8-1985

ATP defendent Na+ transport and the characterization of Na+ / NH4+ exchange in basolateral vesicles of the blue crab, Callinectes sapidus, gill

Linda Ann Fuhrman

ATP DEFENDENT Na^+ TRANSPORT AND THE CHARACTERIZATION OF Na^+/NH_4^+ EXCHANGE IN BASOLATERAL VESICLES OF THE BLUE CRAB, CALLINECTES SAPIDUS, GILL

BY

LINDA ANN FUHRMAN

APPROVED:

CHAIRMAN, THESIS COMMITTEE

MEMBER, THESIS COMMITTEE

MEMBER, THESIS COMMITTEE

EXAMINING COMMITTEE

ATP DEPENDENT Na^+ TRANSPORT AND THE CHARACTERIZATION OF Na^+/NH_4^+ EXCHANGE IN BASOLATERAL VESICLES OF THE BLUE CRAB, CALLINECTES SAPIDUS, GILL

BY

LINDA ANN FUHRMAN

A THESIS PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF RICHMOND IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

AUGUST 1985

TABLE OF CONTENTS

ABSTRACT

ATP dependent Na^+ transport was demonstrated in purified basolateral vesicles of the blue crab (Callinectes sapidus) gill. Active Na^+ uptake was dependent upon the intravesicular presence of either K^+ or NH_4^+ as a counterion. Final membrane preparations consist of inside-out basolateral vesicles, evidenced by intravesicular ouabain (1 mM) and extravesicular vanadate (1 mM) $^{22}Na^+$ uptake inhibition. Na^+/NH_4^+ exchange rates were investigated by determining the NH_4^+ concentration in the recovered extravesicular medium and by using the kinetic capabilities of a DU7 spectrophotometer. These purified basolateral membranes, rich in Na^+,K^+-ATPase activity, provide a useful in vitro tool for examining the stoichiometry of Na^+/NH_4^+ transport in the blue crab gill.

ACKNOWLEDGMENTS

I would like to express my gratitude and appreciation to my mentor, Dr. David W. Towle, Professor of Physiology and Genetics at the University of Richmond, for his patience, guidance, support and expertise throughout the research and preparation of this manuscript and without whom it would not have been possible.

To my associate and friend, Brent Stansbury, now a graduate of the University of Richmond, special thanks for his skilled help in the laboratory. His uplifting spirit offered invaluable relief during this project.

I would also like to bestow my thanks upon my husband, William A. Jennings, Esq., who has been unfailing in his warm support, encouragement, understanding and forbearance.

I am also extremely grateful to my mother, Janet C. Fuhrman, for her indispensable contribution of a perfect typing job.

My acknowledgment would not be complete without my special thanks to all in the Biology Department of the University of Richmond. They have been extraordinarily generous and helpful to me over a long period of time. Their thoughtfulness will not be forgotten.

INTRODUCTION

The blue crab Callinectes sapidus occupies a variety of habitats, including the sea, brackish water, and fresh water. Its capacity for regulating the osmotic concentration of body fluids over such a broad salinity range is well documented (Neufeld et al., 1980; Towle, 1974; Shaw, 1964; Williams, 1965; Aldridge et al., 1979; Bliss, 1982; Cameron et al., 1978; Cantelmo, 1977). The animal maintains a hyperosmotic hemolymph in salinities below approximately 26 PPT, above which point its hemolymph conforms to the external salinity (Mangum and Towle, 1977). Salt regulation appears to be achieved in part by the Na^+,K^+-ATPase enzyme, which exchanges intracellular sodium for extracellular potassium (or ammonium ion) at the expense of energy derived from the hydrolysis of ATP. The specific activity of this enzyme changes under conditions of osmotic stress, and thus enables the organism to make physiological adjustments, minimizing the consequences of environmental change. Towle et al. (1976) observed that the specific activity of the enzyme in gill microsomes prepared from crabs acclimated to 5 PPT salinity is nearly twice that of crabs acclimated to 34 PPT. The increased activity at low salinity is accompanied by the excretion of additional NH_4^+ ions by the gill, suggesting that these NH_4^+ ions may provide the physiological counterion to balance the absorption of Na^+ at the gill (Mangum and Towle, 1977). The hypothesis that excess ammonia in the blood plays an important role in osmoregulation is supported by these findings (Mangum et al., 1976).

Of the eight pairs of gills present in the blue crab, Na^+,K^+-ATPase specific activity is highest in the posterior sixth and seventh gills (Neufeld et al., 1980). A cross section of the crab gill lamella

demonstrates the existence of salt-absorbing cells concentrated around the afferent vessel (Copeland and Fitzjarrell, 1968). Maximal Na^+,K^+-ATPase activity occurs in medial portions of the gill (Neufeld et al., 1980), regions in which ultrastructure also shows increased development of salt-transporting cells. In the process of acclimation, both short term activation and long term synthesis of the enzyme may be important (Neufeld et al., 1980).

Because of the blue crab's relatively simple gill structure and well documented osmoregulatory ability, this organism provides an excellent system for the investigation of the properties of Na^+,K^+-ATPase-mediated transport. The present study was conducted to examine the kinetics of Na^+ and NH_4^+ transport, utilizing purified basolateral membranes of the blue crab gill, with emphasis on attempts to determine the mechanisms of Na^+/NH_4^+ exchange and to test the hypothesis that NH_4^+ effectively substitutes for K^+ in ATPase-mediated Na^+ transport.

MATERIALS AND METHODS

Membrane Preparation

Specimens of the blue crab (Callinectes sapidus) were obtained from Newport News and Hampton, Virginia seafood markets and were maintained in aerated aquaria of 5-8 PPT salinity. Crabs were killed by penetration of anterior carapace, and posterior gills six and seven were excised. The darkly colored ion-transporting side of the gill lamellae was separated from the remaining portion by coarse dissection. The tissue fragments were blotted dry, weighed to the nearest 0.1 gram, and placed in an ice cold

30-ml glass homogenizer. Homogenizing medium (6 mM EDTA, 20 mM imidazole, 250 mM sucrose, pH 6.8: 5 ml/gram of tissue) and sodium deoxycholate (10%: 0.05 ml/gram tissue) were then added to the homogenizing tube, and the tissue homogenized with a teflon pestle at 1,800 rpm. The homogenate (5 ml) was then placed on a sucrose density gradient (28 ml of 10-40% sucrose with 4 ml 60% sucrose cushion) and centrifuged in a Beckman SW27 rotor at 17,000 rpm for 60 minutes. Approximately 5 ml of the resultant vesicular membranes, characteristically white in appearance, were removed from the upper third of the gradient and diluted thirty times ("vesicular loading") with buffered sucrose solutions containing 10 mM Tris-HEPES pH 7.4 and various potassium or ammonium chloride concentrations by the method of Boumendil-Podevin and Podevin (1983). Membrane dilutions were transferred to centrifuge tubes, centrifuged in a Beckman 30 rotor at 28,000 rpm for 60 minutes, removed from the rotor and immediately placed on ice. The supernatant was removed, and the tubes were dried with cotton swabs. Care was taken not to disturb the basolateral membrane pellet. Pellets were resuspended in 30-45 µl of the appropriate buffered intravesicular medium, mixed with a stirring rod, transferred to individual 16x100 mm test tubes, and mixed again. Membranes were incubated in an agitating water bath (37°C) for 30 minutes and then set on ice for no more than half an hour.

Vesicular ^{22}Na Transport Determination

Final membranes (5 µl) were incubated in an extravesicular medium containing ^{22}NaCl (4 mM), $MgCl_2$ (2 mM), sucrose (300 mM), Tris-HEPES (10 mM, pH 7.4), with and without Tris-ATP (4 mM), in a method adapted from Boumendil-Podevin and Podevin (1983). After addition of membranes, tubes were vortexed slightly and incubated for two minutes in an agitating water bath (37°C). Transport was terminated by the addition of 1 ml ice cold

wash buffer (150 mM NaCl, 10 mM Tris-HEPES pH 7.4), and the vesicles were separated from the extravesicular medium by filtration on Schleicher & Schuell BA 80, 0.15 micron filters. Filters were washed with 4 ml wash buffer and placed in scintillation vials. Aquasol (10 ml) was added, and vesicular content of ^{22}Na determined in a liquid scintillation counter. In some experiments, the filtered supernatant was recovered for ammonium ion determinations. The effect of extravesicular vanadate (1.0 mM) and amiloride (0.1 mM) on sodium uptake was determined by their separate incorporation into the incubation medium. The effect of both intravesicular and extravesicular ouabain (1 mM) was also determined.

Ammonium Ion Transport Determinations

Ammonium ion transport was ascertained from measurements of the NH_4^+ concentration in the extravesicular medium. Two methods of measurement were utilized. First, by collecting the extravesicular filtrate, the concentration of NH_4^+ transported from the intravesicular medium to the extravesicular medium was determined using an ammonia assay kit supplied by Sigma Chemical Company. The difference between the NH_4^+ concentrations of filtrates resulting from reactions occurring for a duration of 0 min and 2 min should depict the contribution of NH_4^+ from active Na^+/NH_4^+ exchange. NH_4^+ concentrations were determined as follows: Initial absorbances of ammonia assay solution (3ml: Sigma #170-10) with 200 ul of distilled water (blank), 200 ul ammonia control solution (Sigma #170-5: control) or 200 µl of the appropriate sample, were read at 340 nm using distilled water as reference. L-Glutamic dehydrogenase (20 µl: Sigma GDH 2009) was then added, solutions mixed by gentle inversion, and final absorbances read at 340 nm. The decrease in absorbance at 340 nm is due to the oxidation of

NADH, and is proportional to the ammonium ion concentration according to the following reductive amination reaction:

α- ketoglutarate + NH_4^+ + NADH (high A_{340}) $\xrightarrow{\text{Glutamate dehydrogenase}}$ Glutamate + NAD (low A_{340})

The second method of determination involved the measurement of ammonium ion transport utilizing the kinetic capabilities of a Beckman DU-7 spectrophotometer and QS 1.000/282 microcuvettes. To a cuvette containing α- ketoglutarate (3 µl, 200 mM pH 7.4), NADH (3 µl, 12 mM), extravesicular medium without Na^+ (300 µl) and glutamic dehydrogenase (20 µl: Sigma GDH 2009), a 5 µl membrane sample was added. Without sodium, NH_4^+/Na^+ transport should not take place. The introduction of 5 µl of the appropriate intravesicular medium would demonstrate the concentration of NH_4^+ that exists inside the vesicles. The addition of Na^+ (5 µl, 0.3 mM NaCl) initiates active transport and the spectrophotometer is programmed to read and record the changes in absorbance at 340 nm ten times per minute, thus continually detecting NH_4^+ ions as they are being transported to the extravesicular medium. When stabilization occurs, 5 µl of Triton-X (7.5%) is added to disrupt the vesicular membrane, and thus determine the effects of membrane turbidity on absorbance and to determine the intravesicular NH_4^+ concentration.

The above method was devised by varying concentrations of all reaction compounds, including their reconstituting media, and by adjusting sample sizes of each compound in an attempt to achieve optimum transport and detection conditions.

Protein Determination

Protein concentrations of the final membrane suspensions were determined by the Bio-Rad method (Bradford, 1976). A standard curve was

constructed using bovine serum albumin (1 mg/ml). Membrane samples (5 µl) were added to 3 ml of diluted Bio-Rad concentrate (4:1), vortexed and let stand for 5 minutes to equilibrate. Absorbance (595 nm) was read, using distilled water (10 µl) in 3 ml diluted concentrate as a blank, and protein concentrations were extrapolated from the standard curve.

RESULTS

Uptake of $^{22}Na^+$ into gill basolateral vesicles reached an equilibrium during the first minute of incubation (Figure 1). Transport was ATP-dependent and comprised approximately 86% of total $^{22}Na^+$ uptake. Increasing concentrations of potassium inside the vesicles promoted Na^+ uptake (Figure 2). $^{22}Na^+$ uptake was ATP-dependent and showed saturation kinetics with respect to counterion (K^+) concentration (Figure 2). The requirement for an intravesicular counterion in Na^+ transport was also fulfilled by NH_4^+. Figure 3 shows that the intravesicular presence of NH_4^+ also enhanced ATP-dependent $^{22}Na^+$ uptake, with the exception of intravesicular NH_4^+ concentrations of 50 mM and 75 mM.

Intravesicular ouabain (1 mM) inhibited ATP dependent uptake of $^{22}Na^+$ by approximately 30%, in comparison to 5% inhibition when ouabain (1 mM) was present in the extravesicular medium (Figure 4). The Na^+,K^+-ATPase inhibitor vanadate, unlike ouabain, is effective at the catalytic site of the enzyme. With inside-out vesicles, this inhibitor should retard ATP-dependent $^{22}Na^+$ uptake when present in the extravesicular medium. Vanadate (1 mM) inhibited ATP-dependent Na^+ transport by approximately 45%. Lack of Na^+ transport inhibition by the diuretic amiloride (0.1 mM; Figure 4), suggests that apical membrane contamination is minimal.

Attempts to determine Na^+/NH_4^+ exchange rates using the filtration method were unsuccessful. The recovery of filtrate (i.e. the extravesicular medium) revealed inconsistent results in a series of seven different experiments (Table I), suggesting that the NH_4^+ ions are being caught on the filter apparatus and therefore not consistently detectable in the filtrate. Investigation of Na^+/NH_4^+ exchange directly by use of microcuvettes and the kinetic capabilities of a DU7 spectrophotometer was thus employed to eliminate the use of filtration and the loss of NH_4^+ ions through perhaps membrane adherence of surface charge interactions. The detection of minute amounts of NH_4^+ ions that were being transported to the extravesicular medium was difficult. As Figures 5 and 6 demonstrate, however, the optimum conditions necessary for the detection of more concentrated NH_4^+ concentrations, i.e in resuspension media, have been achieved. The addition of a 5 ul sample of resuspension medium of 0 mM, 25 mM and 50 mM NH_4^+Cl decreased the absorbance at 340 nm proportional to their NH_4^+ concentrations, 0.017, 0.201, and 0.405, respectively. Using the decrease in absorbance associated with 0 mM NH_4^+ resuspension medium as control, decreased absorbances of 25 mM and 50 mM NH_4^+ resuspension medium represent 125 nmoles/ml and 250 nmoles/ml of NH_4^+ respectively. Approximately 78 nmoles NH_4^+/ml were incorporated into 25 mM NH_4^+-loaded vesicles, and 82 nmoles NH_4^+/ml were incorporated into the 50 mM-loaded vesicles. Thus, the efficiency with which NH_4^+ was incorporated into these vesicles varies with the NH_4^+ concentration, i.e 37.6% of the NH_4^+ in the 25 mM resuspension medium does not exist inside the vesicles, whereas 67% of the NH_4^+ in the 50 mM resuspension medium does not exist inside the vesicles. The increase in Na^+ transport associated with 25 mM concentrations of ammonium, in comparison to the Na^+ transport of 50 mM NH_4^+ loaded vesicles, demonstrates this.

Experiments using 25 mM- and 50 mM-loaded vesicles showed that the addition of 5 ul 0.3 M NaCl increased the concentration of NH_4^+ in the extravesicular medium by 7 nmoles/ml and 3 nmoles/ml, respectively. Changes in the NH_4^+ component of this order of magnitude demonstrate further the difficulty in NH_4^+ detection with the methods employed.

Membrane addition (5 ul) induced an increase in absorbance at 340 nm due to turbidity and interfered in the accurate detection of NH_4^+ ion presence and/or expulsion from the intravesicular medium. However, the addition of a 5 ul membrane sample to a solution of 300 ul extravesicular medium without Na^+ (with and without ATP), 3 ul NADH (12mM), 3 ul-ketoglutarate (200 mM, pH 7.4), and 20 ul glutamate dehydrogenase (Sigma GDH 2009) provided a sufficient environment for ammonium ion detection. Perhaps the utilization of a more sensitive detection system might enable us to determine the kinetics of the Na^+/NH_4^+ transport system more effectively.

DISCUSSION

In Callinectes sapidus, the absorption of salts against a concentration gradient appears to occur at the gill, the most likely possibilities for sodium uptake being Na^+/K^+ exchange, Na^+/NH_4^+ exchange, Na^+/H^+ exchange, and possibly electrogenic Na^+ transport (Cameron, 1978; Mantel, 1967). Ninety-nine percent of the total ammonia excretion also occurs across the gills, the antennal gland having no appreciable role in either nitrogen excretion or salt conservation (Kormanik and Cameron, 1981; Cameron et al. 1978). Little is known about the mechanism by which Na^+ is translocated across the gill epithelium, in which Na^+,K^+-ATPase is located predominantly if not exclusively in the basolateral membrane (Mangum and

Towle, 1977; Towle, 1983; Fuhrman et al. 1983). The utilization by the Na^+,K^+-ATPase of NH_4^+ as an effective substitute for K^+ may afford indirect evidence for basolateral Na^+/NH_4^+ exchange, especially in situations of dilute salinity where additional NH_4^+ is formed and Na^+ absorption becomes a potential problem. The fact that ammonia output in the blue crab increases only when ion transport is activated suggests that the rate of NH_4^+ excretion depends on the activity of the transport ATPase (Mangum and Towle, 1977). The purification and isolation of Na^+, K^+-ATPase rich basolateral membrane vesicles from the blue crab gill provides an in vitro tool for investigating the stoichiometry and the kinetics associated with Na^+/K^+ and Na^+/NH_4^+ exchange.

The effective substitution of low concentrations of NH_4^+ for K^+ in ATP-dependent Na^+ uptake supports the idea that Na^+/NH_4^+ exchange takes place across basolateral membranes in blue crab gill. The existence of apical vesicles in membrane preparations would interfere considerably in determining only basolateral, ATPase-mediated Na^+ transport due to the fact that apical membranes have been implicated in Na^+/NH_4^+ exchange processes as well. Pressley et al. (1981) demonstrated that apical Na^+ influx is reversibly inhibited by the drug amiloride. The finding that our membrane vesicles showed no response to 0.1 mM amiloride in the extravesicular medium (Figure 4) suggests minimal apical membrane contamination.

With the methods employed in this experiment, the final basolateral membrane preparation consists of sealed, inside-out vesicles. A greater inhibition of ATP dependent Na^+ uptake by intravesicular ouabain compared with extravesicular ouabain indicates that the vesicles are indeed inside-out. Lack of complete inhibition by ouabain is perhaps due to the low sensitivity of the blue crab ATPase to this inhibitor (Neufeld et al.,

1980). In addition, when vanadate was present in the extravesicular medium, Na^+ uptake was inhibited by 45%. Because vanadate inhibits such uptake by interfering at the catalytic site of the enzyme, this observation reemphasizes the existence of inside-out vesicles due to the fact that the catalytic site is present on the vesicular outer face.

The existence of Na^+ uptake into vesicles in our membrane preparations is unquestionable, yet development of direct techniques for examining Na^+/NH_4^+ basolateral gill transport, mediated by the Na^+,K^+-ATPase enzyme, has proved a difficult task. Perhaps the elucidation of this mechanism and support for the Na^+/NH_4^+ exchange hypothesis can be sought by disproving alternative theories, that is, Na^+/H^+ exchange and NH_3^+ diffusion. One can test for the presence of a Na^+/H^+ exchange system by monitoring pH changes in the membrane suspension after sudden addition of Na^+ (Murer et al, 1976). Acridines have also been used to examine pH gradients in several vesicular systems (Warnock et al., 1982). These methods use fluorescent intensity and record the change in extravesicular probe concentration that results from uptake of the probe into the intravesicular space. The use of furosemide, a Na^+/H^+ transport blocker, would also provide information useful in determining what contribution this system makes to total active uptake of Na^+ in the blue crab gill basolateral vesicles. In any case, the present work does suggest that intravesicular NH_4^+ can substitute effectively for K^+ in serving as a counterion for Na^+ uptake, supporting the existence of Na^+/NH_4^+ exchange at the basolateral membrane.

Ideally, this information can be integrated into a theoretical model of ion transport by the Callinectes sapidus gill. This investigation is based on the hypothesis that, on adaptation to dilute media, electrical neutrality across the gill is maintained by the transepithelial exchange of

Na_3 for NH_4^+ and possibly the exchange of Cl^-/HCO_3^-. Upon introduction and acclimation to dilute waters, amino acid deamination appears to occur in a variety of tissues as a means of cell volume regulation, and excess NH_3 is formed (Mangum and Towle, 1977). Excess NH_3 increases the pH of the hemolymph by combining with H^+ to form NH_4^+, which can then serve as an effective counterion for the basolateral ATPase in the gill. Further work is required to elucidate the stoichiometry of Na^+/NH_4^+ exchange in basolateral vesicles of crab gill.

REFERENCES

Aldridge, J.B. and Cameron, J.N. 1979. CO_2 Exchange in the Blue Crab, Callinectes sapidus (Rathbun). J. Exp. Zool.: 207, 321-328.

Bliss, Dorothy E. ed. 1982. The Biology of Crustacea, Vol, I and V. New York, Academic Press.

Boumendil-Podevin, E.F. and Podevin, R.A. 1983. Effects of ATP on Na^+ Transport and Membrane Potential in Inside-Out Renal Basolateral Vesicles. Biochim. Biophys. Acta.: 728, 39-49.

Bradford, M.M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-dye Binding. Anal. Biochem.: 72, 248-254.

Cameron, J.N. 1978. NaCl Balance in Blue Crabs, Callinectes sapidus, in Fresh Water. J. Comp. Physiol.: 123, 17-135.

Cameron, J.N. and Batterton, C.V. 1978. Antennal Gland Function in the Freshwater Blue Crab, Callinectes sapidus: Water, Electrolyte, Acid-Base and Ammonia Excretion. J. Comp. Physiol.: 123, 143-148.

Cantelmo, A.C. 1977. Water Permeability of Isolated Tissues From Decapod Crustaceans-1. Effect of Osmotic Conditions. Comp. Biochem. Physiol.: 58A, 343-348.

Copeland, D.E. and Fitzjarrell, A.T. 1968. The Salt Absorbing Cells in the Gills of the Blue Crab, Callinectes sapidus (Rathbun) With Notes on Modified Mitochondria. Z. Zellforsch.: 92, 1-22.

Fuhrman, L.A., Stansbury, B.R. and Towle, D.W. 1983. ATP-Dependent Na^+ Transport by Basolateral Membrane Vesicles from Crab Gill. Amer. Zool.: 23, 953.

Kormanik, G.A. and Cameron, J.N. 1981. Ammonia Excretion in the Seawater Blue Crab (Callinectes Sapidus) Occurs by Diffusion, and Not Na^+/NH_4^+ Exchange. J. Comp. Physiol.: 141, 457-462.

Mangum, C.P. and Towle, D.W. 1977. Physiological Adaptation to Unstable Environments. Amer. Sci.: 65, 67-75.

Mangum, C.P., Silverthorn, S.U., Harris, J.L., Towle, D.W. and Krall, A.R. 1976. The Relationship Between Blood pH, Ammonia Excretion and Adaptation to Low Salinity in the Blue Crab Callinectes sapidus. J. Exp. Zool.: 195, 129-136.

Mantel, L.H. 1967. Asymmetry Potentials, Metabolism, and Sodium Fluxes in Gills of the Blue Crab, Callinectes sapidus. Comp. Biochem. Physiol.: 20, 743-753.

Murer, H., Hopfer, V. and Kinne, R. 1976. Sodium/Proton Antiport in Brush-Border Membrane Vesicles Isolated From Rat Small Intestine and Kidney. Biochem J.: 154, 597-604.

Neufeld, G.J., Holliday, C.W. and Pritchard, J.B. 1980. Salinity Adaption of Gill Na, K-ATPase in the Blue Crab, Callinectes sapidus. J. Exp. Zool.: 211, 215-224.

Pressley, T.A., Graves, J.S. and Krall, A.R. 1981. Amiloride-Sensitive Ammonium and Sodium Ion Transport in the Blue Crab. Amer. J. Physiol.: 241, R-370-R378.

Shaw, J. 1964. The Control of Salt Balance in the Crustacea. Symp. Soc. Exp. Biol.: 18, 237-254.

Towle, D.W. 1983. ATP-Dependent Sodium Uptake By Basolateral Membrane Vesicles From the Gill of Green Crab, Carcinus meanas. MDIBL Bulletin.: 23, 10-12.

Towle, D.W. 1974. Equivalence of Gill Na^+ + K^+-ATPases From Blue Crabs Acclimated to High and Low Salinity. Amer. Zool.: 14, 1259.

Towle, D.W., Palmer, G.E. and Harris, J.L. 1976. Role of Gill Na^+ + K^+-Dependent ATPase in Acclimation of Blue Crabs (Callinectes sapidus) to Low Salinity. J. Exp. Zool.: 196, 315-321.

Warnock, D.G., Reenstra, W.W. and Yee, V.J. 1982. Na^+/H^+ Antiporter of Brush Border Vesicles: Studies with Acridine Orange Uptake. Amer. J. Physiol.: 242, F733-F739.

Williams, Austin B. 1965. Marine Decapod Crustaceans, Volume 65 #1. Washington, D.C., U.S. Government Printing Office.

TABLE I

Na^+/NH_4^+ exchange determinations by filtration and supernatent recovery method, in nmoles/ mg protein, using basolateral vesicles of the blue crab, Callinectes sapidus.

NH_4^+ Concentration Used in Vesicular Loading	nmoles Na^+/ mg protein	nmoles NH_4^+ / mg protein
0 mM +ATP	7.6	6.7
0 mM -ATP	7.9	1.3
25 mM +ATP	15.1	15.4
25 mM -ATP	14.6	11.1
125 mM +ATP	34.0	1.3
125 mM -ATP	15.7	3.6
125 mM +ATP	64.3	27.2
125 mM -ATP	21.5	23.3

Figure 1. The effect of membrane incubation time on Na^+ uptake into 100 mM K^+ loaded basolateral membrane vesicles from Callinectes sapidus gill, with (●) and without (o) 4 mM Tris-ATP. Mean ± S.E.

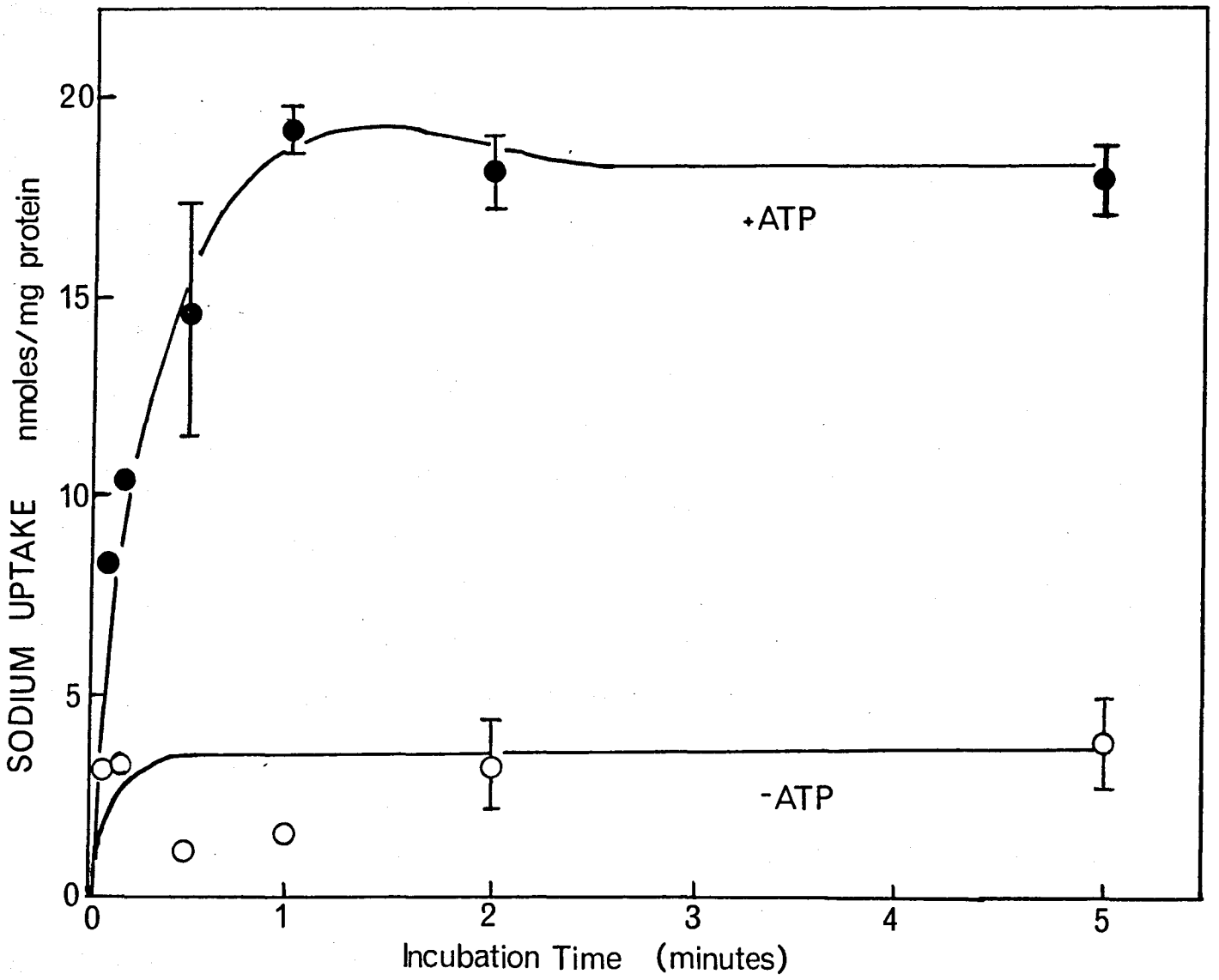

SODIUM UPTAKE nmoles/mg protein
20
15
10
5
0
+ATP
-ATP
0
1
2
3
4
5
Incubation Time (minutes)

Figure 2. The effect of intravesicular K^+ concentration on Na^+ uptake into basolateral membrane vesicles from Callinectes sapidus gills, with (●) and without (o) 4 mM Tris-ATP. Incubation time, 2 minutes. Mean ± S.E.

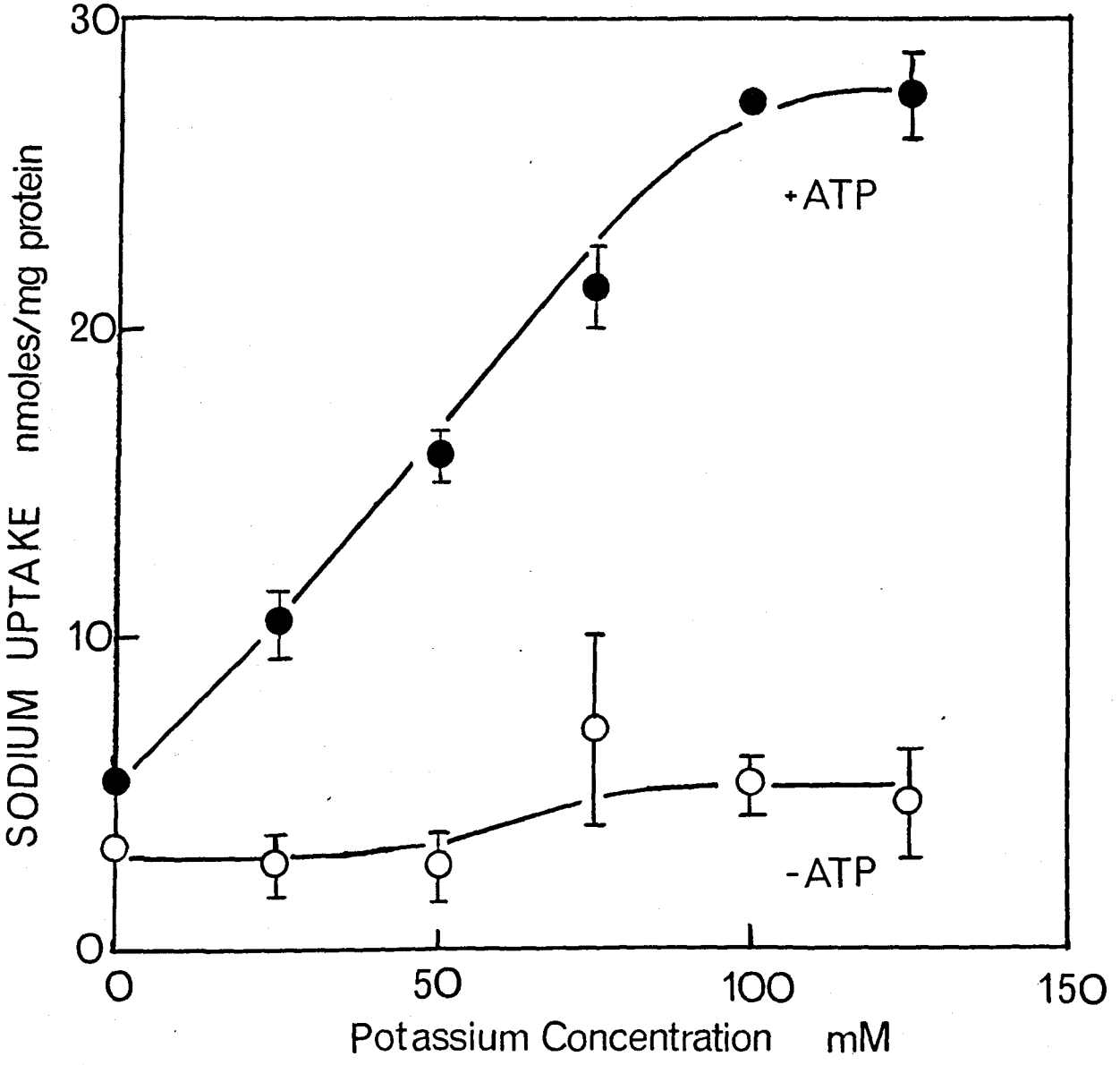

30
20
10
0
SODIUM UPTAKE nmoles/mg protein
+ATP
-ATP
0
50
100
150
Potassium Concentration mM

Figure 3. The effect of intravesicular NH_4 concentrates on Na^+ uptake into basolateral membrane vesicles from Callinectes sapidus gill, with and without 4 mM Tris-ATP. Mean ± S.E.

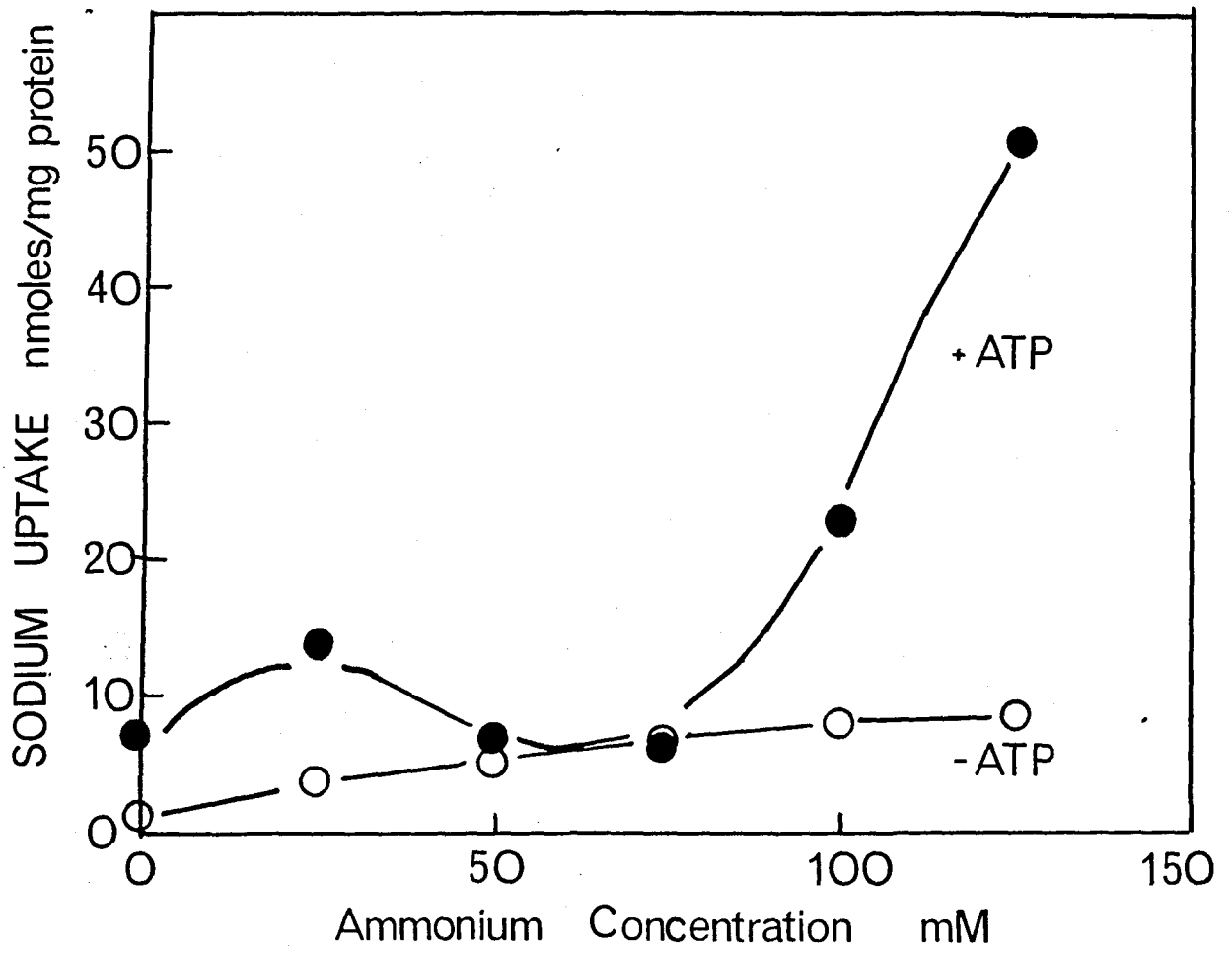

SODIUM UPTAKE nmoles/mg protein
50
40
30
20
10
0
+ ATP
- ATP
0
50
100
150
Ammonium Concentration mM

Figure 4. The effect of ouabain, vanadate and amiloride on sodium uptake in basolateral vesicles of the blue crab, Callinectes sapidus.

i = intravesicular

o = extravesicular

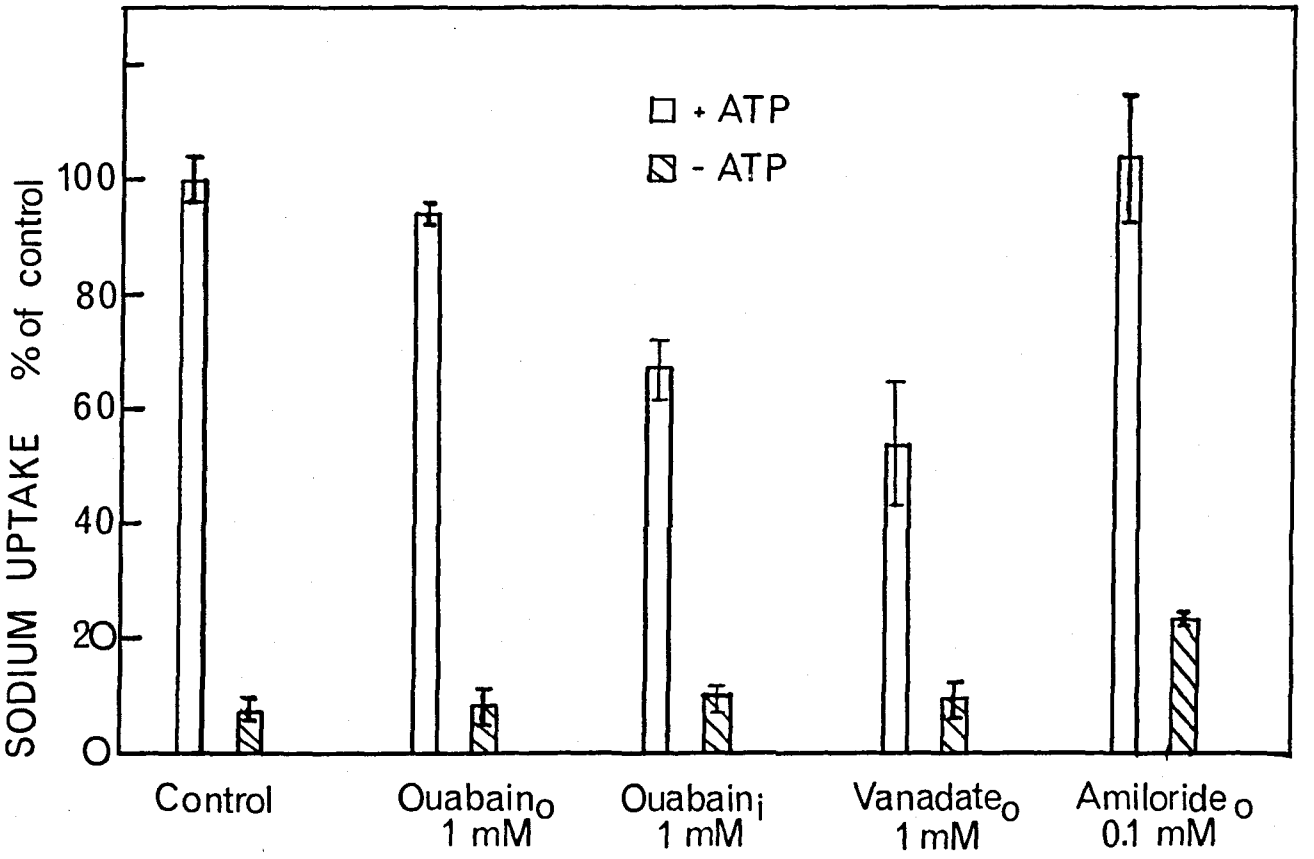

□ + ATP
▨ - ATP
SODIUM UPTAKE % of control
100
80
60
40
20
0
Control
Ouabain$_o$ 1 mM
Ouabain$_i$ 1 mM
Vanadate$_o$ 1 mM
Amiloride$_o$ 0.1 mM

Figure 5. Absorbance changes in Na^+/NH_4^+ transport determination of 25 mM NH_4^+ loaded vesicles from Callinectes sapidus gill, at 340 nm.

(a) 300 ul extravesicular medium without Na^+, with ATP.
3 ul NADH (12 mM)
3 ul ∝- ketoglutarate (200 mM, pH 7.4)

(b) Addition of 20 ul glutamate dehydrogenase (23.3 mg/0.2 ml).

(c) Addition of 5 ul 0 mM NH_4^+ resuspension medium.

(c') Curve in graph indicates gradual stabilization in absorbance.

(d) Addition of 5 ul 25 mM NH_4^+ loaded membrane vesicles.

(e) Addition of 5 ul 0.3 M NaCl.

(f) Addition of 5 ul 7.5% Triton-X.

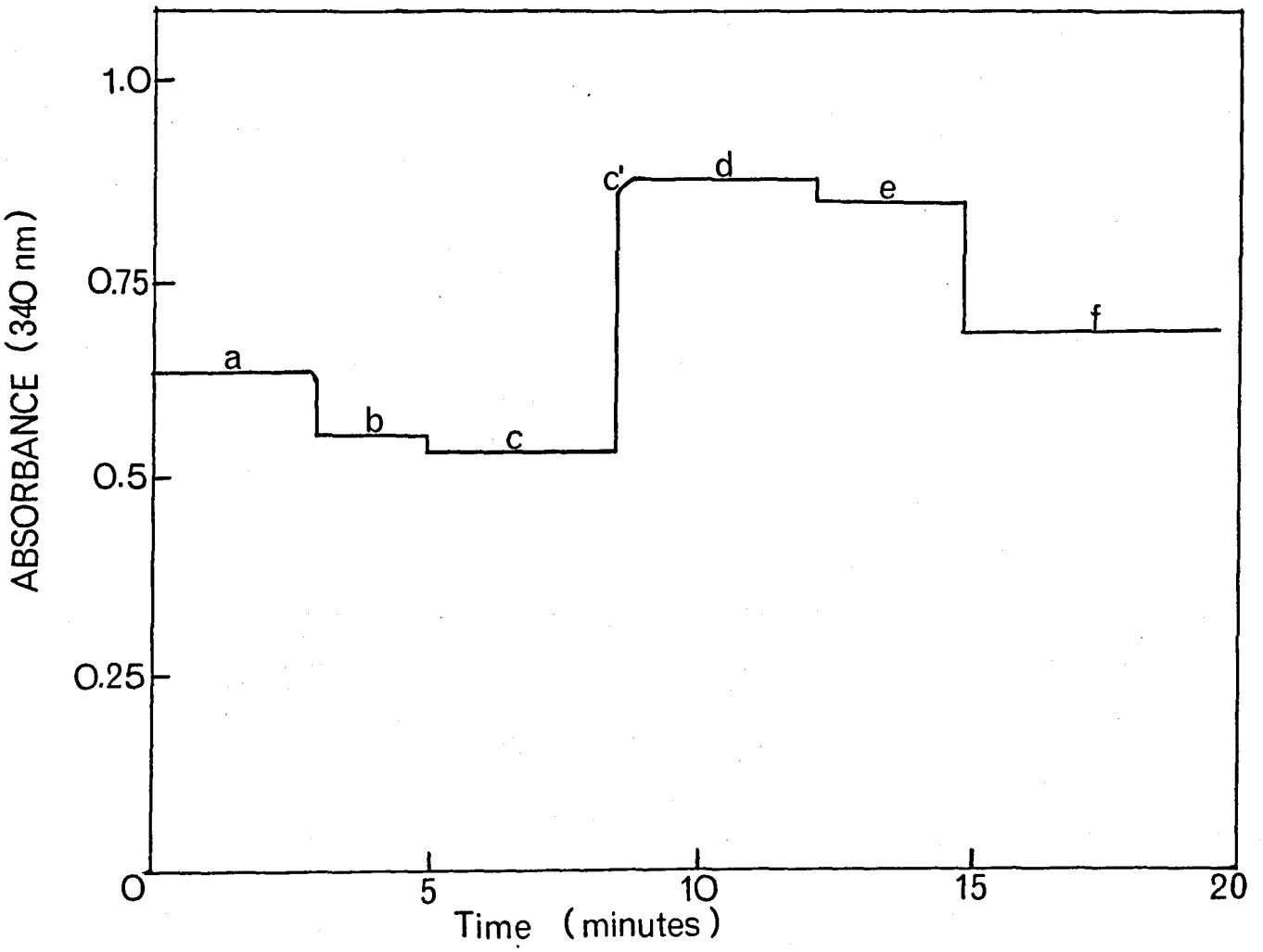

1.0
0.75
0.5
0.25
0
5
10
15
20
ABSORBANCE (340 nm)
Time (minutes)
a
b
c
c'
d
e
f

Figure 6. Absorbance changes in Na^+/NH_4^+ transport determination of 50 mM NH_4^+ loaded vesicles from Callinectes sapidus gill, at 340 nm.

(a) 300 ul extravesicular medium without Na^+, with ATP.

3 ul NADH (12 mM)

3 ul ∝ - ketoglutarate (200 mM, pH 7.4)

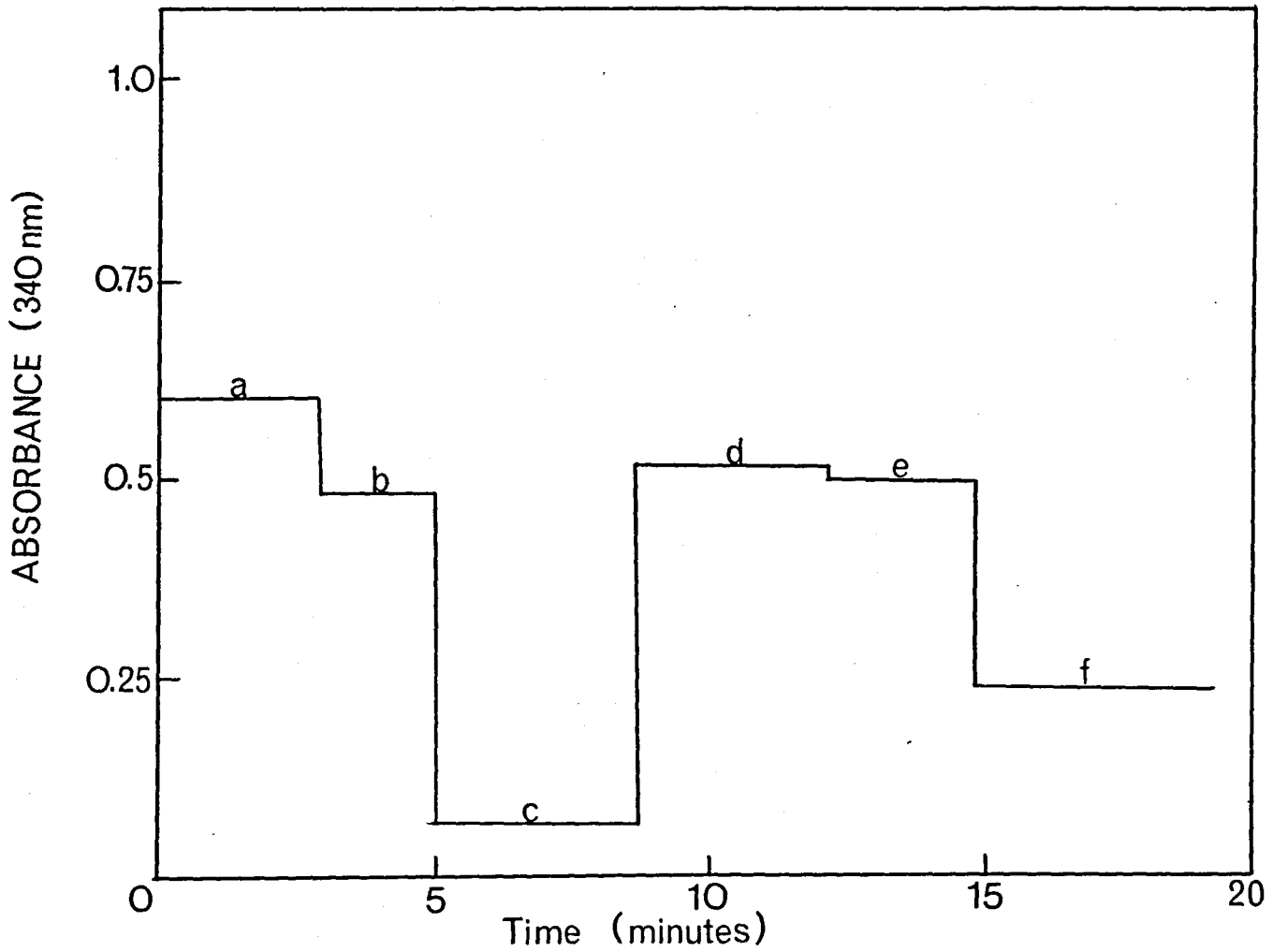
(b) Addition of 20 ul glutamate dehydrogenase (23.3 mg/0.2 ml).

(c) Addition of 5 ul 50 mM NH_4^+ resuspension medium.

(d) Addition of 5 ul 50 mM NH_4^+ loaded membrane vesicles.

(e) Addition of 5 ul 0.3 M NaCl.

(f) Addition of 5 ul 7.5% Triton-X.


ABSORBANCE (340 nm)
1.0
0.75
0.5
0.25
0
5
10
15
20
Time (minutes)
a
b
c
d
e
f

VITA

Linda Ann Fuhrman was born on Novmeber 30, 1960, in Flushing, New York. She attended public schools in Huntington, New York, and graduated from Cold Spring Harbor High School in June, 1978. She attended the University of Richmond in Richmond, Virginia, and received the Bachelor of Science degree with a major in biology in May, 1982. She completed the requirements for the Master of Science degree in biology at the University of Richmond in August, 1985. She was a member of Phi Eta Sigma, Beta Beta Beta, Gamma Sigma Epsilon and the American Society of Zoologists. She was elected an associate member of Sigma Xi while at the University of Richmond. She will begin study towards her Doctor of Veterinary medicine degree at the Virginia-Maryland Regional College of Veterinary Medicine in September of 1985.