RESORPTION OF PHOSPHATE, CALCIUM, AND MAGNESIUM IN THE *IN VITRO* LOCUST RECTUM

by

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ABSTRACT

The ability of the locust rectum *in vitro* to resorb Ca^{++}, Mg^{++}, and PO_4 was studied. The rectum has a low permeability to Ca^{++}, but Ca^{++} is not accumulated against concentration differences. Mg^{++} was not accumulated in the basal compartment of the rectal sac even when Mg^{++} concentration gradients were favourable for net diffusion. Phosphate was found to be accumulated by the rectum against a three-fold concentration difference.

The rectal tissue incorporated inorganic phosphate into organic forms, but phosphorus transferred into the basal compartment was found to be in the form of inorganic phosphate. Uptake into the basal compartment can be described by Michaelis-Menton saturation kinetics. The resorption of water by the rectum did not increase (by solute drag) the amount of PO_4 accumulated in the basal compartment, except at very high PO_4 concentrations in the apical bathing medium. Metabolic poisons such as KCN and IAA inhibited PO_4 accumulation in the basal compartment but did not inhibit PO_4 entry into the tissue from the apical bathing medium. Arsenate, a competitive inhibitor of PO_4 uptake in other systems, inhibited PO_4 entry into the tissue.

A mechanism for PO_4 uptake into the tissue is proposed.

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LIST OF ABBREVIATIONS

B/A	basal/apical side
µl	microliter
ml	milliliter
nM	nanomoles
mM	millimoles
P.E.	polyethylene tubing
mOsm	milliosmoles
mV	millivolts
hr	hour
mg	milligram
D.W.	dry weight
S.E.	standard error
P.D.	potential difference
K_t	substrate concentration for half-maximal unidirectional flux
V_{max}	maximal rate of unidirectional flux

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INTRODUCTION

The role of ionic regulation, osmotic regulation, and metabolic waste removal falls mainly upon the Malpighian tubule-rectal complex of most terrestrial insects [reviewed by Maddrell, 1971]. The process of Malpighian tubule secretion and rectal resorption is analogous to the glomerular filtration and tubular resorption of the vertebrate kidney [Phillips, 1965].

The Malpighian tubules produce a primary fluid that is nearly iso-osmotic with, but not necessarily having the same concentrations or proportions of ions and organic molecules as that of the hemolymph [Ramsay, 1956; Phillips, 1964c; Maddrell and Klunsuwan, 1973]. The main driving force responsible for the production of primary fluid is the active pumping of K^+ and/or Na^+ into the tubule lumen. This transport of ions and the accompanying flow of water set up electrochemical gradients across the tubule which favor movement of other substances into the tubule lumen [Maddrell, 1971]. Small organic molecules (sugars, some amino acids) may be brought into the lumen by solvent-solute or solute-solute drag, while large organic molecules (organic acids, nitrogenous wastes) may be actively secreted [Maddrell, 1971].

The rate of fluid secretion by the Malpighian tubules of most insects seems to be directly related to the K^+ concentration of the basal bathing medium [Ramsay, 1956; Berridge, 1968; Maddrell and Klunsuwan, 1973]. K^+ and Na^+ produce the highest rate of secretion,

but anions also influence secretion. The rate of secretion is inversely related to the hydrated radius of the secreted anion [Berridge, 1969]. Phosphate is an exception since it can support a high rate of secretion completely disproportionate to its hydrated radius [Berridge, 1969].

The primary fluid, containing both metabolic wastes and physiologically important solutes, flows out of the Malpighian tubules into the midgut-hindgut junction where it then flows to the rectum via the anterior part of the hindgut. The concentration of the constituents of the primary fluid are altered to a minor degree by the distal portions of the Malpighian tubules in some insects and by the anterior portion of the hindgut of others. The bulk of the resorption, however, occurs in the rectum of most insects [Ramsay, 1971].

Phillips [1964c] found the rectum capable of resorbing over 95% of the primary fluid. Many of the hemolymph constituents are actively resorbed [monovalent ions -- Phillips, 1964b, c; amino acids -- Balshin and Phillips, 1971; water -- Phillips, 1964a]. The rectal epithelium is covered on the apical side by a chitinous cuticle. This cuticle acts as a molecular sieve that prevents the uptake of large molecules including metabolic wastes [Phillips and Dockrill, 1968]. Recycling of fluid thus results in the accumulation of metabolic wastes in the rectum due to the action of the intima.

Since the Malpighian tubule-rectal complex also controls osmotic pressure and ionic concentrations of the hemolymph, the rate of Malpighian tubule secretion is closely correlated with rectal reabsorption rates [Phillips, 1964c]. Phillips [1964 a, b, c] studied Na^+, K^+, Cl^-, and water secretion and subsequent resorption in the

locust and found that if the balance of ions in the hemolymph was perturbed, the relative secretion rates of ions by the Malpighian tubules will be out of phase with their relative rates of reabsorption in the rectum; thus ions in excess will accumulate in the rectum and ions in short supply will be completely reabsorbed. Water was found to be reabsorbed rapidly under all experimental situations.

Na^+, K^+, Cl^-, and water have been the focus of attention in most of the investigations to date. PO_4, Mg^{++}, and Ca^{++} have been less intensely studied, and then only from the point of view of secretion by the Malpighian tubules.

Since PO_4 can affect the rate of production of primary Malpighian fluid [Maddrell, and Klunsuwan, 1973] and is also a metabolic regulator [Levinson, 1971], it would seem that the locust should be able to regulate the secretion and reabsorption of PO_4 to some extent. Speight [1967] and Maddrell and Klunsuwan [1973] found that PO_4 was more concentrated in the primary Malpighian fluid than in the hemolymph or in the external bathing medium. Maddrell and Klunsuwan [1973] found that in vitro preparations of locust Malpighian tubules were capable of producing a lumen to hemolymph PO_4 ratio of 1.71 to 1.0. Berridge [1969] found that 1.0 mM/l of arsenate, when added to incubation media containing PO_4 as the most abundant anion, greatly inhibited primary fluid production by *Calliphora* Malpighian tubules. Arsenate, aside from being a metabolic poison, is also a competitive inhibitor for PO_4 carrier systems [Rothstein, 1963; Levinson, 1971]. This led Maddrell and Klunsuwan [1973] and Berridge [1969] to suppose that PO_4

translocation may be facilitated by some type of carrier process.

The divalent cations Mg^{++} and Ca^{++} do not seem to be actively secreted by the Malpighian tubules. Ramsay [1956], while working with *in vitro* preparations of the Malpighian tubules of the stick insect, found Mg^{++} and Ca^{++} to be excreted at 1/10 to 1/5 of the concentration of the two ions in the bathing medium. Concentrations of Mg^{++} and Ca^{++} above 10 mM/l in the bathing media inhibited the rate of fluid secretion by the Malpighian tubules [Berridge, 1968]. The hemolymph, however, contains Ca^{++} and Mg^{++} ions at concentrations more than forty times greater than in mammalian blood [Clark and Craig, 1953]. The same authors concluded that much of the Ca^{++} and Mg^{++} is not physiologically active in the hemolymph and is probably sequestered by proteins and other organic compounds. Calcium carbonate is found in the Malpighian tubules of many insects [Clark, 1958], and is usually eliminated as a suspension of granules. The high concentrations of potentially physiologically active Mg^{++} and Ca^{++} in the hemolymph may not necessitate the reclamation of these ions by the rectum, hence excretion as precipitates.

The present study was undertaken with two main objectives in mind. These were:

1. To determine if Ca^{++}, Mg^{++}, and PO_4 are reabsorbed in the locust rectum, as are monovalent ions, and to compare rates of resorption with rates of secretion of these ions by the Malpighian tubules. An *in vitro* preparation, which is known to transport monovalent ions and water at rates comparable to those *in vivo*, was used because of its greater potential for the study of rates under various controlled conditions.

2. To find the mechanism (diffusion, facilitated diffusion, active transport) of net absorption of any of these ions.

After doing a brief study on the uptake of the three ions, I concentrated on the study of PO_4 since it seemed the most promising as far as accumulation was concerned. The bulk of the thesis is concerned with the mechanism and localization of the uptake system for PO_4 in the locust rectum *in vitro*.

MATERIALS AND METHODS

Animals

Adult male and female locusts (*Schistocerca gregaria* Forskal), from a colony maintained at 28°C and 60% relative humidity, and fed a diet of bran and lettuce, were used in all experiments. Locusts were used from one to five weeks after their final molt. Locusts were starved for 36 hours before an experiment to permit excretion of feces, thus facilitating cannulation of the rectum.

Preparation of the Everted Rectal Sacs

Cannulated, everted rectal sacs were prepared according to the method of Goh [1971]. The locust, after being secured on its side upon a plasticine block beneath a dissecting microscope, was anaesthetized with a mixture of CO_2 and ether. A U-shaped, dorsal-lateral incision was made from the fifth to the seventh abdominal segment. The resulting flap of cuticle was pinned back revealing the rectum with associated tracheae and fat bodies. These structures were carefully dissected away from the rectum. The slightly flared end of a cannula (4 cm of P.E. 90 tubing) was inserted through the anus and pushed forward until the flared end was just anterior to the rectal pads. A ligature of clean human hair was used to secure the rectum to the flared end of the cannula. The hindgut anterior to the cannula was severed from the

rectum, and the cannula was slowly withdrawn through the anus until the ends of the rectal pads emerged. The everted rectum was then severed from the body of the locust.

The external surface of the cannulated rectum was rinsed in 10 ml of Ringer solution (Table 1), and the internal surface was flushed with about 0.25 ml of Ringer solution injected through P.E. 10 tubing attached to a syringe. This procedure removed faecal material or hemolymph adhering to the rectum. Another hair was used to ligature the open end of the rectum to form a sac attached to a cannula. A syringe attached to P.E. 10 tubing was used to remove any fluid in the sac. The head of a bent insect pin was inserted into the end of the cannula, thus forming a hook by which the rectal sac could be suspended during incubation and weighing, and preventing evaporation of water. The sac was blotted dry with filter paper and weighed.

Twenty ul of Ringer solution were injected into the sac with a 'Hamilton Syringe' and the sac was reweighed to obtain the weight at zero-time of incubation.

Occasionally a few recta were checked for leakage by leaving them overnight in an amaranth solution. Since amaranth cannot penetrate the intima [Phillips and Dockrill, 1968] its appearance in a sac would indicate damage to the rectum. No leakage was observed.

Incubation Procedure

The rectal sacs were incubated in a water bath at a constant temperature (30 ± 0.5°C), and were aerated with a mixture of 95% O_2 and

5% CO_2 (Figure 1). The recta were incubated in 3 - 25 ml of apical (external) bathing medium. Twenty ul of media were used to bath the basal (internal) surface of the rectal preparation. For experiments of short duration, sacs were pre-incubated for one hour to let them achieve a steady state [Goh, 1971; Balshin, 1973]. At the end of one hour the contents of the sacs were removed and 20 ul of fresh basal bathing media were added. The sac was then reweighed and transferred to fresh apical bathing medium.

To determine if any water was taken up by swelling of the rectal tissue, weights of the empty sacs at zero and final time (t) were compared. If any changes occurred, the difference was either added or subtracted from the amount of water taken up in the basal compartment. In this way it was possible to determine if net water transfer across the rectal sac occurred during the experiment.

In all experiments, the weighing procedure consisted of removing the sac from the bathing media, blotting it dry (with Whatman No. 1 filter paper), then weighing it on a 200 mg 'August-Sauter' torsion balance.

Composition of Bathing Media

A Na^+-Ringer (Table 1) was used as the basal bathing medium and a K^+-Ringer (Table 1) was used as the apical bathing medium in most experiments. The concentration of the ion under study was altered in some experiments; such changes are noted in describing the results. The two Ringers were used in order to roughly approximate in vivo

Figure 1 Diagram of experimental set-up to study rectal absorption *in vitro*.

The everted rectal sac, containing 20 ul of experimental media in the basal compartment was incubated in 3-5 ml of apical medium. Mixing and aeration was achieved by bubbling 95% O_2 and 5% CO_2 through apical medium.

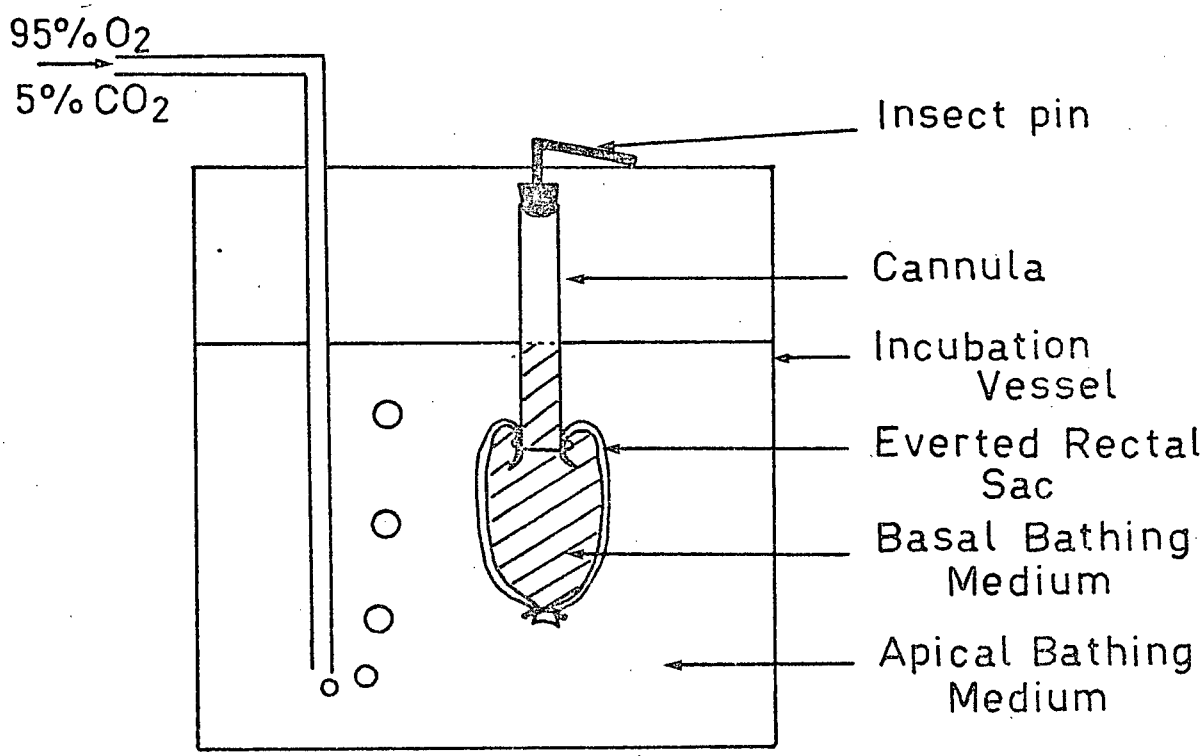

95% O_2
5% CO_2
Insect pin
Cannula
Incubation Vessel
Everted Rectal Sac
Basal Bathing Medium
Apical Bathing Medium

TABLE 1

Composition of Bathing Media

Constituent	Concentration (gm/l)	
	Na^+-Ringer	K^+-Ringer
NaCl	9.82	0.376
KCl	0.48	12.52
$MgCl_2 \cdot 6H_2O$	0.73	0.73
$CaCl_2 \cdot 2H_2O$	0.315	0.315
Dextrose	3.0	3.0
$NaHCO_3$	0.18	0.18
$NaH_2PO_4 \cdot 1H_2O$	0.84	0.84
Sucrose*	126.17	168.80

*Only added to external media when net water movement was to be prevented.

conditions [Phillips, 1964b]. For the same reason, the pH of the external media was set at 5.5 and the internal media was set at pH 7.0 [Speight, 1967; Phillips, 1964a]. A 'Radiometer Model 25' was used to make all pH readings. The pH of the apical bathing (external) medium during the course of experiments did not vary from the zero-time reading.

Measurement of Ion Concentrations in Media and Tissue

Ten ul aliquots were removed from the apical and basal bathing media and were analyzed for the desired ion.

Inorganic PO_4 was measured by the method of Gomori [1942] and the method of Ernster et al. [1950]. The latter technique was used for tissue analysis, because it did not dephosphorylate labile organic phosphates as readily as did the method of Gomori [Martin and Doty, 1949]. A 'Spectronic 20' was used for the colorimetric determinations.

Samples for Mg^{++} determination were put into 3 ml of 0.75% Na^{+}-EDTA and concentrations were determined with a 'Techtron 120AA' flame spectrophotometer [as per Unicam Method Sheet, 1967].

Calcium-45 samples were counted on a 'Nuclear Chicago Mark 1' liquid scintillation counter. 1-3 ul samples were collected with 'Drummond Microcaps' micropipettes. These samples were put into 10 ml of either Bray's solution [Bray, 1960] or Aquasol (New England Nuclear) for counting.

Fluid samples (1-3 ul) containing ^{32}P were placed on planchets, dried and then counted on a 'Nuclear Chicago Model 1042' automatic planchet counter.

Tissues to be analyzed for ^{32}P were first rinsed with iso-osmotic mannitol and were blotted dry with filter paper. The rectal tissue was then spread out evenly on a planchet and was dried slowly under an infra-red lamp. The planchet with the dried tissue was counted on a 'Nuclear Chicago Model 1042' planchet counter.

Tissues analyzed for ^{45}Ca content were rinsed with iso-osmotic mannitol, blotted, put into weighed platinum boats, and were weighed. The tissues were dried for 48 hours at 80°C in a drying oven. The dry weights were found, and then the tissues were dry ashed in a muffle furnace at 460°C for 24 hours [Phillips, 1964b]. The ash was dissolved in 1 ml of distilled water and 3-25 ul samples were taken with Lang-Levy pipettes. These samples were put into 10 ml of scintillation fluid and counted as described above.

Tissues to be analyzed for Mg^{++} were ashed by the same procedure as for Ca^{++}. The ash was dissolved in 3 ml of 0.75% Na^{+}-EDTA and the Mg^{++} content was read on the flame spectrophotometer ('Techtron 120AA') in the atomic absorption mode of operation.

Inorganic phosphate (Pi) was extracted from a tissue homogenate with ice cold 10% TCA. The precipitate was centrifuged, washed with more ice cold 10% TCA, then centrifuged again. The supernatant was decanted and analyzed for Pi by the method of Ernster et al. [1950].

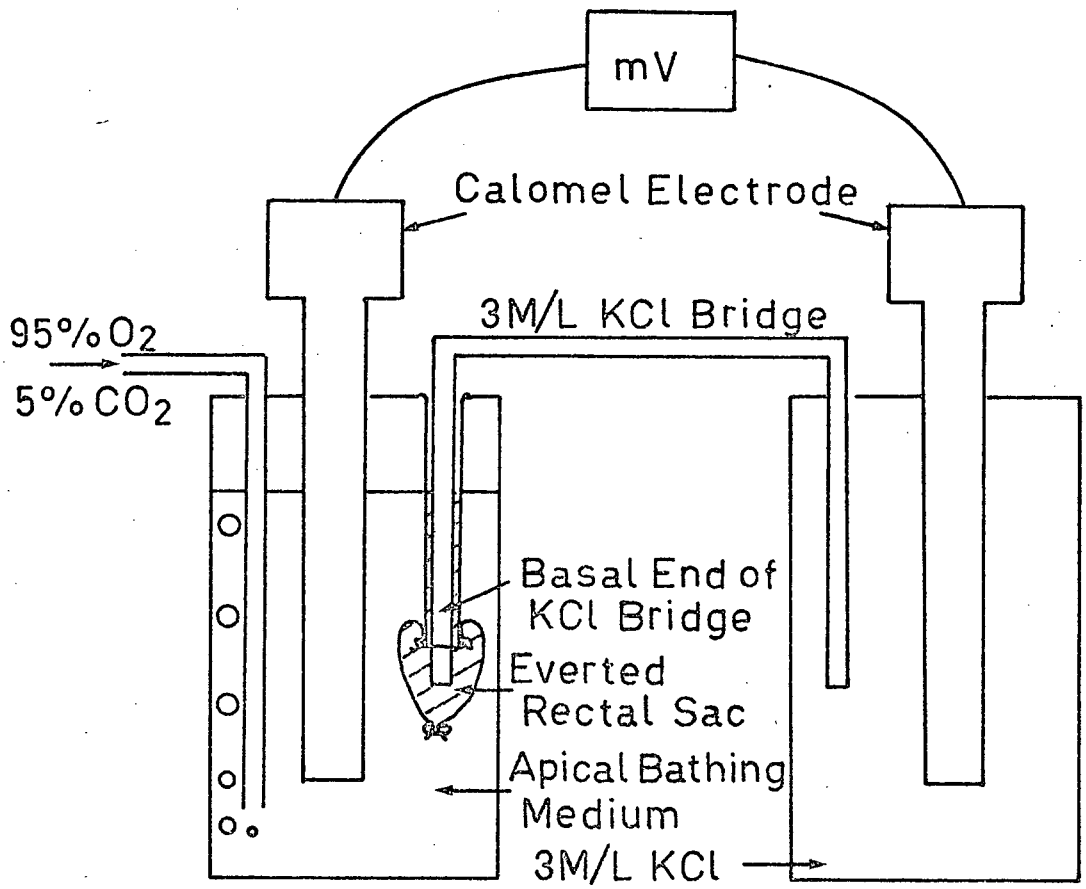
Electropotential Differences Across the Rectal Sac

In order to determine the direction and the magnitude of the potential difference (P.D.) across the rectal sac, the apparatus shown in Figure 2 was used . A 'Keithly Model 602' electrometer was used

Figure 2 Apparatus used for measurement of transrectal potentials.

The asymmetry potential was obtained by immersing the the basal end of the KCl bridge into the apical medium.

KEITHLY MODEL 602 ELECTROMETER



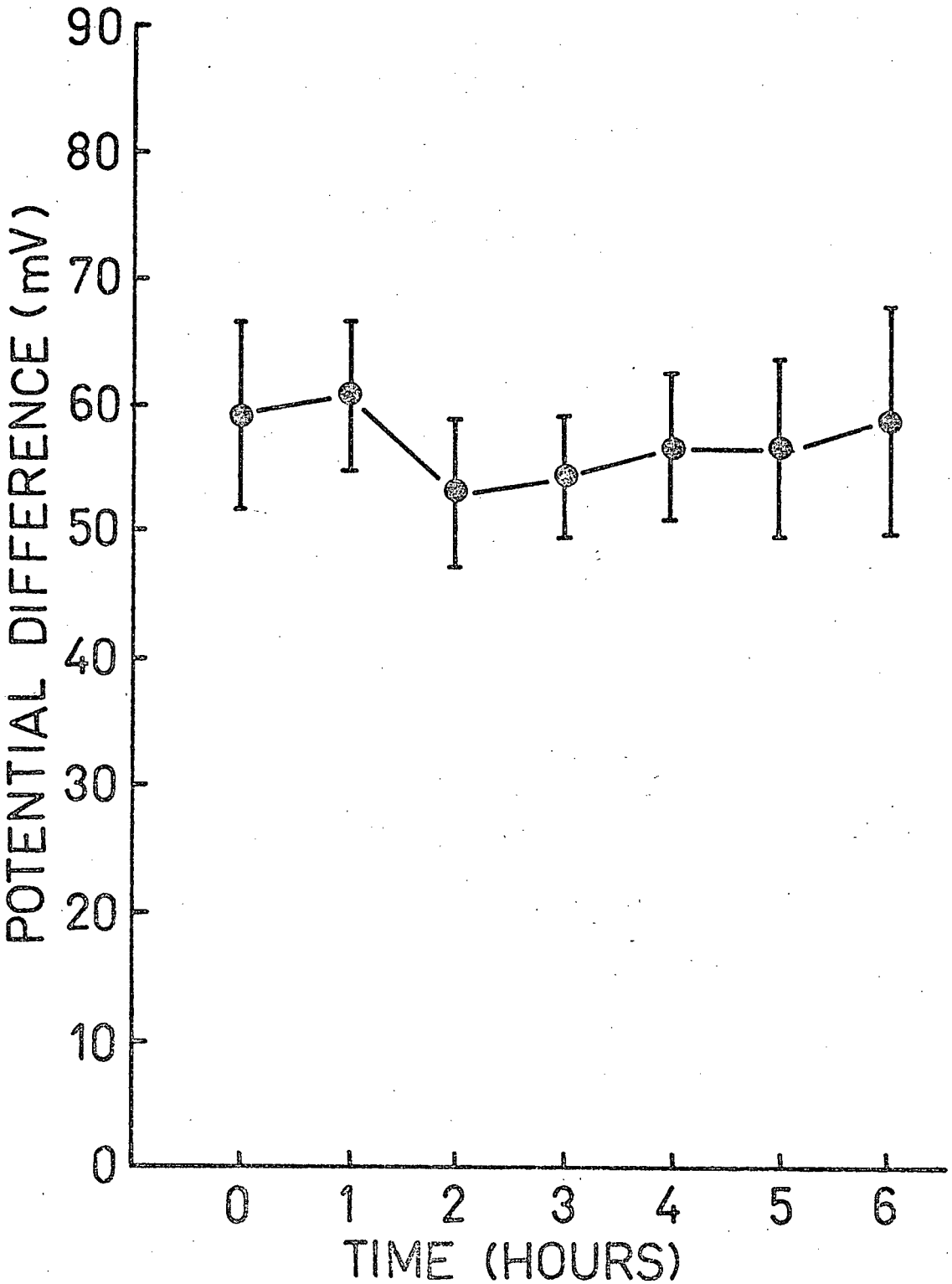
to measure potential differences. 'Radiometer' calomel electrodes in series with a 3 M/l KCl-agar bridge made up in P.E. 10 tubing completed the circuit. The asymmetry potential difference was found by inserting the agar bridge, shown on the basal side, into the vessel containing the apical bathing medium. The asymmetry potential was subtracted from the measured trans-rectal potential differences.

Treatment of Results

The Student's t-test was used to statistically test for significance of the observed differences. Unless the probability level is specifically stated, the term "significantly different" has a probability less than or equal to 0.05.

Figure 3 Trans-rectal potential differences (apical side positive) across everted rectal sacs with time after preparation.

Na-Ringer was present in both compartments (apical and basal) and 420 mOsm/l of sucrose was added to the apical medium to prevent water movement. The vertical bars represent ±S.E. of the mean (7 preparations).


90
80
70
60
50
40
30
20
10
0
POTENTIAL DIFFERENCE (mV)
0
1
2
3
4
5
6
TIME (HOURS)

RESULTS

Viability of the in vitro Rectal Preparation

Goh [1971] evaluated the type of *in vitro* preparation used in this study. His results were comparable to those observed *in vivo* by Phillips [1964b] with respect to the active transport of Na^+, K^+, Cl^-, and H_2O. Balshin [1973] used the same type of everted *in vitro* preparation to demonstrate active transport of amino acids. He used the electropotential difference across the rectal wall, and the uptake rate of H_2O to assess the stability of the preparation with time. A steady state condition was attained after one hour of preincubation and was maintained for at least 6 hours. The preparations used in this study exhibited the same degree of stability with regard to P.D. (Figure 3) and H_2O uptake rate (Figure 4) for at least 6 hours, the longest period of time during which experiments were conducted.

Water movement into the rectal sac was blocked or decreased in some experiments by the addition of sucrose (a non-permeating molecule [Phillips and Dockrill, 1968] to the apical bathing medium. Na^+-Ringer requires 420 mOsm/l of sucrose in the apical bathing medium to block H_2O movement [Balshin, 1973], but K^+-Ringer requires 586 mOsm/l of sucrose to block H_2O movement (Figure 4). This seems to indicate that the preparation can remove H_2O more effectively from a K^+-Ringer than from a Na^+-Ringer. Water is also absorbed at a slightly higher rate

from K^+-Ringer (9.8 ul/hr/rectum, Figure 4) as compared to Na^+-Ringer (7.2 ul/hr/rectum, Balshin, 1973] in the absence of an osmotic difference across the rectal sac.

The initial potential difference fell slightly after the first hour of incubation and then remained between 50 and 60 mV, lumen positive, for the next 5 hours. The observed P.D. was of the same polarity, but greater than that observed *in vivo* [Phillips, 1964b]. Goh [1971] showed that *in vivo* rectum was capable of producing a P.D. of the magnitude observed *in vitro* under certain conditions. The stability and magnitude of the observed P.D. (50-60 mV, lumen positive) agrees closely with that measured *in vitro* by Balshin [1973] during the 5 hour steady-state period.

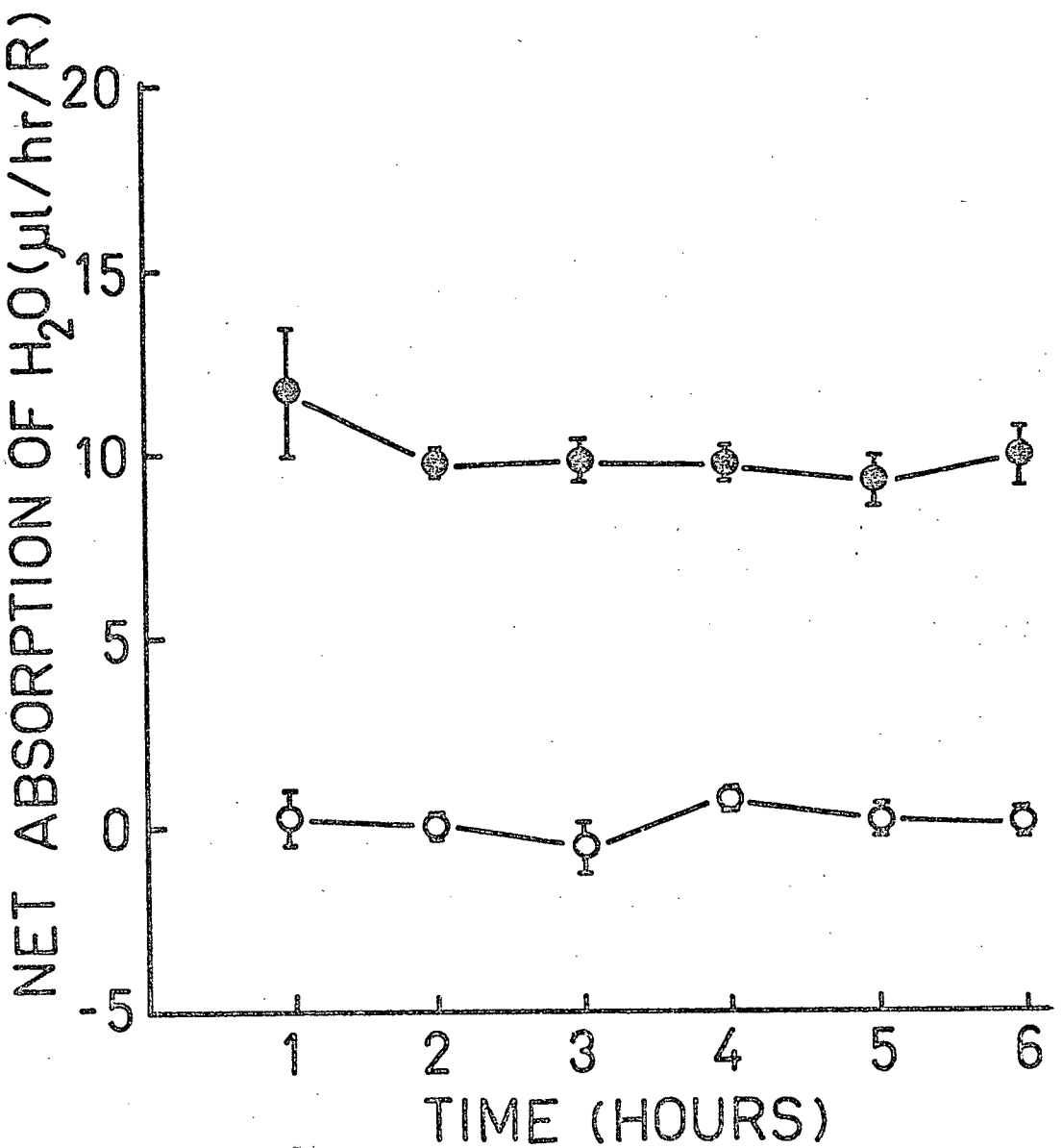
Test for Accumulation of Ca^{++}, Mg^{++}, and PO_4—

To see if these inorganic ions accumulated across the rectal wall, equal concentrations of the ion were placed on both sides of the everted rectal sacs at zero-time. Accumulation of ions with time would indicate some driving force other than concentration difference. Factors such as solvent drag and P.D. could then be examined to see if they could account for the accumulation. If solvent flow is prevented and the P.D. can not account for an observed accumulation, active transport might then be postulated.

The concentrations of free (non-precipitated) Mg^{++} and Ca^{++} in the urine of adult locusts have not been measured, but are expected to be low [1mm Ca^{++}/l, 9mm Mg^{++}/l for the stick insect; Ramsey, 1956].

Figure 4 The net rate of water movement across the rectal wall in the absence and presence of an osmotic gradient.

The bathing medium in the apical compartment was K^+-Ringer and Na^+-Ringer was present as the basal medium. (○) 586 mOsm/l of sucrose was present in the apical medium; (◍) no sucrose was present in the apical medium. Vertical bars represent ±S.E. of the mean (4 preparations).


NET ABSORPTION OF H_2O (µl/hr/R)
20
15
10
5
0
-5
1
2
3
4
5
6
TIME (HOURS)

The Ringer concentrations (Ca^{++} 2.14 mM/l, Mg^{++} 3.59 mM/l) of these ions were therefore considered appropriate to test for net transfer.

Net Ca^{++} Movement

Basal to apical concentration ratios (B/A) of Ca^{++} did not vary from unity after 3 hours of incubation in the absence of water movement (Figure 5). Experiments were conducted at two other media concentrations (0.2 and 0.02 mM/l of Ca^{++}) but again B/A ratios did not exceed unity, indicating only a minimum of Ca movement (Figure 5). When ^{45}Ca was placed initially only in the apical medium (unidirectional flux experiment), a small amount (0.92 ± 0.2 nm/mg D.W. rectal tissue) of Ca^{++} was observed in the basal medium after 3 hours of incubation, indicating that the rectal wall is only slightly permeable to Ca^{++} from the apical side (Table 2). When the recta are incubated in 0.2 mM/l of Ca^{++}, with tracer on both sides, 4 times more ^{45}Ca enters the rectal tissue than under similar conditions when tracer is placed only on the apical side. This indicates that Ca^{++} enters the rectum from the basal side much more rapidly than from the apical side (Table 2).

Net Mg^{++} Accumulation

In these experiments the Mg^{++} concentration in the basal bathing medium was initially 3.6 mM/l while the Mg^{++} concentration in the apical bathing medium was varied over a 100-fold range. The B/A concentration ratios were calculated from the average hourly rates of

Figure 5 The net movement of Ca^{++} and water across the rectal wall, after 3 hours of incubation in Ringer containing various amounts of Ca^{++}.

K^{+}-Ringer was present as the apical bathing medium (with 586 mOsm/l of sucrose), and Na^{+}-Ringer was present as the basal bathing medium. At zero-time the Ca^{++} concentrations were the same in both the apical and basal bathing media (0.02, 0.2, or 2.0 mM/l of Ca^{++}). Preparations were pre-incubated for one hour in the experimental media. The vertical bars represent ±S.E. of the mean (5 preparations).

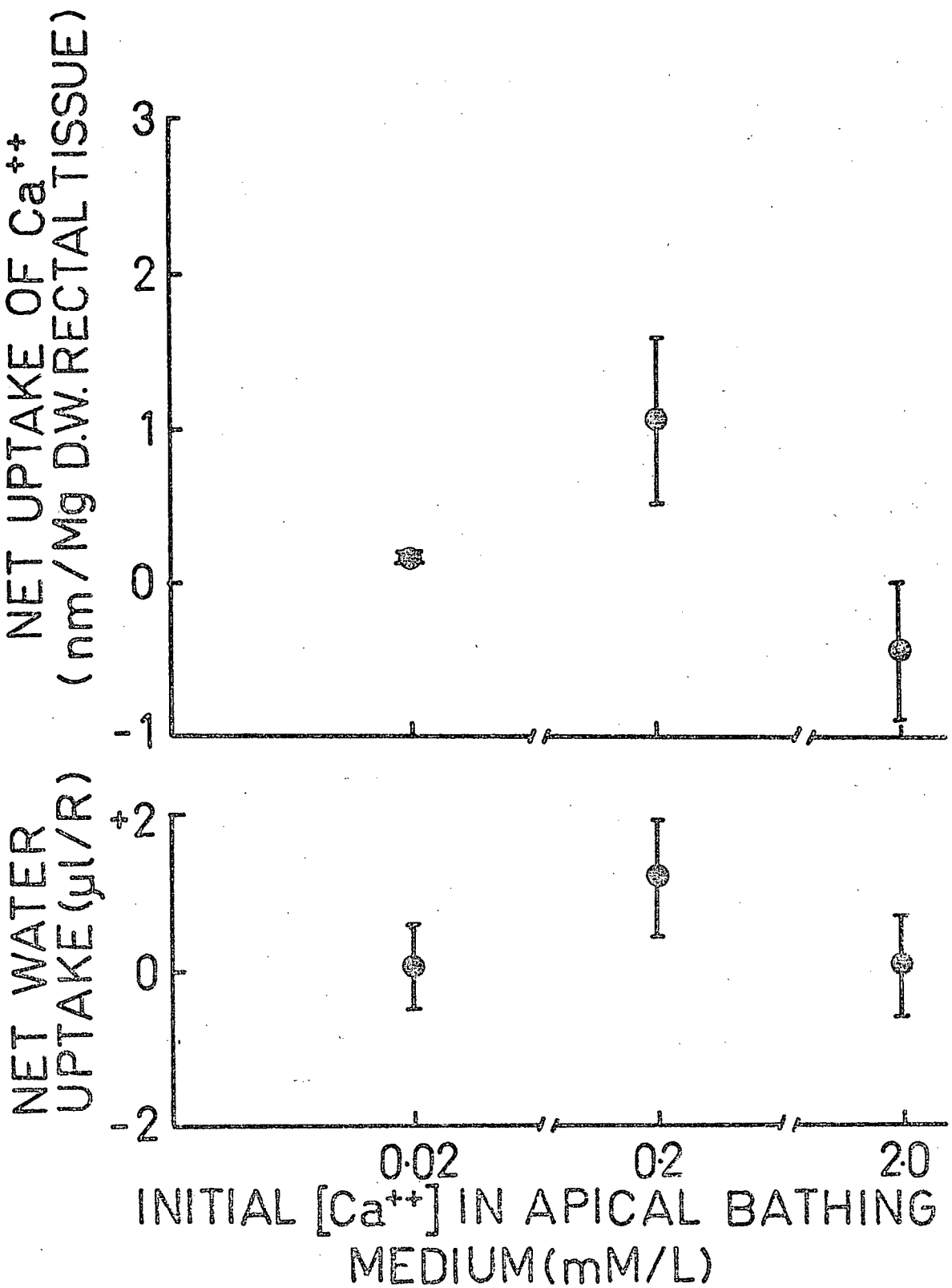

NET UPTAKE OF Ca++
(nm/Mg D.W. RECTAL TISSUE)
3
2
1
0
-1
NET WATER
UPTAKE (µl/R)
+2
0
-2
0·02
0·2
2·0
INITIAL [Ca++] IN APICAL BATHING
MEDIUM (mM/L)

TABLE 2

The Net Movement of Ca^{++} into the Rectal Tissue and Into the Basal Bathing Medium, Over 3 Hours of Incubation in Media Containing Various Amounts of Ca^{++}

K^+-Ringer with various Ca^{++} concentrations was the apical bathing medium and Na^+-Ringer, with initial Ca^{++} concentration identical to that of the apical bathing medium, was the basal bathing medium. 586 mOsm/l of sucrose was added to the apical bathing medium to prevent water movement. Recta were pre-incubated for one hour in the experimental media. The results are expressed as the mean ±S.E. (at least 5 preparations).

Initial Apical and Basal Ca^{++} Concentration (mM/l)	Net Accumulation of Ca^{++} (nM/mg D.W. Rectal Tissue)	
	In Basal Compartment	In Rectal Tissue
0.02	0.04 ± 0.02	0.5 ± 0.1
0.2	1.08 ± 0.6	6.6 ± 1.1
2.0	-1.5 ± 1.0	17.9 ± 3.5
0.2 * (unidirectional flux)	0.9 ± 0.2	1.4 ± 0.1

*unidirectional flux -- 0.2 mM/l of Ca^{++} was present in the apical and basal bathing medium, but tracer (^{45}Ca) was present only in the apical bathing medium.

net uptake (Figure 6). Since these rates were so small, the B/A ratio barely exceeded unity, indicating virtually no basal Mg^{++} accumulation. Two other observations of interest are evident from Figure 6. Firstly, the influx of water has no significant effect on the Mg^{++} uptake rate except at 36 mM/l of Mg^{++} in the apical bathing medium. Secondly, a tenfold apical to basal concentration difference has no significant effect on the uptake of Mg^{++} in the basal compartment when water movement is prevented.

Figure 7 indicates that the rectal tissue content of Mg^{++} is relatively independent of Mg^{++} concentration in the apical bathing medium. These measurements were made at the end of four hours of incubation. Freshly extirpated, recta contain 4.8 ± 1.5 nm Mg^{++}/mg D.W. rectal tissue.

Net PO_4 Accumulation

Speight [1967] measured the PO_4 concentration of hindgut fluid (made up mostly of Malpighian tubule fluid), and deproteinized hemolymph of locusts. She found concentrations of 14.6 ± 5.6 and 6.2 ± 1.3 mM/l of PO_4 (mean ±S.D.) respectively. Rectal concentration of PO_4 was 42 mM/l.

With these in vivo PO_4 concentrations in mind, rectal preparations were incubated in Ringers containing either 4, 12 or 42 mM/l of PO_4 in both apical and basal bathing media. In Figures 8 and 9 the change in PO_4 concentration ratios (Basal/apical side; B/A) is plotted against time, and against PO_4 concentration in the apical

Figure 6 The net movement of Mg^{++} and H_2O across the rectal wall at various Mg^{++} concentrations in the apical bathing medium, with and without net water movement across the rectal wall.

K^+-Ringer with various Mg^{++} concentrations was present as the apical bathing medium, with 586mOsm/l of sucrose (○), or without sucrose (◉). Na^+-Ringer was present as the basal bathing medium. The initial Mg^{++} concentration in the basal compartment (3.6 mM/l) was the same in all experiments. The rates are an average of hourly rate measurements taken over 3 hours. Preparations were preincubated for one hour in the experimental media. The vertical bars represent ±S.E. of the mean (4 preparations).

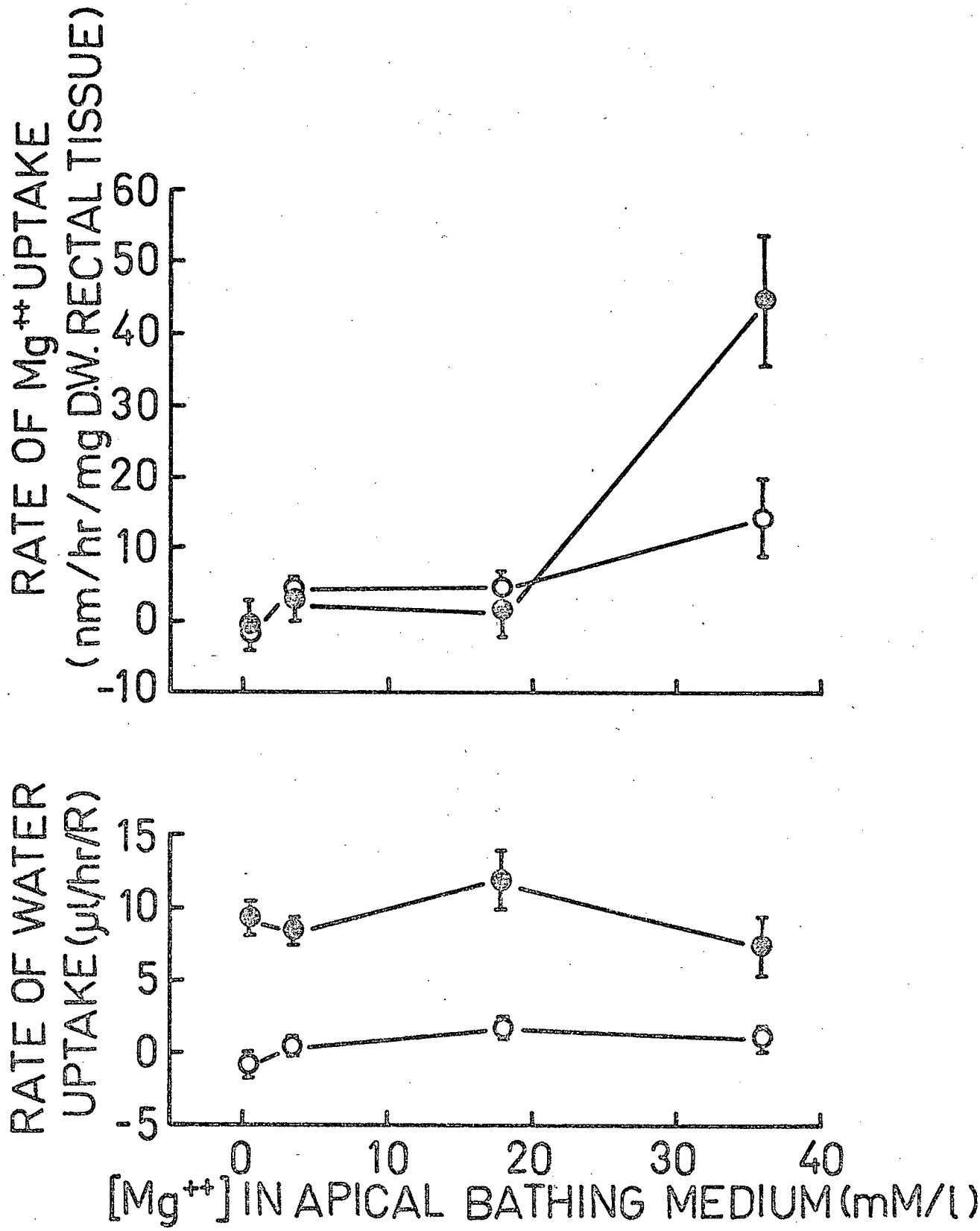
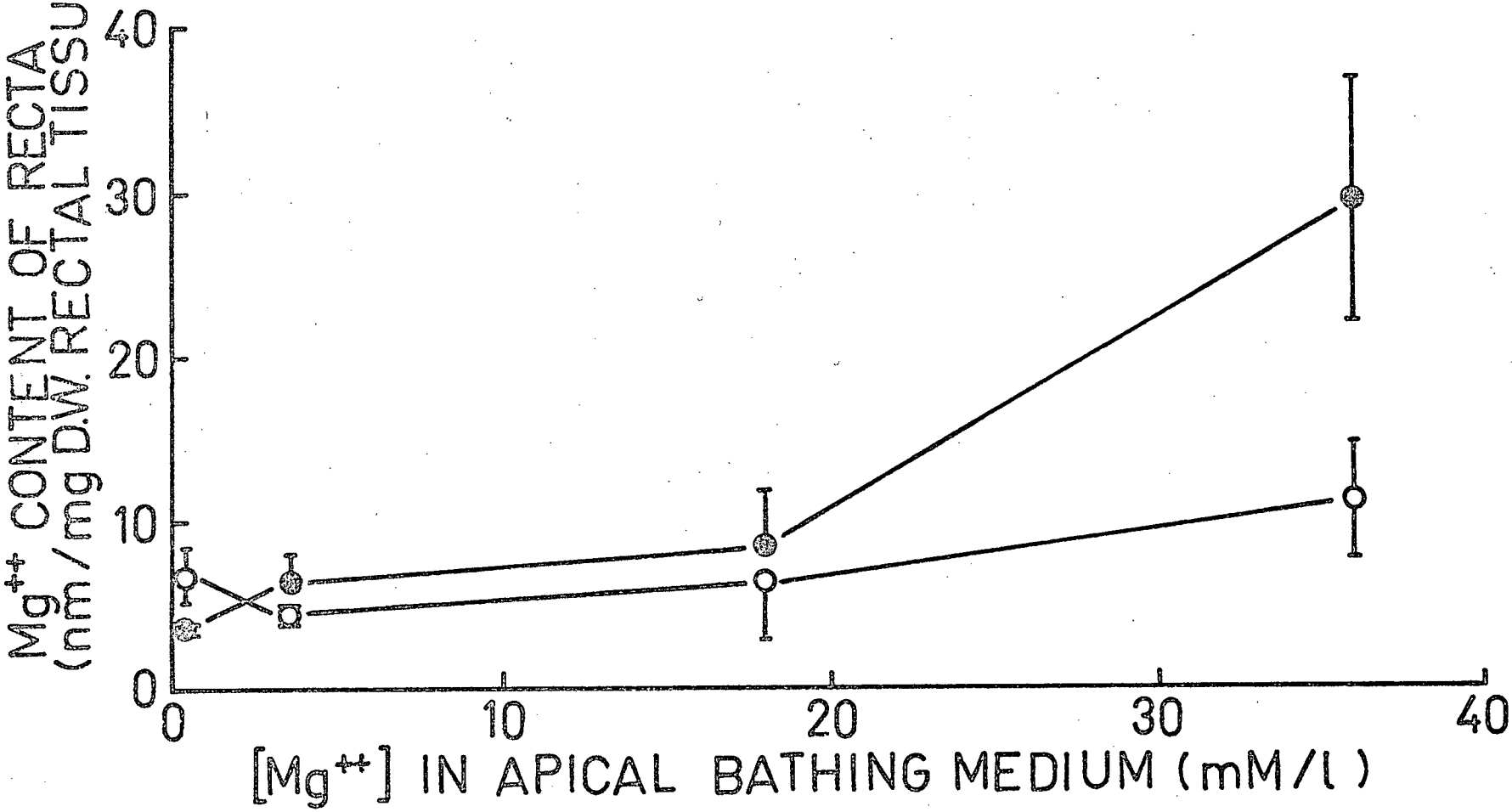

RATE OF Mg^{++} UPTAKE
(nm/hr/mg D.W. RECTAL TISSUE)
60
50
40
30
20
10
0
-10
RATE OF WATER
UPTAKE (µl/hr/R)
15
10
5
0
-5
0
10
20
30
40
[Mg^{++}] IN APICAL BATHING MEDIUM (mM/l)

Figure 7 The total amount of Mg^{++} in rectal tissue after 4 hours of incubation, with and without sucrose present in the apical bathing medium.

K^+-Ringer with various Mg^{++} concentrations was the apical bathing medium, with 586 mOsm/l of sucrose (○) or without sucrose added (●). Na^+-Ringer was the basal bathing medium, with an initial concentration of 3.6 mM/l of Mg^{++} in all experiments. The vertical bars represent ±S.E. of the mean (4 preparations).


Mg^{++} CONTENT OF RECTA
(nm/mg D.W. RECTAL TISSUE)
0
10
20
30
40
0
10
20
30
40
[Mg^{++}] IN APICAL BATHING MEDIUM (mM/l)

bathing medium, respectively. The highest B/A ratio (ca. 3) was obtained in 2 hours and was maintained for at least 4 more hours in an experiment where 4 mM/l PO_4 was placed in both apical and basal bathing media at zero-time. Preparations incubated in both 12 and 42 mM/l of PO_4 showed a gradual increase in B/A with time, but neither concentration could produce a B/A ratio as great as that observed when 4 mM/l of PO_4 was present.

Tissue Contribution to Uptake of PO_4 in the Basal Compartment

In order to determine how much, if any, PO_4 was contributed to the basal compartment by the tissue, preparations were incubated for 6 hours in PO_4-free medium with 586 mOsm/l of sucrose in the apical bathing medium. During this period 114.8 ± 14.7 nm PO_4 rectum (mean ±S.E.) accumulated in the basal compartment. This was almost as much as the total accumulation of PO_4 (139.0 ± 25.3 nm/retum) at 6 hours in the basal compartment when the recta were incubated in apical and basal bathing media containing initially 7 mM/l of PO_4. In another experiment when 6.0 mM/l of PO_4 was present in the basal bathing medium and 0.6 mM/l of PO_4 was present in the apical bathing medium, a total of 60 nM/rectum of PO_4 accumulated in the basal compartment after 6 hours of incubation. It seems, that when the basal bathing medium contains little or no PO_4, the rectal tissue can contribute substantial amounts of PO_4 to the basal compartment. Since the PO_4 accumulation in the basal compartment increases with increasing PO_4 concentration in the apical bathing medium, when the PO_4 concentration in the basal

Figure 8 The basal: apical (B/A) concentration ratio of PO_4 across *in vitro* rectal sacs with time.

K^+-Ringer was the apical bathing medium, and Na^+-Ringer was the basal bathing medium. The apical and basal bathing media both contained initially either 4(●), 12 (○), or 42 (△) mM/l of PO_4. The apical bathing medium contained 586 mOsm/l of sucrose to prevent water movement. The vertical bars represent ±S.E. of the mean (4 preparations).

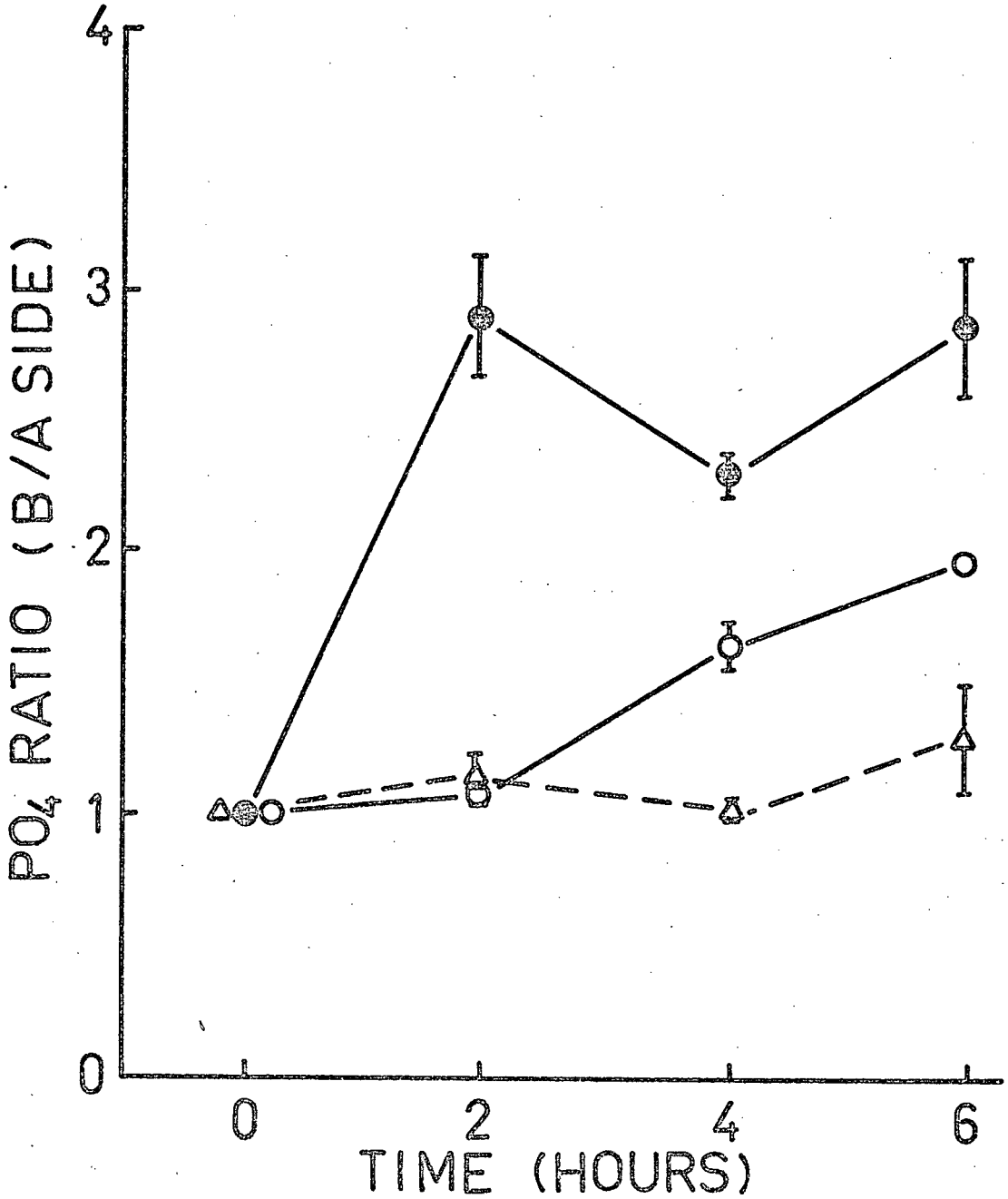
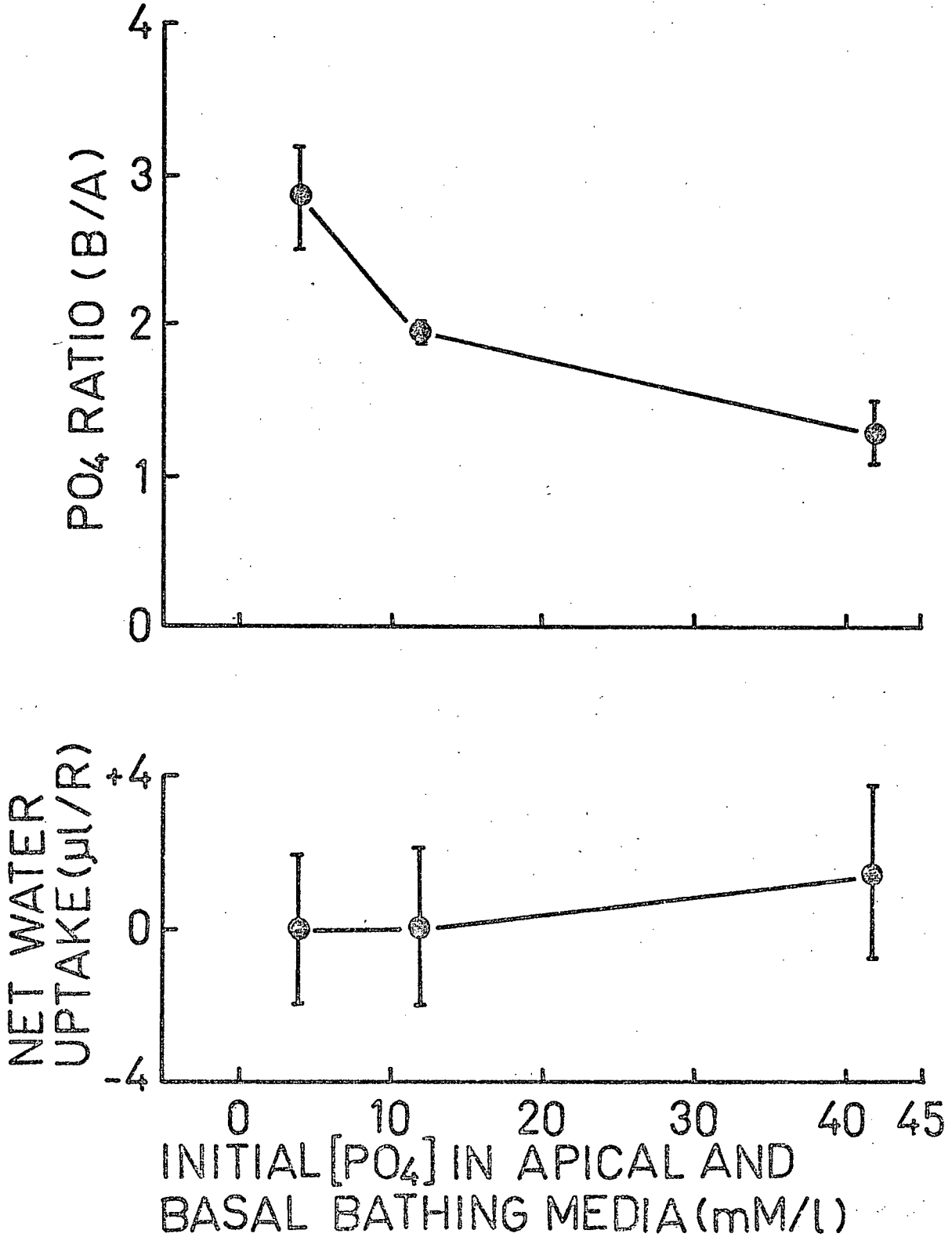

4
3
2
1
0
PO4 RATIO (B/A SIDE)
0
2
4
6
TIME (HOURS)

Figure 9 The final PO_4 ratio (B/A) and the net movement of water across the rectal sacs (after 6 hours of incubation) when the PO_4 concentration of the bathing media was varied.

K^+-Ringer was the apical bathing medium and Na^+-Ringer was the basal bathing medium. The apical and basal bathing media both contained initially either 4, 12, or 42 mM/l of PO_4. The apical bathing medium contained 586 mOsm/l of sucrose. The vertical bars represent ±S.E. of the mean (4 preparations).


4
3
2
1
0
PO4 RATIO (B/A)
+4
0
-4
NET WATER UPTAKE (µl/R)
0
10
20
30
40
45
INITIAL [PO4] IN APICAL AND BASAL BATHING MEDIA (mM/l)

compartment is initially held constant, at least part of the accumulation is due to transepithelial movement of PO_4 against a large gradient.

Type of PO_4 (Inorganic and/or Organic) in the Basal Compartment

It was of interest to see if the PO_4 accumulating in the basal compartment was all inorganic. The method of Ernster et al. [1950] was used to measure the Pi present in an aliquot drawn from the basal bathing medium. Another aliquot from the same medium was hydrolyzed with hot acid, thus cleaving PO_4 from acid-labile organic phosphates. The PO_4 was then measured by the method of Ernster et al. [1950]. The amount of PO_4 did not increase over the amount of inorganic phosphate measured in the first aliquot; hence, all the measurable PO_4 in the basal bathing medium was present as inorganic phosphate.

A similar test was conducted when ^{32}P was used to estimate the rate of accumulation of PO_4 in the basal compartment [Ernster et al. 1950]. The method of Ernster et al. [1950] was used to remove all the Pi (including ^{32}Pi) from an aliquot taken from a sample of basal bathing medium. The same aliquot with the ^{32}Pi removed was then counted on a clanchet counter. Only the background reading was observed, indicating that no ^{32}P was present in an organic form.

Effect of Water Uptake on PO_4 Accumulation in the Basal Medium

To test the effect of H_2O uptake on the accumulation of PO_4 in the basal compartment, recta were incubated in Ringer with no sucrose present, hence no osmotic difference existed between the apical and basal compartments. No difference in the rate of PO_4 uptake in the basal compartment was observed between recta incubated in the absence or presence of an osmotic gradient, when the PO_4 concentration in the apical bathing medium was low (Figure 10). However, at the highest PO_4 concentration in the apical medium (61 mM/l), H_2O uptake has a significant ($p < 0.001$) effect on the rate of PO_4 transfer into the basal compartment.

Kinetics

Saturation kinetics are obtained when the net rate of PO_4 uptake in the basal compartment is plotted against a 100-fold external concentration range (0.6 - 61.0 mM/l) of PO_4 (Figure 10). The zero-time concentration of PO_4 in the basal compartment was 6.0 mM/l of PO_4 in all experiments. The data fit Michealis-Menton kinetics with K_t of 5.0 mM/l and V_{max} of 52.6 nM/hr/mg D.W. rectal tissue (Figure 11).

Saturation kinetics is a phenomenom associated with carrier mediated processes. If PO_4 was diffusing into the basal compartment, a linear relationship (Fick's Law) should occur when rate is plotted against the PO_4 concentration in the apical bathing medium. This was not the case. Stein [1967] considers saturation as "relatively strong" evidence for carrier-mediated systems.

Figure 10 The net rate of PO_4 appearance in the basal compartment and the net water movement across the rectal sac for a range of PO_4 concentrations in the apical bathing medium.

K^+-Ringer with varied PO_4 concentrations was the apical bathing medium, with 420 mOsm/l of sucrose present (○) and without sucrose present (●). Na^+-Ringer was the basal bathing medium, that contained initially 6.0 mM/l of PO_4 in all experiments. Net rate of PO_4 appearance in the basal compartment was measured over a period of 1 hour. The vertical bars represent ±S.E. of the mean (at least 4 preparations).

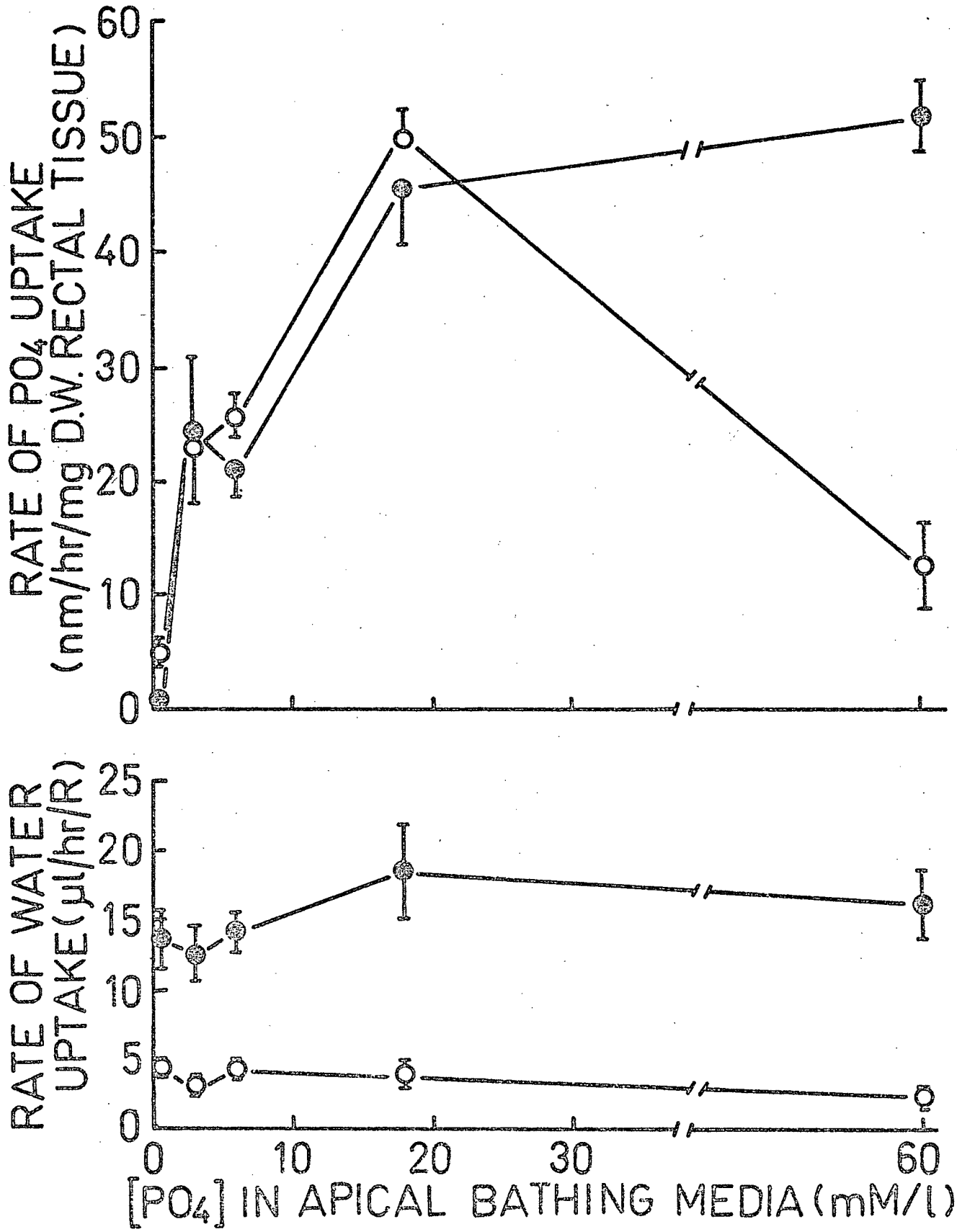

RATE OF PO_4 UPTAKE
(nm/hr/mg D.W. RECTAL TISSUE)
60
50
40
30
20
10
0
RATE OF WATER
UPTAKE (µl/hr/R)
25
20
15
10
5
0
0
10
20
30
60
[PO_4] IN APICAL BATHING MEDIA (mM/l)

Figure 11 Double reciprocal plot of the net rate of PO_4 entry into the basal compartment as a function of the PO_4 concentration in the apical bathing medium.

This plot was drawn from the values obtained from Figure 10. Water movement was restricted with 420 mOsm/l of sucrose in the apical bathing medium.

1/ NET PO4 UPTAKE
(1/nm/mg D.W. RECTAL TISSUE)
.20
.15
.10
.05
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
1/[PO4] (mM/l)

Inhibition of Phosphate Transfer

Another test for carrier mediated systems involves uptake rate measurements in the presence of substrate analogs. Analogs compete with a natural substrate for a carrier site and this competition shows up as a decreased rate of uptake of the natural substrate. Rothstein [1963] demonstrated that arsenate was a competitive inhibitor for the active PO_4 uptake system in yeast cells. Berridge [1969], found that arsenate inhibited PO_4 driven secretion by the Malpighian tubules of an insect, and postulated that the arsenate was competing (with PO_4) for a carrier site on the membrane.

Recta were incubated in Ringer containing 8 mM/l of PO_4 and 8 mM/l of AsO_4 in the apical and basal bathing media. 586 mOsm/l of sucrose was present in the apical bathing medium to prevent H_2O movement. The total amount of ^{32}P that entered into the tissue over a 6 hour period was measured after incubation in the absence and in the presence of AsO_4. Twice as much PO_4 accumulated in the control tissue ($p < 0.01$) indicating AsO_4 inhibits PO_4 uptake (Table 3). However, as well as being a competitive inhibitor for PO_4 uptake systems, AsO_4 is also a metabolic poison; so at this point it was not possible to tell whether the AsO_4 was inhibiting a PO_4 carrier, or whether the AsO_4 was affecting cellular energy metabolism and hence PO_4 uptake.

A combination of 2 mM/l of potassium cyanide and 2 mM/l of iodoacetic acid with 5 mM/l of PIPES buffer (piperazine-N, N-bis (2-ethane sulfonic acid) monosodium monohydrate) and 50 mOsm/l of sucrose in the apical bathing medium at pH 6.6 was used to abolish water uptake in the recta [Balshin and Phillips, 1971]. The control media

TABLE 3

Net ^{32}P Uptake in Either Control or Poisoned Tissue, After 6 Hours of Incubation

In experiment 1, K^+-Ringer with 586 mOsm/l of sucrose, 8 mM/l of PO_4, and 8 mM/l of AsO_4 was the experimental bathing medium (pH 5.5), and Na^+-Ringer with 8 mM/l or PO_4 was the basal bathing medium (pH 7.0). The bathing media were the same for the control except for the absence of AsO_4. In experiment 2, Na^+-Ringer with 5 mM/l of PIPES buffer, and 8 mM/l of PO_4 was present as the apical and basal bathing medium (control). The apical bathing medium contained 420 mOsm/l of sucrose. The experimental bathing media were the same as the control bathing media with the addition of 2 mM/l of KCN and 2 mM/l of IAA to both bathing media. The apical bathing medium (pH 6.6) contained 50 mOsm/l of sucrose. The results are expressed as the mean ±S.E (6 preparations).

Experiment	Apical Bathing Medium	Sucrose in Apical Bathing Medium (mOsm/l)	Tissue Uptake of ^{32}P (nM PO_4/rectum)	Net H_2O Movement (µl/rectum)
1	K^+-Ringer	586	107.3 ± 8.6	+1.4 ± 3.4
1	K^+-Ringer plus 8mM/l of AsO_4	586	56.9 ± 4.9	-5.4 ± 6.0
2	Na^+-Ringer	420	64.6 ±10.2	+2.6 ± 1.2
2	Na^+-Ringer plus 2mM/l of KCN and IAA	50	47.4 ± 6.7	-0.2 ± 2.4

contained 420 mOsm/l of sucrose in the apical bathing medium to abolish H_2O uptake. After 6 hours of incubation (no pre-incubation), no statistical difference ($p > 0.1$) in tissue ^{32}P uptake could be observed between the control and test preparations. Seemingly, PO_4 entry into the rectal tissue from the apical bathing medium is not affected by the presence of KCN and IAA in the apical bathing medium.

Since the ^{32}P accumulation was measured in the tissue, the apical membrane is the only barrier between the tissue and the apical bathing medium. Therefore, unless AsO_4 was interfering with intracellular PO_4 binding, the experiments with AsO_4 and the metabolic inhibitors KCN and IAA may indicate that the location of the carrier is in the apical membrane.

Further Characterization of the System

The amount of PO_4 taken up into the basal compartment, when measured chemically, exceeded that estimated by ^{32}P uptake by about a factor of 4 (Table 4). This observation prompted an investigation into the tissue incorporation of PO_4 into organic forms during incubation in ^{32}P labelled PO_4.

The Pi and the acid-labile organic-PO_4 content of freshly extirpated unincubated recta were determined using the method of Ernster et al. [1950]. Another group of recta were then incubated in Na-Ringer with 5 mM/l of PIPES buffer at pH 6.6. This modified Na-Ringer was the apical and basal bathing medium with the addition of ^{32}P to the

TABLE 4

Various Fractions of PO_4, Determined by Chemical Analysis and Radiotracer Estimation (^{32}P), in Incubated and Unincubated Recta

Recta were incubated for 6 hours. Na^+-Ringer with 5 mM/l of PIPES buffer and 8 mM/l of PO_4 was the incubation medium in both the apical and basal compartments for control tissue. In addition, the apical bathing medium (pH 6.6) contained 420 mOsm/l of sucrose to prevent net water movement. The incubation media for the poisoned incubated tissues were the same as for the control tissues with the addition of 2 mM/l of KCN and 2 mM/l of IAA to both bathing compartments. The incubation medium on the apical side (pH 6.6) contained 50 mOsm/l of sucrose. Results are expressed as the mean ±S.E. (6-12 preparations).

Method of PO_4 Determination	Treatment of Recta	Inorganic PO_4(Pi) (nM/rectum)	Acid Labile Organic-PO_4 (nM/rectum)	Acid Labile Organic-PO_4 plus Pi (nM/rectum)	Non-Acid Labile Organic-PO_4 (nM/rectum)	Net Basal Uptake of PO_4 (nM/rectum)
	unincubated	—	—	—	—	—
^{32}P Estimation	incubated control	20.5±2.1	45.6	66.1±6.9	16.2±0.8	24.0±2.8
	incubated poisoned	44.8±6.9	7.8±1.1	—	—	29.8±3.1
	unincubated	52.2±4.5	66.0	118.2±5.8	—	—
Chemical Analysis	incubated control	115.2±6.1	149.0	264.2±11.0		94.9±8.1
	incubated poisoned	173.2±11.7	—	—	—	65.0±4.4

apical bathing medium only. After being incubated for 6 hour, the tissues were analyzed for Pi and acid labile organic-PO_4. The chemical determinations showed a significant 2-fold increase in both Pi and acid labile organic-PO_4 over the unincubated tissues.

The values derived for ^{32}P influx indicate that roughly three-quarters of the PO_4 taken up by the rectal tissue (61.8 nM/rectum) goes into organic-PO_4, while one-quarter (20.5 nM/rectum) remains as Pi. This amount of ^{32}P accounts for about one-third of the increase in chemically determined Pi over the amount obtained from unincubated recta. The additional amount may occur as a by-product of energy metabolism, and possibly as a release of previously sequestered unlabelled PO_4.

If preparations are incubated in the presence of 2 mM/l of KCN and 2 mM/l of IAA, the chemically determined tissue Pi increases more than 3 fold (173.2 ± 11.7 nM/rectum) over the unincubated tissue, and about 1.5 times (115.2 ± 6.08 nM/rectum) over the amount of Pi in the control tissue. The ^{32}P estimate more than doubles (44.8 ± 6.9 nM/rectum) over the control value (20.5 ± 2.1 nM/rectum). The ^{32}P estimate of acid-labile organic-PO_4 (7.78 ± 1.1 nM/rectum) in the poisoned tissue is about one-sixth of the amount of organic-PO_4 in the control tissue (45.6 nM/rectum), indicating that the metabolic inhibitors have interferred with the metabolic incorporation of PO_4 into organic forms, but have not interferred with the entry of PO_4 into the tissue.

The chemically determined amount of PO_4 in the basal compartment was significantly greater for the control recta (94.9 ± 8.1 nM/rectum) than for the poisoned recta (65.0 ± 4.4 nM/rectum). However, the ^{32}P

estimations of the amount of PO_4 accumulated in the basal compartments of poisoned recta were almost identical with the estimations of the amount of PO_4 accumulated by the control recta (also determined by ^{32}P estimation). This observation seems to implicate metabolism in the accumulation of PO_4 in the basal compartment. Metabolic release of PO_4 leads to a decrease in $^{32}PO_4$ specific activity in the tissue, hence it is not surprising that chemical and isotopic estimations of PO_4 accumulation in the basal compartment do not agree.

DISCUSSION

In this study an *in vitro* preparation was used to determine whether the locust rectum is possibly a site of Mg^{++}, Ca^{++}, and PO_4 reabsorption and hence regulation. Ca^{++} and Mg^{++} (at low concentrations) do not appear to be transported by the *in vitro* rectum. PO_4 is accumulated in the basal compartment against a sizeable electrochemical gradient.

These findings are perhaps not surprising when observations from the literature are considered. Urate, carbonate, oxalate, and phosphate salts of the divalent cations are found as precipitates in the lumen of the Malpighian tubules of many insects, including Orthopterans [reviewed by Clark, 1958]. Ramsay [1956], using an *in vitro* preparation of stick insect (Orthopteran) Malpighian tubules, found that both Mg^{++} and Ca^{++} are present at much lower concentrations in Malpighian tubule fluid than in the hemolymph. In this study, it was found that the *in vitro* locust rectum has a very low permeability to Ca^{++} from the apical side, but Ca^{++} can enter the rectal tissue from the basal side much more readily (Table 2). The latter observation may suggest that Ca^{++} is exchanged between the storage sites within tissues and the hemolymph, thus maintaining a dynamic balance of free Ca^{++} within the hemolymph of the locust. The amount of unbound Ca^{++} in the hemolymph is ultimately regulated by the Malpighian tubules that secrete Ca^{++} at low rates. Ca^{++} may be continuously absorbed (actually concentrated as is Mg^{++}; Wyatt, 1961) from digested food material in such large

quantities as to insure a steady supply of this divalent cation. In effect, excess of Ca^{++} may be the normal situation, and deficiencies exceptionally rare.

The apparent lack of Mg^{++} uptake (at low Mg^{++} concentrations) by *in vitro* locust recta may reflect the *in vivo* situation, since regulation (as suggested for Ca^{++}) perhaps occurs at the tissue and Malpighian tubule levels. At the hemolymph level a dynamic balance between bound (a fairly large amount; Wyatt, 1961) and physiologically active Mg^{++} could control the amount of free Mg^{++} present throughout quite a wide range of total Mg^{++} fluctuation. The Malpighian tubules excrete only a small amount of the total Mg^{++} present in the hemolymph [Ramsay, 1956]. This observation coupled with the observation that almost all insects, concentrate Mg^{++} from their food [Wyatt, 1961] may not necessitate active retention of Mg^{++} by the rectum.

PO_4 does seem to be recycled because it is resorbed by the *in vitro* locust rectum. PO_4 is accumulated as Pi in the basal compartment, and the rate of uptake is affected by the phosphate concentrations in the apical and basal bathing media. This uptake is against both electrical and chemical gradients, cannot be explained by solvent drag and is partially inhibited by the presence of KCN and IAA. Harrison and Harrison [1961] studied the ability of rat small intestine (*in vitro*) to concentrate phosphate in the basal bathing medium. They found that their preparations were capable of producing B/A phosphate ratios of 4.2 - 5.4 to 1 after 3 hours of incubation. They termed the uptake "true transport" of PO_4, but did not characterize the transport mechanism further.

An important consideration in the amount of Pi appearing in the basal compartment is the amount contributed by the tissue. The rectal

tissue is capable of contributing a substantial amount of PO_4 to the basal compartment, in the absence of PO_4 in the bathing medium. This contribution by the rectal tissue decreases as the initial concentration of PO_4 in the basal bathing medium increases. Phillips [1964b, c] found that the in vivo locust rectum decreased its rate of uptake of Na^+, K^+, and Cl^- when the hemolymph concentration of these ions was increased. The observations on PO_4 uptake in this study may reflect a similar mechanism for control of phosphate uptake by the locust rectum. However, it is also quite possible that PO_4 moves by simple diffusion from the rectal tissue to the basal compartment. As the concentration of phosphate in the basal bathing medium is increased, the magnitude of the diffusion gradient from the tissue to the basal compartment is decreased, and this reduced gradient is observed as a decreased rate of PO_4 uptake in the basal compartment. Although diffusion offers a plausible explanation of how PO_4 moves from the tissues to the basal compartment, diffusion does not explain why poisoned recta, with large amounts of Pi in the tissues, contain less PO_4 in the basal compartment than unpoisoned recta with small amounts of Pi in the tissues (Table 4) or why accumulation in the basal compartment is dependent upon the PO_4 concentration in the apical bathing medium when the initial levels of PO_4 in the basal compartment are constant (Figure 10). Harrison and Harrison [1961] also noticed a tissue contribution to PO_4 accumulation in the basal compartment of rat small intestine, but did not indicate how much, or how, phosphate moved from the tissue to the basal compartment.

It was of interest to determine whether some of the phosphate that was found in the basal compartment was organic or not, because

hemolymph of some insects contains 20 - 30 mM/l of acid-soluble organic-PO_4 [Wyatt, 1961]. The rectal tissues perhaps contribute organic phosphates to the hemolymph; however, all measurable phosphates which were observed to be accumulated in the present experiments appeared as inorganic PO_4.

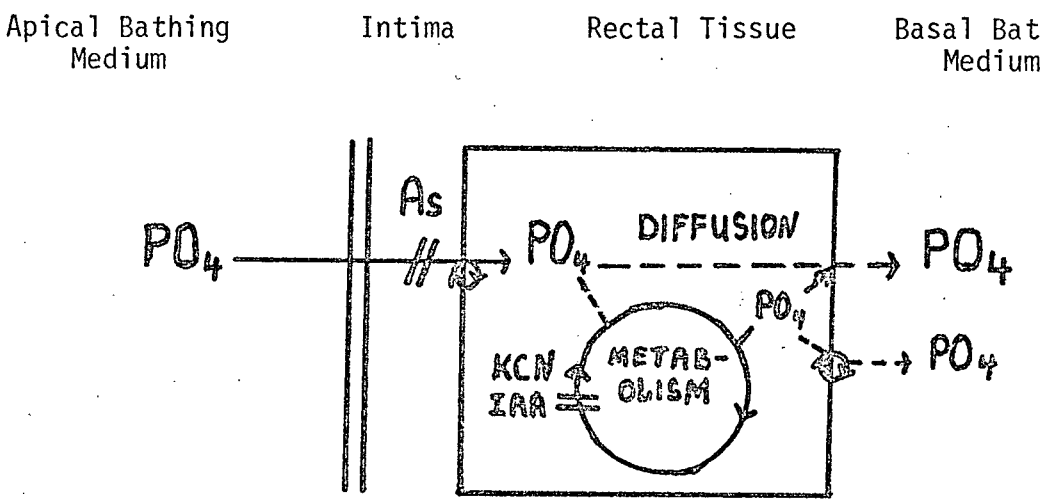
The tracer experiments indicate that ^{32}P is incorporated into organic forms by rectal tissues and is also transferred as ^{32}Pi to the basal compartment. The dilution of tracer PO_4 by a large pool of unlabelled PO_4 in the tissue means that tracer studies of transepithelial transport of PO_4 are difficult to interpret.

The effects of KCN and IAA, arsenate, and water uptake on accumulation of PO_4 by rectal tissue and within the basal compartment, support the idea of a carrier on the apical border of the rectum, but do not exclude a carrier on the basal border as well. KCN and IAA inhibit metabolic incorporation of PO_4 in the rectal tissues, but do not seem to inhibit the tissue uptake of Pi from the apical bathing medium (Table 3 and 4). Arsenate, a competitive inhibitor for PO_4 uptake systems, decreases the tissue uptake of PO_4 from the apical bathing medium. Together, the above two observations seem to indicate that a passive carrier system for PO_4 uptake into the tissue exists on the apical border of the rectum. If PO_4 uptake in the basal compartment is measured in the presence of water influx, the amount of PO_4 found in the basal compartment does not increase when the PO_4 concentration in the apical bathing medium is increased from 18 to 61 mM/l of PO_4 (top curve of Figure 10). This observation indicates that the saturable carrier is the rate limiting step for PO_4 uptake in the rectum; however, where

this carrier is located on the rectum is not obvious. From a theoretical point of view it would seem advantageous for the locust to be able to control the amount of PO_4 entering the animal from the environment; hence being able to control the amount of PO_4 entering the rectum may be a case of having an apical membrane surface impermeable to phosphate except at specific carrier sites. Balshin [1973] found an active carrier for amino acids on the apical border of the locust rectum. PO_4 carriers have also been postulated for both the apical and basal border of an Orthopteran's Malpighian tubules [Maddrell, 1971].

The large quantity of ^{32}Pi incorporated into organic phosphates by rectal tissue may indicate that phosphorylation is a key step in PO_4 uptake, by lowering the activity of Pi in the tissue. Goodman and Rothstein [1957] found that glyceraldehyde-dehydrogenase may be important in esterifying PO_4 as an aid to its entry into yeast cells. Organelles, such as mitochondria, which are heavily concentrated at the apical border of the rectum, perhaps also aid in lowering Pi activity in this part of the cell. Metabolic activity is important in determining the amount of PO_4 entering the basal compartment, because recta poisoned with KCN and IAA showed a marked decrease in PO_4 accumulation in the basal compartment (Table 4). However, KCN and IAA do not greatly affect the total amount of PO_4 entering the rectal tissue from the apical bathing medium (Table 3). It is possible that PO_4 uptake by the rectum is a two step process, consisting of an entry step from the apical bathing medium mediated by a facilitated diffusion mechanism, followed by a translocation step across the cell into the basal compartment, mediated by metabolic incorporation of PO_4.

When all the observations are considered it is possible to put together a tentative model for PO_4 uptake by the *in vitro* locust rectum. Although this model is consistent with the findings of this study, it is by no means the only explanation for the observations, but is of value in so far as the experiments it suggests.



Salient features of the Model:

(a) carrier that transports PO_4 into the rectum by facilitated diffusion located on the apical border. This carrier is inhibited by arsenate. Entry is increased by high PO_4 concentration in the rectal lumen caused by more rapid water reabsorption [Speight, 1967].

(b) metabolic incorporation of PO_4 in the rectal tissue. This step is inhibited by KCN and IAA.

(c) PO_4 transfer from the rectal tissue into the basal compartment is mediated by either a carrier on the basal membrane or by simple diffusion (or both). Transfer by either mechanism (carrier or diffusion) would be influenced by metabolic poisons because the latter cause large changes in tissue levels of inorganic PO_4.

The facilitated entry step on the apical border of a cell, followed by metabolic incorporation of the permeant is a process which facilitates entry of substrates in other organisms. Scarborough [1970], studying glucose uptake in *Neurospora crassa*, found a facilitated diffusion entry step for glucose. Glucose, upon entry into the cells, was phosphorylated and then shunted into the general metabolism of the cell. When a non-metabolizable analog (3-O-methyl-D-glucose) was used as the substrate for the carrier, the concentration in the cells equilibrated with the bathing medium, but did not exceed that of the bathing medium (cell/medium glucose concentration ratio = 1). Jain [1972] terms this type of uptake "loosely coupled energized transport," and although it may not be an accurate description of PO_4 transport in the locust rectum, the process seems to be analogous.

Because of the presence of hormones and other factors in vivo [Mordue, 1969], in vitro systems do not necessarily accurately reflect in vivo mechanisms, but it is still of value to extrapolate in vitro findings to the whole animal.

Values are available for calculating the rate of PO_4 secretion by the Malpighian tubles of the locust. Speight [1967] found that

primary Malpighian fluid contains 14.6 ± 5.65 nM/ul (mean ±S.D.) of PO_4 (*in vivo* measurement). Maddrell and Klunsuwan [1973] found that an *in vitro* preparation of locust Malpighian tubules produced a primary fluid containing 12 nM/ul of PO_4. Phillips [1964c] found that the Malpighian tubules of the locust secreted at a rate of 8 ul/hour. The estimated Malpighian tubule secretion rates for PO_4 are 8 x 12 = 96 nM of PO_4/hour, and 8 x 14.6 = 116.8 nM of PO_4/hour. If the rate of PO_4 uptake into the basal compartment by recta incubated in apical bathing medium containing 18 mM/l of PO_4 is taken from Figure 10, uptake values of 99.3 ± 7.65 nM of PO_4/hour/rectum (water flow across the rectal wall prevented), and 80.4 ± 11.2 nM of PO_4/hour/rectum (water flow across the rectal wall not inhibited)(values are mean ±S.E.) are obtained. The values for the Malpighian tubule excretion rates and rectal reabsorption rates for PO_4 correspond quite closely. All of the PO_4 excreted by the Malpighian tubules can be resorbed by the rectum.

The locust rectum, therefore, has the resorptive capability expected of a site responsible for PO_4 regulation in the locust. PO_4 is rapidly accumulated when hemolymph levels are low, and is more slowly accumulated when hemolymph levels are higher. The saturation mechanism for PO_4 uptake would allow excess PO_4 to be voided with the feces should internal levels become too high.

SUMMARY

1. Net transfer of calcium or magnesium across the *in vitro* locust rectum was not observed.
2. Phosphate is accumulated in the basal compartment of the *in vitro* locust rectum against large concentration difference.
3. The rectal tissue is capable of contributing a substantial amount of inorganic PO_4 to the basal compartment in the absence of PO_4 in the basal bathing medium.
4. Only inorganic phosphate (no organic phosphates) is accumulated in the basal compartment.
5. Water uptake (solvent drag) does not increase the amount of PO_4 accumulated in the basal compartment.
6. The PO_4 uptake fits Michaelis-Menton kinetics with K_t of 5.0 mM/l and V_{max} of 52.6 nM/hr/mg D.W. rectal tissue.
7. PO_4 entry into the basal compartment is inhibited by 2 mM KCN/l and 2 mM IAA/l. Arsenate inhibits PO_4 entry from the apical bathing medium into the rectal tissue.
8. A possible mechanism for PO_4 uptake by the rectum is proposed.

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