRANSMURAL POTENTIAL USING EITHER GLUCOSE OR 3-O-METHYL GLUCOSE. Δ REPRESENTS THE MEAN OF THE DIFFERENCE BETWEEN THE CONTROL VALUE AND THE EXPERIMENTAL VALUE. A NEGATIVE VALUE REPRESENTS A DECREASE IN MAGNITUDE AND A POSITIVE VALUE INDICATES AN INCREASE. * INDICATES A VALUE THAT IS SIGNIFICANTLY DIFFERENT FROM THE CONTROL AT THE 5% LEVEL.

SUGAR: Glucose

OUABAIN LOCATION	Δ NA FLUX	± SEM	Δ SUGAR FLUX	± SEM	Δ pH	± SEM	Δ POTENTIAL	± SEM
M & S	-.44	.06	-.13	.02	.39	.12	.12	.04
M	-.22	.08	-.03	.01	.37	.10	.07	.04
S	-.59	.12	-.29	.05	.38	.09	.13	.03
S + M&S	-.51*	.12	-.21*	.05	.38*	.08	.12	.05

SUGAR: 3-O-methyl glucose

OUABAIN LOCATION	Δ NA FLUX	± SEM	Δ SUGAR FLUX	± SEM	Δ pH	± SEM	Δ POTENTIAL	± SEM
M & S	-.16*	.03	-.10*	.01	.28*	.06	.33	.18
M	-.11	.06	-.09	.05	.25	.12	.38	.20
S	-.96*	.16	-.08*	.03	.24*	.05	.35	.18
S + M&S	-.56*	.11	-.14*	.02	.26*	.06	.34	.19

DISCUSSION

Cryptochiton stelleri is a marine member of one of the most primitive classes of the phylum Mollusca. The mollusc gut had hitherto been considered little more than a semi-permeable membrane with respect to nutrient absorption, and in the case of the chiton, absorption had been attributed to the digestive gland (85). The initial finding by Lawrence and Mailman (106) of a potential difference across the chiton gut was indicative of ion transport, and other studies by Lawrence and Lawrence (104) revealed that active nutrient absorption occurred in the anterior gut of this animal.

With these initial studies in mind, the ion replacement experiments were performed in order to obtain an overall look at the effect of various ionic environments upon the potential difference across the anterior chiton gut to determine which ions were responsible for the potential. The observed effects of ion replacement could be the result of two processes, 1) the effect upon active transport mechanisms and 2) trans-cellular diffusion potentials. Ignoring active transport mechanisms for the time being and considering the high permeability of the chiton intestinal tissue to sodium and water at both the luminal and contraluminal surfaces (155), any net effect of ion replacement at both surfaces could be due to a large diffusion potential imposed across the tissue,

giving rise to a net potential. This idea is supported by a sodium ion removal resulting in an increase in the magnitude of the potential, which is inconsistent if a transcellular sodium pump is involved but is consistent with a slightly greater permeability of the luminal membrane to sodium. Likewise, the mucosal surface would seem to be more permeable to chloride and potassium ions, since their removal results in a decrease in the magnitude of the potential with a chloride free Ringer solution and an increase with a potassium free Ringer solution. Thus the mucosal surface appears to be more permeable to all three of the ions under consideration. This fact is further substantiated by the total replacement of all ions by isosmotic sucrose, which resulted in an extremely large increase in the magnitude of the potential, indicating also that there is a great deal of shunting of the current due to the movement of all three ions. Figure 23 seems to be consistent with the observed data and the high permeability of the chiton gut.

Following the ion replacement studies, specific experiments were designed to further elucidate the results of sodium, chloride, and potassium ion repacement by examining the fluxes of sodium, sugar, and water using labeled compounds in order to propose a mechanism of transport for salt, water, and organic nutrients in the anterior chiton intestine.

That sodium ion is actively transported by the intestinal

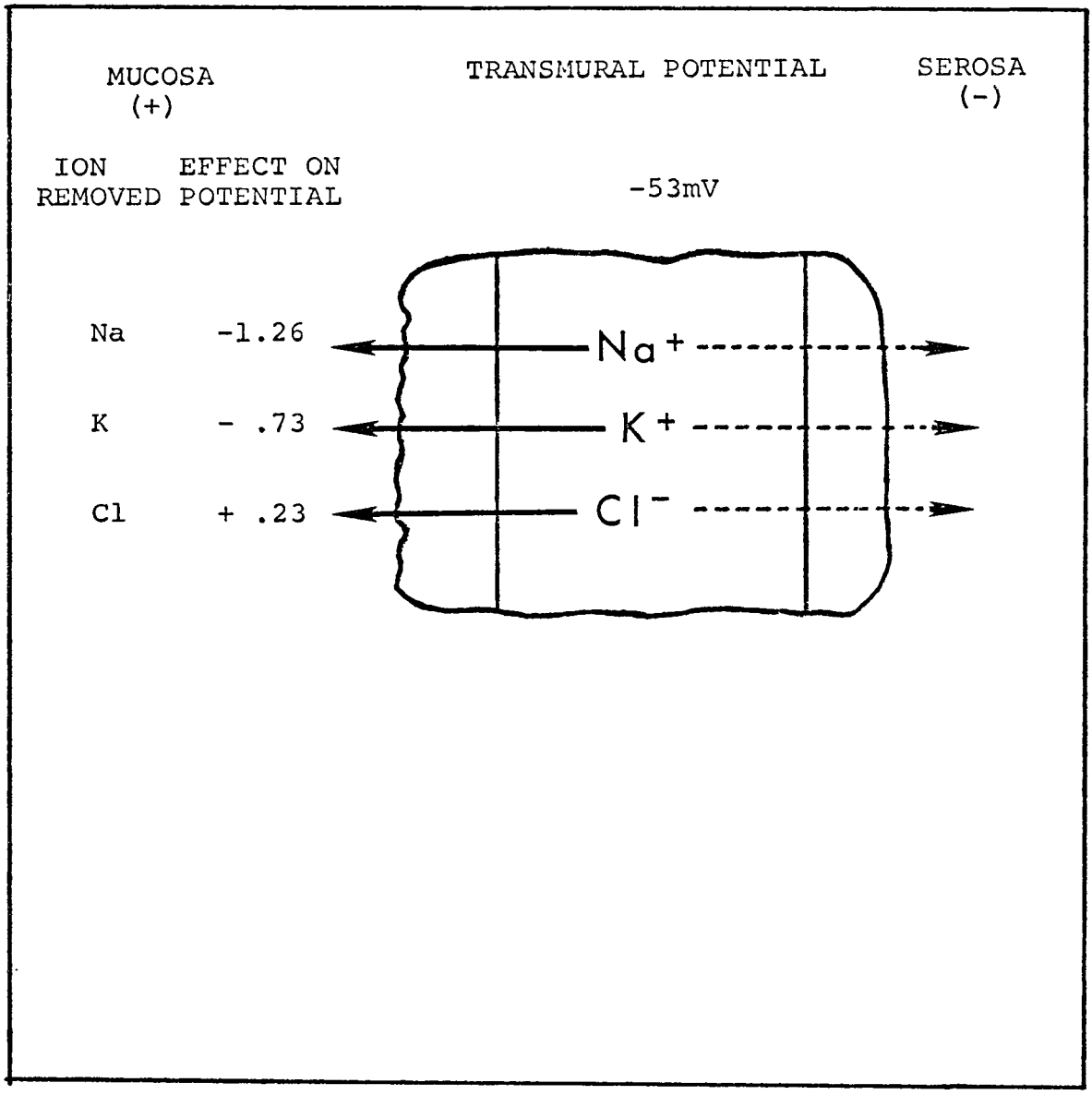


FIGURE 23 A MODEL OF THE CHITON ANTERIOR INTESTINAL EPITHELIAL CELL ILLUSTRATING THE INCREASED PERMEABILITY OF THE MUCOSAL SURFACE TO SODIUM, POTASSIUM, AND CHLORIDE WHEN THESE IONS ARE ABSENT FROM THE BATHING MEDIUM. THE RESULTING EFFECT OF ION REMOVAL UPON THE TRANSMURAL POTENTIAL IS ALSO SHOWN.

epithelium in all species studied to date is well documented and has been noted by almost every worker in the area of intestinal transport. The hypothetical "pump" responsible for net sodium movement into the serosal compartment and a serosal positive potential is postulated to be located at the basal membrane of the epithelial cell, since ouabain, a known ATPase inhibitor, strongly inhibits the flux of sodium and hence decreases the potential difference across the tissue only when placed on the serosal side (57, 58, 109, 137).

Data from this study indicates that sodium ions are also actively transported in the anterior chiton gut. This transport is probably from the mucosal to the serosal compartment, with a simultaneous and equal passive movement in the opposite direction. However, due to the negative serosal potential in the anterior chiton gut, the sodium flux from the mucosa to the serosa is in the direction of the electrochemical potential and cannot be considered active in the absence of a net flux. Indeed, the serosa to mucosa flux would more likely seem to be active, since it is against an electrochemical gradient. The difficulty in determining the active flux is due to the high permeability of the chiton gut tissue.

The evidence for an active sodium transport in the chiton gut is a strong inhibition of the sodium flux at the serosal

surface and a simultaneous increase in the negativity of the potential difference with ouabain in the serosal compartment and a nonsignificant change in sodium flux with ouabain in the mucosal compartment. The sodium flux is also strongly inhibited during anaerobic conditions. Decreasing the sodium concentration caused a linear decrease in the sodium flux (Figure 10). A linear relationship between sodium concentration and sodium flux is consistent with either passive diffusion or a transport system which is well below saturation. Considering the effects of anaerobiosis and ouabain, the latter is more likely.

Because of the aforementioned negative serosal potential in the anterior chiton gut, the situation is quite different from the vertebrate gut, where the active movement of sodium into the serosal compartment gives rise to a positive serosal potential. In the chiton, the active transport of sodium could not be responsible for the negativity of the serosal surface. The increase in negativity of the potential in the serosal compartment during the partial sodium and ouabain experiments, however, indicates that sodium ion does contribute to the potential difference across the tissue, probably by being pumped into the serosal compartment and making the potential less negative. The unidirectional sodium flux from the mucosa to the serosa (and serosa to mucosa), then, decreases despite an increase in negativity at the serosal

surface, indicating that the sodium flux is not completely dependent upon the electrochemical gradient and supporting evidence for a mucosa to serosa directed sodium "pump".

The negative serosal potential in the anterior chiton gut results from a large active movement of another cation in the opposite direction of sodium ion movement, that is, from the serosa to the mucosa, or of an anion in the same direction as sodium ion movement. There is, however, no evidence of any net movement of either potassium or chloride ions. Since the lumen of the chiton anterior gut is acidic with an average pH of 5.0 (85), and because the serosal compartment became basic with an average pH of 7.8 during each control period, there is some evidence for an active movement of hydrogen ions from the serosal to the mucosal compartment. Also, in non-everted gut segments, the gut sac contents, in this case the mucosal compartment, became more acidic (pH 6.4), the more proximal anterior intestinal segments attaining a lower pH than those more distally located. Other apparent evidence for such a hydrogen "pump" is the following:

1) In the partial sodium experiments, the pH increased (became more alkaline) in the serosal compartment and the potential increased in negativity.
2) Under anaerobic conditions, the pH decreased (became more acidic) in the serosal compartment and

the potential decreased in negativity.

3) Using ouabain, whether on the serosal side, the mucosal side, or on both sides simultaneously, the result was that the pH increased (became more alkaline) in the serosal compartment and the potential increased in negativity.

The above suggests that there is an energy requiring but ouabain insensitive "pump" which moves hydrogen ions from the serosal to the mucosal compartment, giving rise to a negative serosal potential. The magnitude of this potential is opposed by the movement of sodium ions in the opposite direction, but because of the nearly equal unidirectional fluxes of sodium, that is, an active flux from mucosa to serosa and a passive flux in the opposite direction, and the shorting effect of potassium and chloride ion movement, as opposed to the net flux of hydrogen ions into the mucosal compartment, the resulting negative serosal potential is maintained. A possible explanation of the increase in pH using ouabain and partial sodium is that either more ATP is available for the movement of hydrogen ions or there is a hydrogen-cation exchange mechanism which is stimulated by an inhibition of the sodium flux. The relative decrease in pH during anaerobic conditions could be due to a decreased pumping of hydrogen ions out of the serosal compart-

ment resulting in their accumulation and, thus, a decreased pH in relation to control. Also possibly reinforcing the results is the presence of the Pasteur effect (104).

Parsons and McHardy (122), Swallow and Code (150), and Sladen and Dawson (142) have suggested a hydrogen-sodium ion exchange in the anterior vertebrate intestine, resulting in a slightly acidic mucosal solution in the jejunum due to the secretion of hydrogen ions. This ability to secrete hydrogen ions diminishes aborally along the small intestine, the rise in pH causing a marked increase in the net absorption rate of water and electrolytes in the ileum (32, 150). Indeed, such a decreasing gradient of hydrogen ions along the intestinal tract of the chiton has been observed (85). However, the chiton is apparently in osmotic equilibrium with its environment (155) and the gut is not operational for the net absorption of water and electrolytes. Electrolyte balance has been shown to be a function of the gills in marine cephlapods (155), and it would be interesting to speculate that a similar function exists in the chiton.

Also of interest here is that the anterior chiton gut is not clearly delineated from the stomach by a spincter (85, 93, 156) as is the case in the vertebrate intestinal tract. Further, the epithelial tissue of the anterior chiton gut is histologically similar to that of the stomach

(85) and apparently is operational like that of the vertebrate stomach for the secretion of hydrogen ions into the intestinal lumen, providing an optimal environment for the function of digestive enzymes and for the preparation for nutrient absorption. Indeed, the chiton anterior intestine should possibly be considered as a specialized region of the stomach.

It is of interest also to note that although D-glucose, 3-O-methyl D-glucose, and the purines and pyrimidines thymine, guanine, and uracil (91) are actively transported in the anterior chiton gut, most absorption of organic nutrients, including D-galactose (104), those amino acids which have been tested (129), and some purines and pyrimidines, particularly thymine, guanine, and hypoxanthine (91), occurs in the posterior intestine, which is separated from the anterior by a sphincter. The differences in the types of nutrients absorbed may be a result of the differences in the mucosal ionic environments of the two gut regions.

The fluxes of sodium were in all cases greater in magnitude by a factor of almost two in the anterior intestine as opposed to the posterior intestine (unpublished data). These large and nearly equal fluxes of sodium across the anterior chiton gut suggest a function similar to that observed in the anterior vertebrate intestine by Visscher (154), Parsons and McHardy (122), and Code and Hindle (35). That is, the jejunum is unfitted for the net absorption of sodium and

rather serves the "equilibrating" function of maintaining the contents of the lumen in an isotonic state necessary for optimal sugar absorption.

Lawrence and Lawrence (104) established that the active transport of D-glucose and 3-O-methyl-D-glucose did indeed occur in the anterior intestine of the chiton, resulting in a net flux of sugar from the mucosal to the serosal compartment. The criteria for this active sugar transport was as follows:

1) The accumulation of the sugar occurs against a concentration gradient; i.e., S/M ratios > 1.
2) The transport depends upon metabolic energy; i.e., is inhibited by anoxia and low temperature.
3) Transport is inhibited by phlorizin, a known competitive inhibitor of actively transported sugars (unpublished data).
4) Transport is specific in that, of those sugars studied, only D-glucose and 3-O-methyl-D-glucose are actively transported.

The studies contained herein have shown that this active sugar transport in the anterior chiton gut is dependent upon the presence of sodium ion. 3-O-methyl-D-glucose, which is transported but not metabolized, behaved similarly to glucose except for a difference in magnitude which is probably due to either glucose metabolism or the

relative affinity of glucose to the carrier molecule. The evidence for the sodium dependent sugar transport is summarized as follows:

1) A decrease in sodium ion concentration (partial sodium experiments) caused a linear decrease in net sugar flux with both glucose and 3-O-methyl-glucose (see Figure 10).
2) A decrease in net sodium flux (partial sodium experiments) was correlated in a linear fashion with a decrease in net sugar flux with both glucose and 3-O-methyl-glucose (see Figure 12).
3) Ouabain, an inhibitor of active sodium transport, caused a corresponding significant decrease in the net flux of sugar.

Also indicative of a sodium requirement for sugar movement is that during anaerobic conditions, which causes decreased metabolic activity and thus inhibits the sodium "pump", there was a simultaneous significant decrease in the sugar flux.

This information seems to support a model for sugar transport similar to that proposed for the vertebrate intestinal tissue, which is the two stage mobile carrier hypothesis of Crane, consisting of 1) a phlorizin sensitive, sodium dependent energy independent entry into the epithelial cell and 2) an oxygen dependent energy dependent accumulation

step (39, 42).

In this model, the "uphill" movement of sugar into the cell depends upon the inward "downhill" gradient of sodium, which is effective because the rate of carrier movement is dependent upon the interaction with sodium. Once inside the cell, the sugar and sodium dissociate from the carrier. The sodium asymmetry is maintained by its removal by the sodium "pump" either at the luminal surface and/or at the basal membrane, where it results in an increase in potential. Intracellular potassium would be expected to interfere with the sodium interaction for outward movement of the carrier, decreasing the carriers affinity for the sugar and resulting in a greater rate of accumulation of sugar within the cell.

In the vertebrate intestine, the active absorption of sugars occurs only at the mucosal surface, specifically in the brush border. Recent work with the brush border has revealed a highly differentiated subcellular organelle with well developed digestive as well as absorptive functions (45, 46, 47, 51, 52, 82, 117, 153). Sixty Å knob-like structures have been found to protrude luminally from hamster brush border and to contain maltase and sucrase activity (90). Hexoses released from mucosal membrane digestion, then, are probably absorbed at or near the site of hydrolysis. Semenza et al. (140) have reported that sodium ion is also a requirement for optimal sucrase activity and provides a further ad-

vantage to the equilibrating function of the anterior intestine. The current model of intestinal sugar transport as given by Crane (39, 42) is summarized in Figure 24. Data obtained in the experiments described herein seem to suggest that a similar model for active carbohydrate transport exists in the anterior chiton gut since the presence of sodium ion is required for active sugar movement in these animals. However, instead of mucosal surface digestion, as in the vertebrate, the chiton possesses a specialized gland, the sugar gland, which releases carbohydrases into the esophagus (93, 156). These enzymes function optimally at a pH of 5.0 - 5.2, which is the observed pH of the anterior intestine, and result in the formation of glucose for the anterior absorption. Here, then, is an absorptive system which is basically similar to that in the vertebrate intestine and yet is unique to these animals.

The movement of other ions, particularly potassium and chloride, and water appears to be entirely passive in the anterior chiton gut. Similar results have been found in vertebrate tissue (157, 159). However, as previously mentioned, the anterior chiton gut is operational for the early digestion of nutrients and is not fitted for the net absorption of electrolytes and water. In fact, sodium chloride is in equilibrium across chiton epithelial tissue

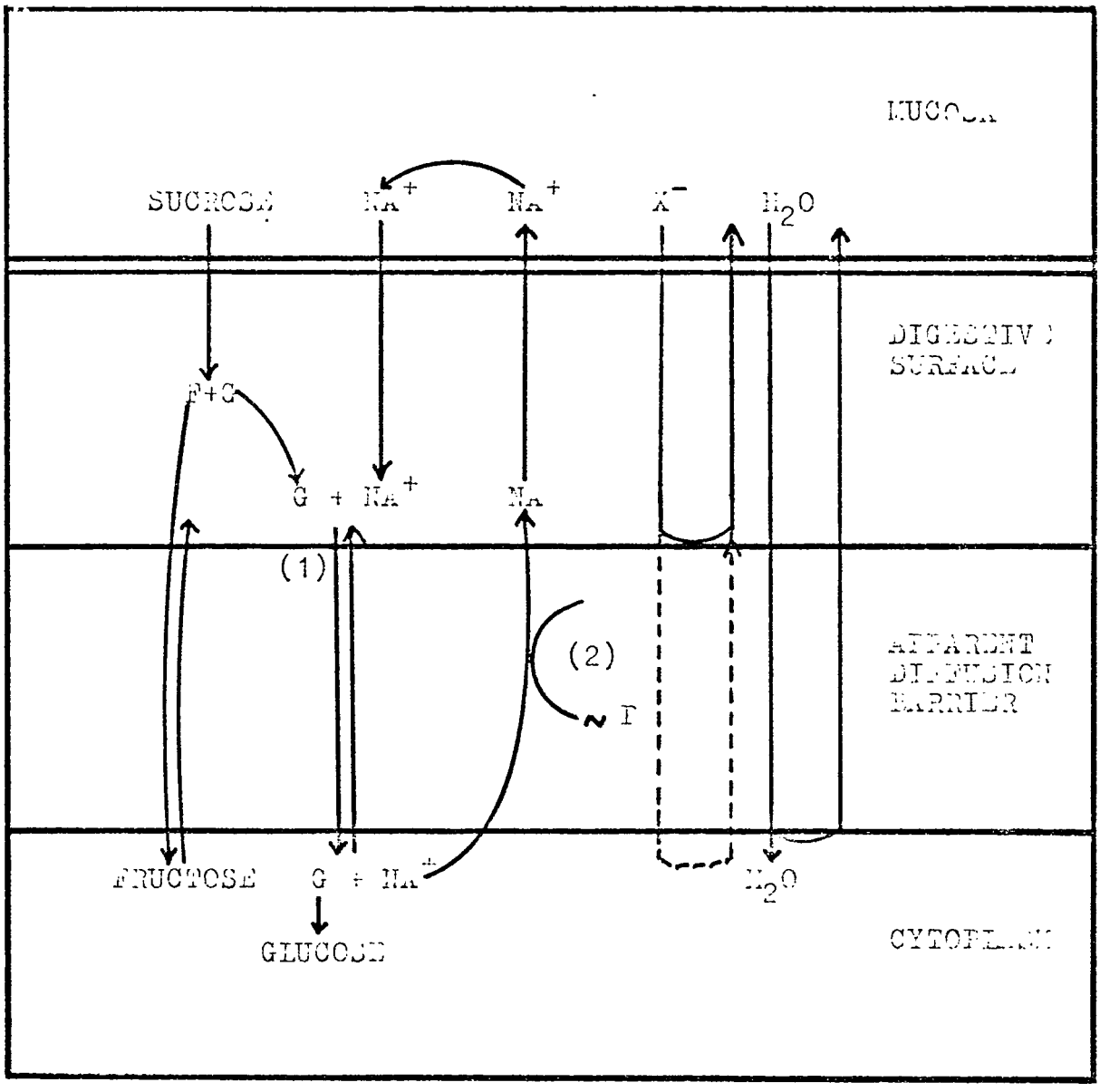


FIGURE 24 THE CURRENT MODEL OF CARBOHYDRATE HYDROLYSIS AND ACTIVE ABSORPTION IN THE BRUSH BORDER OF THE INTESTINAL EPITHELIAL CELL OF THE VERTEBRATE. FROM CRANE (39). (1) REPRESENTS SITE OF SPECIFICITY AND INHIBITION BY PHLORIZIN. (2) REPRESENTS SITE OF INHIBITION BY METABOLIC POISONS AND OUABAIN.

(155). Hence, the fluxes of water were equal in both directions across the gut tissue. Also, there was not net accumulation of potassium or chloride in either the serosal or mucosal compartment during any experimental period. The use of potassium free Ringer solution caused the potential to increase in negativity, indicating that potassium ion may 1) move oppositely to hydrogen ion, possibly in an exchange process, or 2) leak from the cell along its concentration gradient, creating a diffusion potential. Potassium free solution may also inhibit the sodium "pump", which is the case in vertebrate intestinal tissue (133). Chloride free Ringers caused the potential to decrease in negativity, suggesting that chloride might 1) move passively with the active transport of sodium from the mucosa to the serosa, since the same result is obtained by using sodium sulfate as a substitute in the vertebrate gut, the sulfate not being transported to the serosa along with the sodium and giving rise to a more positive (less negative) serosal potential, or 2) leak from the cell, creating a diffusion potential.

In conclusion, the results of these experiments can be seen graphically in Figure 25. In this proposed model for salt, water, and sugar transport in the anterior chiton intestine, the following points are indicated:

1) Sodium ion is actively transported from the mucosa to the serosa. This transport occurs at the basal membrane, is

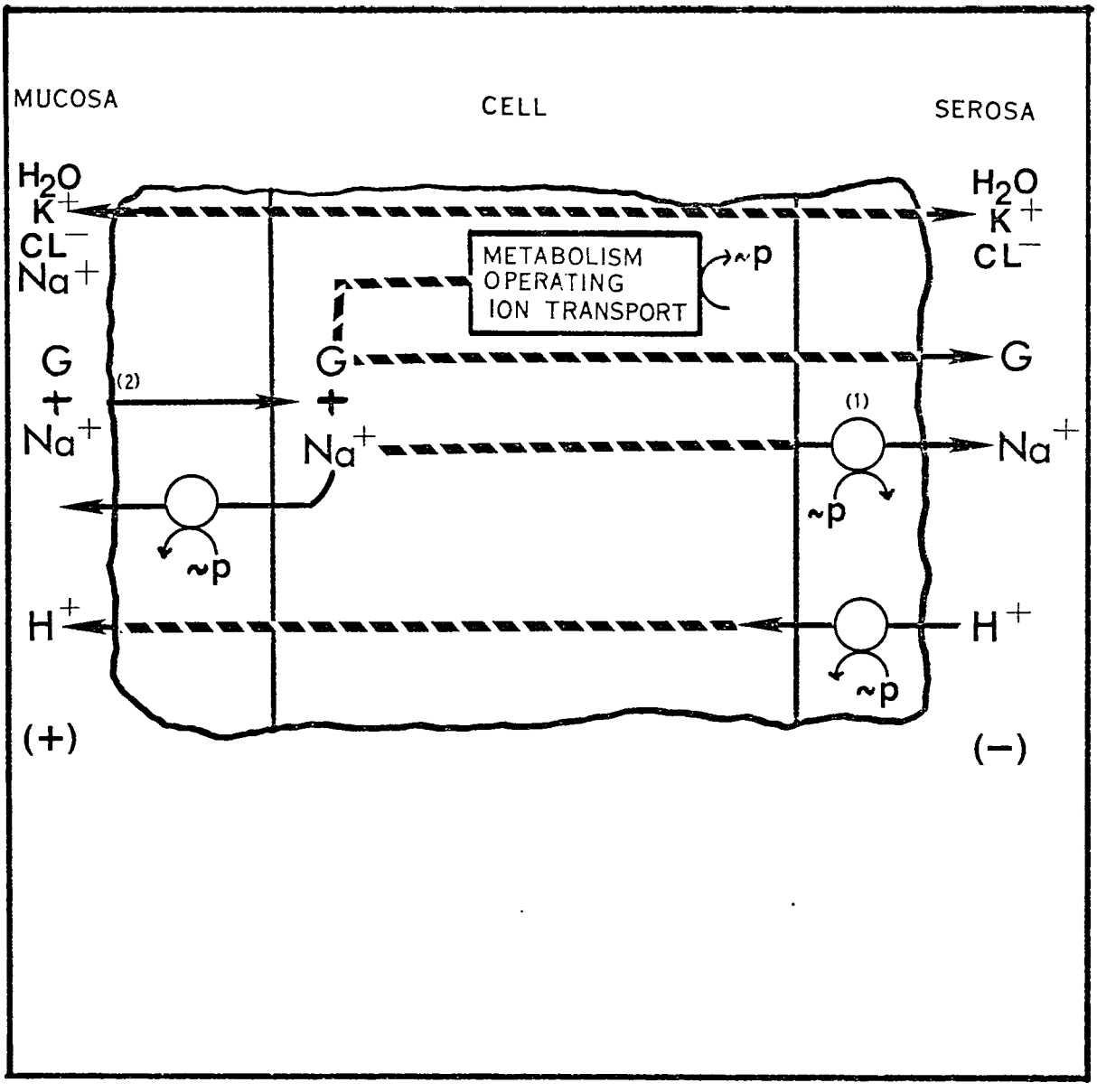


FIGURE 25 THE PROPOSED MODEL FOR SALT, WATER, AND CARBOHYDRATE TRANSPORT IN THE ANTERIOR INTESTINE OF C. stelleri. (1) REPRESENTS SITE OF INHIBITION BY OUABAIN. (2) REPRESENTS THE SITE OF SPECIFICITY.

ouabain sensitive, and is inhibited by anaerobic conditions.

2) The movement of potassium, chloride, and water is most likely passive.

3) The active transport of D-glucose and 3-O-methyl-D-glucose is dependent upon the presence of sodium ion.

4) The negative serosal potential across the anterior chiton gut and the acidic pH in the lumen appear to be the result of the active transport of hydrogen ion from the serosal to the mucosal compartment. The transport mechanism is ouabain insensitive but is inhibited by anaerobic conditions.

BIBLIOGRAPHY

1. Alvarado, F. D-xylose: a substrate for the process of sugar active transport by the small intestine. Experimentia. 20: 302, 1964.

2. Alvarado, F. Hypothesis for the interaction of phlorizin and phlovetin with membrane carriers for sugars. Biochim. Biophys. Acta. 135: 483, 1967.

3. Alvarado, F. Transport of sugars and amino acids in the intestine: evidence for a common carrier. Science. 151: 1010, 1966.

4. Alvarado, F., and R.K. Crane, Phlorizin as a competitive inhibitor of the active transport of sugars by hamster small intestine in vitro. Biochim. Biophys. Acta. 56: 170, 1962.

5. Alvarado, F. and R.K. Crane. Studies on the mechanism of intestinal absorption of sugars: Phenylglycoside transport and its relationship to phlorizin inhibition of the active transport of sugars by the small intestine. Biochim. Biophys. Acta. 93: 116, 1964.

6. Asano, T. Metabolic disturbances and short circuit current across the intestinal wall of rat. Am. J. Physiol. 207: 415, 1964.

7. Baker, R.D., G.W. Searle, and A.S. Nunn. Glucose and sorbose absorption at various levels of rat small intestine. Am. J. Physiol. 200: 301, 1961.

8. Baker, R.D., M.J. Wall, S. Watson, and J.L. Long. Transmural electrical potential across rat jejunum: effect of eversion and rapid temperature changes. Biochim. Biophys. Acta. 150: 649, 1968.

9. Barnett, J.E.G., W.T.S. Jarvis, and K.A. Munday. Structural requirements for active intestinal sugar transport: the involvement of H bonds at C #1 and C #6 of the sugar. Biochem. J. 109: 61, 1968.

10. Barry, R.J.C. Short-circuit current and solute transfer by rat jejunum. J. Physiol. 181: 410, 1965.

11. Barry, R.J.C. Transfer of glucose and fluid by different parts of the small intestine of the rat. J. Physiol. 157: 279, 1961.

12. Barry, R.J.C., S. Dikstein, J. Mattews, and D.H. Smyth, Potentials in the isolated intestine. J. Physicl. 155: 17, 1961.

13. Barry, R.J.C., S. Dikstein, J. Mattews, D.H. Smyth, and E.M. Wright. Electrical potentials associated with intestinal sugar transfer. J. Physiol. 171: 316, 1963.

14. Barry, R.J.C., J. Eggention, and D.H. Smyth. A neutral sodium pump and the hexose transfer potential in the rat small intestine. J. Physiol. 191: 72, 1967.

15. Barry, R.J.C., J. Eggenton, and D.H. Smyth. Sodium pumps in the rat small intestine in relation to hexose transfer and metabolism. J. Physiol. 204: 299, 1968.

16. Barry, R.J.C., J. Eggenton, D.H. Smyth, and E.M. Wright. Sodium concentration and hexose-dependent potentials in the small intestine. J. Physiol. 182: 40, 1965.

17. Barry, R.J.C., D.H. Smyth, and E.M. Wright. Short circuit current and solute transfer by rat jejunum. J. Physiol. 181: 410, 1965.

18. Benson, J.A. and A.J. Rampone. Gastrointestinal Absorption. Ann. Rev. Physiol. 28: 201, 1966.

19. Bentley, R. Amiloride: a potent inhibitor of sodium transport across a toad bladder. J. Physiol. 195: 317, 1968.

20. Berger, E.Y., G. Kanzaki, M.A. Homer, and J.M. Steele. Simultaneous flux of sodium into and out of the dog intestine. Am. J. Physiol. 196: 74, 1959.

21. Berger, E.Y., G. Kanzaki, and J.M. Steele. Simultaneous flux of potassium into and out of the dog intestine. Am. J. Physiol. 196: 1270, 1959.

22. Berger, E.Y., and J.M. Steele. The calculation of transfer rates in two compartment systems not in dynamic eqvilium. J. Gen. Physiol. 41: 1135, 1957.

23. Bihler, I. and S. Adamic. The effect of lithium on in-

testinal sugar transport. Biochim. Biophys. Acta. 135: 466, 1967.

24. Bihler, I. and R.K. Crane. Studies on the mechanism of intestinal absorption of sugars. Biochim. Biophys. Acta. 102: 423, 1962.

25. Booth, C.C. Sites of absorption in the small intestine. Fed. Proc. 26: 1583, 1967.

26. Budolfsen, S.E. The absorption of sodium chloride in the colon and the distal part of the small intestine. Acta. Physiol Scand. 32: 148, 1954.

27. Capraro, V. The sodium and glucose transport by in vitro intestinal preparations. Experimentia. 19: 347, 1963.

28. Clarkson, T.W. The transport of salt and water across isolated rat ileum. J. Gen. Physiol. 50: 695, 1967.

29. Clarkson, T.W., A.C. Cross, and S. Toole. Dependence on substrate of the electrical potential across the isolated gut. Nature. 191: 501, 1961.

30. Clarkson, T.W., A.C. Cross, and S. Toole. Electrical Potentials across isolated small intestine of the rat. Am. J. Physiol. 200: 1233, 1961.

31. Clarkson, T.W. and A. Rothstein. Transport of monovalent cations by isolated small intestine of the isolated small intestine of the rat. Am. J. Physiol. 199: 898, 1960.

32. Clarkson, T.W., A. Rothstein, and A.C. Cross. Transport of monovalent anious by isolated small intestine of the rat. Am. J. Physiol. 200: 781, 1961.

33. Clarkson, T.W. and S. Toole. Measurement of short-circuit current and ion transport across the ileum. Am. J. Physiol. 206: 658, 1964.

34. Code, C.F., P. Bass, G.B. McClary, Jr., R.L. Newnum, and A.L. Orvis. Absorption of water, sodium and potassium in small intestines of dog. Am. J. Physiol. 199: 281, 1960.

35. Code, C.F. and W. Hindle. Some differences between duodenal and ileal sorption. Am. J. Physiol. 203: 215, 1962.

36. Cooperstein, I.L. and S.K. Brockman. The electrical potential difference generated by the large intestine: its relation to electrolytes and water transfer. J. Clin. Invest. 38: 435, 1959.

37. Cooperstein, I.L. and A.M. Hogben. Ionic transfer across the isolated frog large intestine. J. Gen. Physiol. 42: 461, 1959.

38. Crane, R.K. Absorption of sugars. Handbook of Physiology. The Alimentary Canal. III. 1323-1351, 1968.

39. Crane, R.K. Hypothesis for mechanism of intestinal active transfer of sugars. Fed. Proc. 21: 891, 1962.

40. Crane, R.K. Intestinal absorption of sugars. Physiol. Rev. 40: 789, 1960.

41. Crane, R.K. Sodium dependent transport in the intestine and other animal tissues. Fed. Proc. 24: 1000, 1965.

42. Crane, R.K. Gastroenterology: structure & function of a digestive-absorptive surface. Fed. Proc. 28: 5, 1969.

43. Crane, R.K. Studies on the mechanism of the intestinal absorption of sugars: mutral inhibition, in vitro, between some actively transported sugars. Biochim. Biophys. Acta. 45: 477, 1960.

44. Crane, R.K. Uphill flow of sugar from intestinal epithelial cells induced by the reversal of the sodium gradient: its significance for the mechanism of sodium dependent acture transport. Biochim. Biophys. Res. Commun. 17: 481, 1964.

45. Crane, R.K. and A. Eichholz. Fractionation of membrane enzymes present in brush borders of hamster intestinal epithelial cells. Fed. Proc. 25: 656, 1966.

46. Crane, R.K. and A. Eichholz. Studies on the organization of the brush border in intestinal epithelial cells: tris-disruption of isolated hamster brush borders and density gradient separation of gractions. J. Cell Biol. 26: 687, 1965.

47. Crane, R.K., A. Eichholz, and J. Overton. Studies on the organization of the brush border in intestinal epithelial cells: II. Fine structure of fractions of tris-disrupted hamster brush borders. J. Cell Biol. 26: 693, 1965.

48. Crane, R.K., G. Forstner, and A. Eichholz. Studies on the mechanism of intestinal absorption of sugars: an effect of sodium concentration on the apparent michaelis constauts for intestinal sugar transport, in vitro. Biochim. Biophys. Acta. 109: 467, 1965.

49. Crane, R.K. and P. Mandlestram. The active transport of sugars by various preparations of hamster intestine. Biochim. Biophys. Acta. 45: 460, 1960.

50. Crane, R.K., D.B. McDougal, and K.D. Little. Studies on the mechanism of intestinal absorption of sugars: location of galactose concentration within the intestinal wall during active transport, in vitro.

51. Crane, R.K. and D. Miller. The digestive function of the epithelium of the small intestine: an intracellular locus of disacchavide and sugar phosphate ester hydrolases. Biochim. Biophys. Acta. 52: 281, 1961.

52. Crane, R.K. and D. Miller. The digestive function of the epithelium of the small intestine: an intracellular locus of disacchavide and sugar phosphate ester hydrolases. Biochim. Biophys. Acta. 52: 281, 1961.

53. Crane, R.K. and T.H. Wilson. In vitro method for the study of the rate of absorption of sugars. J. Appl. Physiol. 12: 145, 1958.

54. Csaky, T.Z. Link between active transport of elctrolytes and non-electrolytes. Fed. Proc. 22: 3, 1963.

55. Csaky, T.Z. Significance of sodium in active intestinal transport of non-electrolytes. Am. J. Physiol. 201: 999, 1961.

56. Csaky, T.Z. and G.W. Fernald. Absorption of 3-O-methyl glucose from the intestine of the frog, Rana piprens. Am. J. Physiol. 198: 445, 1960.

57. Csaky, T.Z. and Y. Hara. Inhibition of active intestinal sugar transport by digitalis. Am. J. Physiol. 209: 467, 1965.

58. Csaky, T.Z., Hartzog and G.W. Fernald. Effect of digitalis on active intestinal sugar transport. Am. J. Physiol. 200: 459, 1961.

59. Csaky, T.Z. and P.M. Ho. Effect of mucosal potassium on the intestinal glucose transport pelveger. Arch. Ges. Physiol. 291: 63, 1966.

60. Csaky, T.Z. and P.M. Ho. The effect of potassium on the intestinal transport of glucose. J. Gen. Physiol. 50: 113, 1966

61. Csaky, T.Z. and M. Thale. Effect of ionic environment on intestinal sugar transport. J. Physiol. 151: 59, 1960.

62. Curran, P.F. Ion transport in intestine and its coupling to other transport processes. Fed. Proc. 24: 993, 1965.

63. Curran, P.F. Sodium, chloride, and water transport by rat ileum in vitro. J. Gen. Physiol. 43: 1139, 1960.

64. Curran, P.F. Water absorption from intestine. Am. J. Clin. Nutr. 21: 781, 1968.

65. Curran, P.F., A.M. Goldner, and S.G. Schultz. Sodium and sugar fluxes across the mucosal border of rabbit ileum. J. Gen. Physiol. 53: 367, 1969.

66. Curran, P.F. and J.R. MacIntosh. A model system for biological water transport nature. 193: 347, 1962.

67. Curran, P.F. and G.F. Schwartz. Sodium, chloride, and water transport in rat colon. J. Gen. Physiol. 43: 555, 1960.

68. Curran, P.F. and A.K. Solomon. Ion and water fluxes in the ileum of rats. J. Gen. Physiol. 41: 143, 1958.

69. D'Angostino, A., W. Leadbetter, and W. Schwartz. Alterations in the ionic composition of isotonic saline solutions instilled into the colon. J. Clin. Invest. 32: 444, 1953.

70. Despopoulos, A. Glucose transport in hamster intestine: inhibition by glycosides. Am. J. Physiol. 211: 1329, 1966.

71. Detheridge, J.F., J. Matthews, and D.H. Smyth. The effect of inhibitors on intestinal transfer of glucose and fluid. J. Physiol. 183: 369, 1966.

72. Devroede, G.J. and S.F. Phillips. Conservation of electrolytes and water by the human colon. J. Lab. Clin. Med. 70: 990, 1967.

73. Diamond, J.M. and H. McD. Tormey. Role of long extracellular channels in fluid transport across epithelia. Nature. 210: 817, 1966.

74. Diamond, J.M. and J. McD. Tormey. Studies on the structural basis of water transport across epithelial membranes. Fed. Proc. 25: 1458, 1966.

75. Dubois, M., K.A. Giles, J.K. Hamilton, P.A. Rebers, and F. Smith. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350, 1956.

76. Edmonds, C.J. The gradient of electrical potential difference and of sodium and potassium of the gut contents along the caecum and colon of normal and sodium depleted rats. J. Physiol 193: 571, 1967.

77. Edmonds, C.J. Transport of potassium by colon of normal and sodium depleted rats. J. Physiol. 193: 603, 1967.

78. Edmonds, C.J. Transport of sodium and secretion of potassium and bicarbonate by the colon of normal and sodium depleted rats. J. Physiol. 193: 589, 1967.

79. Edmonds, C.J. and J. Marriott. Electrical potential and short-circuit of an in vitro preparation of rat colon mucosa. J. Physiol. 194: 479, 1968.

80. Edmonds, C.J. and J. Marriott. Factors influencing the electrical potential across the mucosa of the rat colon. J. Physiol. 194: 457, 1968.

81. Edmonds, C.J. and D.E. Nielsen. Transmembrane electrical potential differences and ionic composition of mucosal cells of rat colon. Acta. Physiol. Scand. 72: 338, 1968.

82. Eichholz, A. Structural and functional organization of the brush border of intestinal epithelial cells. Biochim. Biophys. Acta. 135: 475, 1967.

83. Faust, R.G., M.G. Leadbetter, R.K. Plenge, and A.K. McCaslin. Active sugar transport by the small intestine. J. Gen. Physiol. 52: 482, 1968.

84. Fernie, S.M., S.L. Hart, and J.A. Nissim. Glucose binding of homogenates of intestinal mucosa. Nature. 213: 985, 1967.

85. Fretter, V. The structure and function of the alimentary canal of some species of polyplacophora (mollusca). Trans. Roy. Soc. Edin. 59: 119, 1937.

86. Gilles-Baillien, M., and E. Schoffeniels. Bioelectrical potentials in the intestinal epithelium of the Greek tortoise. Comp. Biochem. Physiol. 23: 95, 1967.

87. Gilles-Baillien, M., nad E. Schoffeniels. Origin of bioelectrical potentials of intestinal epithelium of the Greek tortoise. Biochim. Biophys. Acta. 53: 537, 1961.

88. Gilman, A., and E. Koelle. Ion transport in the gut. Circulation. 21: 948, 1960.

89. Gilman, A., E. Koelle, and J.M. Ritchie. Transport of potassium ions in the rat intestine. Nature. 197: 1210, 1963.

90. Gray, G.M. Malabsorption of Carbohydrates. Fed. Proc. 26: 1415, 1967.

91. Hanisch, M.E. Absorption of the natural purines and pyrimidines by the gut of the chiton, cryptochiton stelleri. University of Houston Press, 1970.

92. Huang, K.C., and W.R. Rout. Intestinal transport of sugar and aromatic amino acids in killifish, Fundulus heteroclitus. Am. J. Physiol. 212: 799, 1967.

93. Hyman, L.H. The Invertebrates. Mollusca I. Vol. VI. McGraw-Hill Co. New York. 1967. 70-142.

94. Inglefinger, F.J. and J.S. Fordtran. Absorption of water, electrolytes, and sugars from the human gut. Handbook of Physiology. The Alimentary Canal. III. 1457-1490, 1968.

95. Jackson, M.J. and D.H. Smyth. Role of Sodium in the intestinal active transport of organic solutes. Nature. 219: 388, 1968.

96. Jacobs, F.A., R.C. Flaa, D. Person, and T.J. Pacholl. Influence of solute concentration on intestinal absorption. Fed. Proc. 20: 137, 1961.

97. Kinter, W.B. and T.H. Wilson. Autoradiographic study of sugar and amino acid absorption by everted sacs of hamster intestine. J. Cell. Biol. 25: 19, 1965.

98. Klahr, S., C. Kantrow, and N.S. Bricker. Effect of metabolic inhibitors and of strophanthin on sodium transport and metabolism of the isolated turtle bladder. Comp. Biochem. Physiol. 33: 773, 1970.

99. Kohn, P.G., D.H. Smyth, and E.M. Wright. Effect of dipeptides and disaccharides on the electrical potential across the rat small intestine. J. Physiol. 185: 47, 1966.

100. Krawitt, E.L. In vivo calcium transport by rat small intestine. Am. J. Physiol. 214: 232, 1968.

101. Landau, B.R., L. Bernstein, and T.H. Wilson. Hexose transport by hamster intestine in vitro. Am. J. Physiol. 203: 237, 1962.

102. Lauterbach, F. The effect of cariotonic absorption of actively transported or diffusing substance and concentration on transport of sodium. Biochim. Biophys. Acta. 135: 256, 1967.

103. Lauterbach, F. The effect of cardiotonic steroids on the intestinal absorption of actively transported and diffusing substances and on their relationship to sodium concentration and sodium transport. Biochim. Biophys. Acta. 135: 273, 1967.

104. Lawrence, A.L. and D.C Lawrence. Sugar absorption in the intestine of the chiton, Cryptochiton stelleri. Am. Zoologist. 6: 114, 1966.

105. Lawrence, D.C., A.L. Lawrence, M.L. Greer, and D. Mailman. Intestinal absorption in the sea cucumber, Stichopus parvimensis. Comp. Biochem. Physiol. 20: 619, 1966.

106. Lawrence, A.L. and D.S. Mailman. Electrical potentials and ion concentrations across the gut of Cryptochiton stelleri. J. Physiol. 193: 535, 1967.

107. Levin, R.J. Transmural potentials across the small and large intestine of the bullfrog Rana catesbeiana. Proc. Soc. Exptl. Biol. Med. 121: 1033, 1966.

108. Levinson, R.A. Absorption of sodium, chloride, water, and simple sugars in rat small intestine. Am. J. Physiol. 211: 939, 1966.

109. Lyon, I. and R.K. Crane. Studies on in vitro transmural potentials in relation to intestinal absorption. II. An effect of ouabain on glucose dependent increment of transmural potential of rat small intestine. Biochim. Biophys. Acta. 126: 146, 1966.

110. Lyon, I. and R.K. Crane. Studies on in vitro transmural in relation to intestinal absorption. III. Potassium inhibition of sodium dependent transmural potential of rat small intestine. Biochim. Biophys. Acta. 135: 61, 1967.

111. Lyon, I. Studies on transmural potentials in vitro in relation to intestinal absorption. IV. Phlorizin - sugar interactions in rat gut. Biochim. Biophys. Acta. 135: 496, 1967.

112. Maffley, H. and I.S. Edelman. Relationship of carbohydrate metabolism to sodium transport. Fed. Proc. 20: 137, 1961.

113. Matthews, J. and D.H. Smyth. Two stage transfer of glucose by the intestine. J. Physiol. 154: 63, 1960.

114. McDougal, D.B., K.D. Little, and R.K. Crane. Studies on the mechanism of intestinal absorption of sugars. Biochim. Biophys. Acta. 45: 483, 1960.

115. Nelson, R.A. and R.J. Beargie. Relationship between sodium and glucose transport in canine jejunum. Am. J. Physiol. 148: 83, 1959.

116. Newey, H., B.J. Parsons, and D.H. Smyth. The site of action of phlorizin in inhibiting intestinal absorption of glucose. J. Physiol. 148: 83, 1959.

117. Newey, H., P.A. Sanford, and D.H. Smyth. Location of function in the intestinal epithelium cell in relation to carbohydrate absorption. J. Physiol. 168: 423, 1963.

118. Newey, H., P.A. Sanford, and D.H. Smyth. Some effects of ouabain and potassium on transport and metabolism in rat small intestine. J. Physiol. 194: 237, 1968.

119. Nutbourne, D.M. The effect of small hydrostatic pressure gradients on the rate of active sodium transport across isolated living frog skin membranes. J. Physiol. 195: 1, 1968.

120. Olsen, W.A. and F.J. Inglefinger. The role of sodium in intestinal absorption in man. J. Clin. Invest. 47, 1133, 1968.

121. Parsons, D.S. Salt and water absorption by intestinal tract. Brit. Med. Bull. 23: 252, 1967.

122. Parsons, D.S. and G.J.R. McHardy. The absorption of water and salt from the small intestine of the rat. Quart. J. Exptl. Physiol. 42: 33, 1957.

123. Parsons, D.S. and C.R. Patterson. Fluid and solute transport across rat colonic mucosa. Quart. J. Exptl. Physiol. 50: 220, 1965.

124. Parsons, D.S. and J.S. Prichard. Hydrolysis of disaccharides during absorption by the perfused small intestine of amphibia. Nature. 208: 1097, 1965.

125. Phillips, S.F. and C.F. Code. Sorption of potassium in the small and large intestine. Am. J. Physiol. 211: 607, 1966.

126. Phillips, R.A., A.H.G. Love, T.G. Mitchell, and E.M. Neptune. Cathartics and the sodium pump. Nature. 206: 1367, 1965.

127. Ponz, F. The active transport of sugars through the intestine and its relation to the sodium ion. Arg. Portug. Bioquim. 7: 93, 1964.

128. Powell, D.W. and S.J. Malawer. Relationship between water and solute transport from isosmotic solutions by rat intestine in vivo. Am. J. Physiol. 215: 49, 1968.

129. Puddy, R.E. Ion and carbohydrate absorption in the posterior intestine of Cryptochiton stelleri. University of Houston Press, 1970.

130. Rossi, S., C. Lippe, and V. Capraro. The influence of sodium transport on glucose transport by an in vitro intestinal preparation. Experimentia. 18: 325, 1962.

131. Swada, M. and T. Asano. Effects of metabolic disturbances on potential difference across intestinal wall of rat. Am. J. Physiol. 204: 105, 1963.

132. Schachter, D. and J.S. Britten. Active transport of non-electrolytes and the potential gradients across intestinal segments in vitro. Fed. Proc. 20: 137, 1961.

133. Schultz, S.G. and P.F. Curran. Intestinal absorption of sodium chloride and water. Handbook of Physiology. The Alimentary Canal. III. 1245-1275, 1969.

134. Schultz, S.G., P.F. Curran, and A.M. Goldner. Sodium and sugar fluxes across mucosal border of rabbit ileum. J. Gen. Physiol. 53: 362, 1969.

135. Schultz, S.G., P.F. Curran, and E.M. Wright. Interpretation of hexose-dependent electrical potential differences in small intestine. Nature. 214: 509, 1967.

136. Schultz, S.G. and R. Zalusky. Ion transport in isolated rabbit ileum: short-circuit current and sodium fluxes. J. Gen. Physiol. 47: 567, 1964.

137. Schultz, S.G. and R. Zalusky. The interaction between active sodium transport and active sugar transport in the isolated rabbit ileum. Biochim. Biophys. Acta. 71: 503, 1963.

138. Schultz, S.G. and R. Zalusky. Transmural potential difference, short-circuit current and sodium transport in isolated rabbit ileum. Nature. 198: 894, 1963.

139. Schultz, S.G., R. Zalusky, and A.E. Gass. Ion transport in isolated rabbit ileum: chloride fluxes. J. Gen. Physiol. 48: 375, 1964.

140. Semenza, G., R. Tosi, M.C. Vallotton-delachaux, and E. Mülhaupt. Sodium activation of human intestinal sucrase and its possible significance in the enzymatic organization of brush borders. Biochim. Biophys. Acta. 89: 109, 1964.

141. Skou, J.C. Enzymatic basis for active transport of sodium and potassium across cell membranes. Physiol. Rev. 45: 596, 1965.

142. Sladen, G.E. and A.M. Dawson. Effect of bicarbonate on sodium absorption by the human jejunum. Nature. 218: 267, 1968.

143. Smith, M.W. Sodium-glucose interactions in the goldfish intestine. J. Physiol. 182: 559, 1966.

144. Smith, M.W. The *in vitro* absorption of water and solutes from the intestine of the goldfish, *Carassius auratus*. J. Physiol. 175: 38, 1964.

145. Smyth, D.H. and R. Whittman. Membrane transport in relation to intestinal absorption. Brit. Med. Bull. 23: 231, 1967.

146. Smyth, D.H. and E.M. Wright. Streaming potentials in the rat small intestine. J. Physiol. 172: 61, 1964.

147. Stern, B.K. and W.F. Jensen. Active transport of glucose by suspensions of isolated rat intestinal epithelial cells. Nature. 209: 789, 1966.

148. Stirling, C.E. High-resolution radioautography of phlorizin-^{3}H in rings of hamster intestine. J. Cell Biol. 35: 605, 1967.

149. Stirling, C.E. and W.B. Kinter. High-resolution radioautography of galactose-^{3}H accumulation in rings of hamster intestine. J. Cell. Biol. 35: 585, 1967.

150. Swallow, J.H. and C.F. Code. Intestinal transmucosal fluxes of bicarbonate. Am. J. Physiol. 212: 717, 1967.

151. Taylor, A.E., E.M. Wright, S.G. Schultz, and P.F. Curran. Effect of sugars on ion fluxes in intestine. Am. J. Physiol. 214: 836, 1968.

152. Tidball, C.S. Active chloride transport during intestinal secretion. Am. J. Physiol. 200: 309, 1961.

153. Ugolev, A.M., N.N. Ieusuitora, N.M. Timofeeva, and I.N. Fediushina. Location of hydrolysis of certain disaccharides and peptides in the small intestine. Nature. 202: 807, 1964.

154. Visscher, M.B., R.H. Varco, C.W. Carr, R.B. Dean, and D. Erickson. Sodium movements between the intestinal lumen and the blood. Am. J. Physiol. 141: 488, 1944.

155. Wilbur, K.M. and C.M. Yonge, ed. Physiology of Mollusca. Vol. I. Academic Press. New York and London. 283-308, 1964.

156. Wilbur, K.M. and C.M. Yonge, ed. Physiology of Mollusca. Vol. II. Academic Press. New York and London. 59-61, 77, 1964.

157. Wilson, T.H. Intestinal Absorption. W.B. Saunders and Co. Philadelphia and London. 263, 1962.

158. Wilson, T.H., B.R. Landau, and Jorgensen. A common pathway for sugar transport in hamster intestine. Am. J. Physiol. 200: 111, 1961.

159. Wiseman, G. Absorption from the intestine. Academic Press. London and New York. 564, 1964.

160. Wright, E.M. Diffusion potentials across the small intestine. Nature. 212: 189, 1966.

161. Wright, E.M. The origin of the glucose dependent increase in the potential difference across the tortoise small intestine. J. Physiol. 185: 486, 1966.