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BY

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ABSTRACT

Evidence from this study suggests that $Na^+ + K^+$-activated ATPase is involved in the ionic regulation of sodium in the hemolymph of the blue crab in low salinity environments. The time course of changes in gill $Na^+ + K^+$-ATPase specific activity following transfer of crabs from 30 o/oo to 5 o/oo salinity indicates that a major increase in activity occurred within 2.5-3.0 hr. Apparently, a 8.8-11.1% decrease in hemolymph sodium ion concentration to about 355-360 meq/1 in approximately 2-3 hr was sufficient to activate the ATPase cation transport system in the gills of Callinectes sapidus. Following enzymatic activation, hemolymph sodium concentration gradually approached stabilization at 270-310 meq/1 within 24 hr. Acclimation to high salinity (30 o/oo) occurred within 2 hr after transfer from 5 o/oo salinity.

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INTRODUCTION

Of the environmental parameters which play significant roles in distribution of animals, salinity is of particular importance to aquatic organisms. Animals which can tolerate wide ranges of salinity are, by definition, euryhaline and those which are restricted to narrow ranges are stenohaline. Euryhaline animals may be able to invade and survive in fresh, brackish, and marine environments, either because they can tolerate large changes in the osmotic concentration of body fluids (osmoconformers) or because they can regulate the osmotic concentration of the blood independently of the environment (osmoregulators). In some aquatic animals, osmoregulation is well developed and the concentration of osmotically active substances is maintained at relatively constant levels regardless of the environmental salinity. In others, the osmoregulatory ability is not sufficient to maintain osmotic stability in different salinities and thus their cells and tissues must be able to endure some degree of fluctuation in osmotic concentration.

The osmotic concentration of the blood depends on the total number of solute substances present, irrespective of kind. The Na^+ and Cl^- concentrations are responsible for the bulk of the osmotic pressure exerted by the blood of most organisms (Schoffeniels and Gilles, 1970). Ionic regulation therefore appears to be associated in a significant manner with the mechanism of osmoregulation.

The common blue crab, Callinectes sapidus Rathbun, is both euryhaline and eurythermal, and thus is one of the most successful estuarine species. It is known to inhabit environments ranging from fresh (Gunter, 1938; Odum, 1953; Copeland and Fitzjarrell, 1968; Mangum and Amende, 1972) to hypersaline waters (Gifford, 1962; Hedgepeth, 1967). In Virginia waters, it is found in salinities from 30-32 parts per thousand (o/oo) total salinity to essentially fresh water (0-0.5 o/oo) (Van Engel, 1958; Mangum and Amende, 1972).

The life cycle of the blue crab involves movements across salinity gradients with salinity-related, age, sexual, and seasonal distribution patterns (Ballard and Abbott, 1969; Engel and Eggert, 1974). After hatching, the zoeal larvae of C. sapidus normally develop in salinities between 20-30 o/oo (Sandoz and Rogers, 1944; Costlow and Bookout, 1959). At salinities below 20 o/oo the zoeal larvae do not develop probably because they absorb water at the molt stages and they may be very sensitive to dilution of the medium at this stage of their life cycle (Prosser, 1973). Upon reaching the first crab stage, immature crabs of both sexes migrate toward lower salinity environments. At maturity, females return to the high salinity waters to spawn. Thus, male crabs are predominant in the low salinities and females in the high salinities.

Ionic regulation plays a significant role in the osmoregulatory ability of the blue crab. It has been demonstrated that the blue crab maintains its hemolymph ionic concentration

hyperosmotic to the medium in low salinity (below 28 o/oo) environments and isosmotic at 28-35 o/oo salinity (Tan and Van Engel, 1966; Mantel, 1967; Ballard and Abbott, 1969; Tagatz, 1971; Gerald and Gilles, 1972; Lynch et al., 1973; Weiland and Mangum, 1975). There is evidence that the main site of ion absorption in Crustaceans is through the gills (Nagel, 1934; Krogh, 1939; Lockwood, 1962; Potts and Parry, 1964; Prosser, 1973). The active absorption of sodium ions (Gerard and Gilles, 1972; Colvocoresses et al., 1974; Engel et al., 1974; Mangum et al., 1975) by the gill epithelia (Mantel, 1967; Schoffeniels and Gilles, 1970; Smith and Linton, 1971) appears to be a major mechanism of hemolymph sodium regulation by C. sapidus in hypo-osmotic conditions.

The oxygen consumption of C. sapidus increases by 30-50% when placed in brackish water (King, 1965). Respiration of excised gills is known to be affected by salinity (Engel and Eggert, 1974). Mantel (1967) found that a part of the oxygen consumption by isolated gills is dependent on the presence and/or transfer of Na^+, since respiration is decreased by ouabain and by a Na^+-free medium.

Copeland and Fitzjarrell (1968) have shown a highly developed epithelial layer of cells (patches) located in the respiratory (lamillar) platelets of the gills of C. sapidus. These cells are presumed to provide the mechanism for salt absorption by active transport. The cell patches are much more extensive in gills of animals acclimated to low salinities

(Prosser, 1973).

The membrane-localized enzyme, ouabain sensitive, Na^++K^+-activated adenosine triphosphatase (Na^++K^+-ATPase) is believed to play an essential part in the active exchange of Na^+ for K^+ across the plasma membranes of many animal cells (Skou, 1965; Glynn, 1968; Glynn and Karlish, 1975). According to Quinn and Lane (1966) the activity of the Na^++K^+-ATPase is characterized by its ability to catalyze the hydrolysis of ATP; by the requirement of magnesium (Mg^{2+}); by maximal activity in the presence of both Na^+ and K^+; by ouabain inhibition; and by its presence in the internal cell membranes and plasma membranes. Principal support for the hypothesis that Na^++K^+-ATPase is correlated with active transport comes from ouabain inhibition. Ouabain, a cardiac glycoside, has been shown to inhibit both cation transport in the intact cell (Glynn, 1957) and Na^++K^+-ATPase activity of membrane fragments (Skou, 1965).

Another characteristic of Na^++K^+-ATPase is that NH_4^+ can replace K^+ with little loss of activity in the enzyme system. The ability of NH_4^+ to serve as an effective counterion, coupled with the finding that the output of ammonia in C. sapidus increases in dilute sea water (Mangum et al., 1975), supports the hypothesis that C. sapidus osmoregulates at low salinity by actively exchanging NH_4^+ for Na^+ at the gill (Mangum, et al., 1975; Towle and Harris, 1976). It has been demonstrated that increased sodium uptake in C. sapidus is correlated with increased Na^++K^+-ATPase specific activity of gill microsomes. (Towle, 1974;

Towle and Harris, 1976).

Since sodium ions account for approximately 85.5% of the total cations in the hemolymph of the blue crab (Florkin, 1960), experiments designed to measure the in vivo concentration of this ion would indicate its role in the overall ionic regulation of this species. The present study describes some of the characteristics of the $Na^+ + K^+$-ATPase enzyme system prepared from crabs acclimating to low salinity and reports some results relating the in vitro $Na^+ + K^+$-ATPase to the ionic regulatory mechanism in the intact animal.

Some of the field data representing hemolymph sodium ion concentration of crabs in various salinities are obscure. Colvocoresses et al. (1974) cited a mean serum sodium concentration of 350 milliequivalents per liter (meq/1) for blue crabs found in 21 o/oo salinity at 26° C in the York River. Five days later, a mean serum sodium concentration of 400 meq/1 was recorded for crabs found in the same salinity, temperature, and location. Apparently, the effects of migration and tidal influences were not taken into consideration and thus there are some inconsistances in the data. In the present study, an attempt is made to establish the relative lapse of time incurred in the acclimation of crabs to low salinity. A preliminary report of some of these results has already appeared (Mangum, et al., 1975; Towle and Harris, 1976).

MATERIALS AND METHODS

Experimental animals

Adult intermolt male blue crabs, (Callinectes sapidus Rathbun), were obtained from a seafood distributor in Richmond, Virginia, in June-August 1975. Reportedly, these crabs were caught in pots at Gwynn Island (Mathews, Virginia) on the Chesapeake Bay. Crabs measured 12-14 cm in carapace length (point to point distance between lateral spines) and weighed 170-200 g. Prior to the experiments, crabs were acclimated two days without food in well-aerated recirculating water of desired salinity at 18°C. A 12-hr light-dark photoperiod of fluorescent light was established. All salinity concentrations were prepared by diluting Instant Ocean Synthetic Sea Salts (I.O.S.S.S.) (Aquarium Systems, Inc.) with tap water. Salinities were periodically assayed with a flame photometer for Na^+ concentration and confirmed with a Van Waters and Rogers glass hydrometer. At 34 o/oo (average salinity of oceanic sea water), I.O.S.S.S. has 10.2 o/oo Na^+ concentration. The sodium value cited by Barnes (1954) for natural sea water is 10.56 o/oo.

Several of the crabs had lost some of their appendages. Chelipeds, when present, were secured with rubber bands to prevent cannibalism. Where technically feasible, the same crabs were used throughout the entirety of each separate experiment. Data from crabs that died during experimentation were discarded.

Hemolymph Na^+ analysis

At designated time intervals, a single (0.1 ml) sample of prebranchial hemolymph was extracted from the arthrodial membrane at the base of a "walking leg", within 15 sec after removal of

the crab from water. Hemolymph samples were drawn with (1cc) Stylex disposable plastic syringes with 22-guage ($1\frac{1}{2}$") needles and frozen immediately in the syringes at -20°C until assayed. Each crab was transferred to the experimental acclimation salinity immediately after an initial sample was taken.

Clots in the hemolymph were disrupted when the thawed hemolymph was ejected from the syringe into Microfuge test tubes. After centrifuging the sample in a Beckman/Spinco 152 Microfuge for 5 min. at 25°C, the supernatant serum was used for the determination of Na^+ concentration. A dilution (1:1000) of serum in deionized-distilled water and 0.02% Sterex SE detergent (Harleco) was assayed with a Coleman Model 21 flame photometer and Model 22 Galv-o-meter. A calibration curve was established for conversion of percent transmittance to milliequivalents Na^+/liter, as a direct read-out scale was not available.

Crab gill microsome preparation

The crab gill microsomal fraction was prepared by a modified method of Hendler et al. (1972). All operations were performed at 0-4°C unless otherwise noted. The right-side gills of individual crabs were excised, pooled, and rinsed in a homogenizing solution containing 0.25M sucrose, 6mM disodium ethylene-diamine tetra-acetic acid (EDTA) and 20mM imidazole-HCl (pH 6.8). Following surgical removal of afferent and efferent vessels, the gills were blotted briefly, weighed (nearest 0.1 mg) and placed in a fresh volume (10 ml/g) of homogenizing solution containing 0.1% (w/v) sodium deoxycholate. The tissue was homogenized at 1725 rev/min

in a Teflon-glass Potter-Elvehjem apparatus using 18 complete up and down strokes. The resulting homogenate was strained through two single layers of gauze and centrifuged at 10,800 x gmax for 35 min in a Beckman Model L3-50 Ultracentrifuge. The supernatant was then decanted and centrifuged at 105,000 x gmax for 60 min. After removing the supernatant, the microsomal pellet was resuspended in a volume (in ml) of fresh homogenizing solution (without sodium deoxycholate) equivalent to 5 times the original gill weight (in g). The suspension was homogenized (3-4 strokes) in a Teflon-glass homogenizer and frozen at -20°C in Teflon-capped glass test tubes until assayed. When time did not allow for immediate gill microsomal preparation, pooled gills were frozen at -20°C in 2 ml of homogenizing solution. The gill microsomal fraction was always prepared within 24 hr after excision.

Assay of Na^++K^+-dependent ATPase activity

The ouabain-sensitive, Na^++K^+-dependent ATPase specific activity of the gill microsomes was assayed according to a modified method of Towle and Harris (1976). The microsomal suspensions were thawed in ice water and vortexed briefly. Triplicate 50 µl samples (20-25 µg of microsomal protein) were preincubated at 25°C for 10 min in the total ATPase assay medium containing 20mM imidazole-HCl (pH 7.8), 100mM NaCl, 30mM KCl, and 5mM $MgCl_2$ (final concentrations). Identical triplicate samples were preincubated in the control assay medium which contained 20mM imidazole-HCl (pH 7.8), 130mM NaCl, 5mM $MgCl_2$, and 1mM ouabain. The enzymatic reaction was started by adding 0.2 ml of substrate (50mM disodium

ATP, pH 7.0 with 10% imidazole) to each tube, resulting in a total volume of 2 ml. Following incubation at 25°C for 30 min, the reaction was terminated by adding 2 ml of ice-cold 10% (w/v) trichloroacetic acid to each tube and placing them in an ice water bath. Each tube was then centrifuged at 10,800 x gmax for 10 min. Liberated inorganic phosphate in the supernatant was measured spectrophotometrically in a Baush and Lomb Spectronic 700 according to the phosphorus estimation methods of Fiske and SubbaRow (1925). Protein concentrations were determined by the procedure of Lowry et al. (1951). Ouabain-sensitive, $Na^+ + K^+$-dependent ATPase specific activity was calculated by subtracting the ouabain insensitive (control) value from the total ATPase value yielding µmoles of inorganic phosphate per mg of microsomal protein per min.

RESULTS

Preliminary investigations determined the effects of chelliped binding and other conditions on hemolymph sodium concentration. Haefner's (1971) study demonstrated that claw binding does not interfere with feeding, molting, or survival of captive crabs. The present study revealed that there was no significant variation in hemolymph sodium ion concentration due to autotomy of limbs, frequent hemolymph sampling, or short-term starvation (less than 2.5 days). Removal of hemolymph samples, either by aspiration with a syringe or by severing a pereiopod, had no effect on sodium ion concentration. There was no

difference in fresh serum or frozen serum thawed once.

The sodium concentration of the external medium was approximately 400 milliequivalents per liter (meq/1) for 30 o/oo salinity, 325 meq/1 for 25 o/oo, and 65-70 meq/1 for 5 o/oo salinity (Figure 1). The mean serum sodium concentration varied with the external salinity. It ranged from 380-410 meq/1 at 30 o/oo, 395 meq/1 at 25 o/oo, and 270-310 meq/1 at 5 o/oo (Figure 2). In acclimation from a salinity of 30 o/oo to 5 o/oo, C. sapidus mean serum sodium ion concentration fell from 398 meq/1 to 312 meq/1 in approximately 24 hr (Figure 3). Approximately 2-3 hr after transfer to low salinity, the rate of sodium dilution is slowed at 350-360 meq/1. In another acclimation experiment, transfer from 30 o/oo to 5 o/oo caused the mean serum sodium concentration to fall from 405 meq/1 to 310 meq/1 within 5 hr (Figure 4). The rate of dilution in hemolymph concentration decreased around 350 meq/1 serum sodium concentration after 2 hr acclimation. Upon transferring the same crabs back to high salinity (30 o/oo) after 24 hr adaption, the mean serum sodium concentration rose from 310 meq/1 to 380 meq/1 and stabilized in approximately 2 hr (Figure 5). After transfer from 25 o/oo to 5 o/oo salinity, C. sapidus mean serum sodium drops from 395 to 270 meq/1 in 24 hr (Figure 6). The rate of dilution slowed between 2-3 hr and at this time the mean hemolymph sodium concentration was estimated at 355 meq/1.

Preliminary experiments comparing the $Na^+ + K^+$-ATPase activity

of fresh and frozen gill microsomes from crabs demonstrated that the difference in storage conditions produced less than 1% difference in Na^++K^+-ATPase specific activities. Quinn and Lane (1966) have stored frozen gill (Carcinus maenus) homogenates for 4-6 months with no significant loss in Na^++K^+-ATPase activity. Although the in vitro temperature optimum for Na^++K^+-ATPase specific activity has been shown to be 37-45°C for C. maenus (Quinn and Lane, 1966), assays were performed at 25°C to approach in vivo environmental conditions for C. sapidus. The concentration of microsomes per assay (20-25 µg protein) was well within the linear range of proportional ATPase activity defined for C. sapidus (Towle and Harris, 1976).

From a comparative study, it was revealed that the microsomes of the posterior gills of the blue crab adapted to 30 o/oo salinity possess a Na^++K^+-ATPase specific activity 23% greater than that of the anterior gills. Also, excised gills from crabs in low salinity (5 o/oo) exhibited almost twice as much specific activity as those from crabs adapted to 30 o/oo salinity. A major increase in activity of the Na^++K^+-ATPase occurred within 2.5-3.0 hr following transfer from 30 o/oo to 5 o/oo salinity (Figure 7). The increase in activity at 2.5 hr was 51% greater than the control value (t=0) and 70% greater than the control at 3 hr.

DISCUSSION

The osmoconforming and hyperregulatory abilities of C.

sapidus are well demonstrated by a comparison of sodium concentration of the hemolymph and acclimation medium. The data in this study show that the hemolymph sodium concentration is essentially isionic at 28-30 o/oo and hyperionic from 28-5 o/oo salinity. This follows the trends which have been established for the blue crab by other investigators (Mantel, 1967; Ballard and Abbott, 1969; Smith and Linton, 1971; Engel et al., 1974; Colvocoresses et al., 1974).

The finding that a two-fold increase in Na^++K^+-ATPase specific activity in gills of crabs acclimated to dilute medium is consistent with previous investigations of Na^++K^+-ATPase activity from gills of blue crabs adapted to low and high salinities (Towle, 1974; Towle and Harris, 1976). The increased gill Na^++K^+-ATPase activity in crabs acclimated to 5 o/oo salinity may account for their ability to maintain their hemolymph sodium ion concentration hyperionic to the environment. The possible existence of two different Na^++K^+-ATPases, one predominating in a high salinity environment and another in low salinity, has been discounted previously (Towle, 1974; Towle and Harris, 1976).

There have been several observations that indicate functional differences in the osmoregulatory ability of anterior and posterior gills of euryhaline crabs (Florkin and Schoffeniels, 1969; King and Schoffeniels, 1969). The posterior gills possess large osmophlic patches and the anterior gills possess either smaller patches or none at all (Copeland and Fitzjarrell, 1968).

Also, differences in respiration rates and differences in total ATP levels between anterior and posterior gills have been reported (Engel, et al., 1974). This is in agreement with the present finding that, even at 30 o/oo salinity, the posterior sets of gills possess a 23% greater $Na^+ + K^+$-ATPase activity than the anterior gills.

Evidence from this study suggests that $Na^+ + K^+$-activated ATPase is involved in the osmoregulatory ability, or more specifically, the ionic regulation of sodium in the hemolymph of C. sapidus in low salinity environments. The time course of changes in gill $Na^+ + K^+$-ATPase specific activity following transfer of crabs to 5 o/oo sea water indicates that a major increase in activity occurs within 2.5-3.0 hr. At 3 hr, there is a 70% increase in $Na^+ + K^+$-ATPase specific activity over that of the control. Since the activity of $Na^+ + K^+$-ATPase from low salinity adapted crabs is 80% greater than the activity of the high salinity adapted crabs (Towle, 1974), it followed then that at 3 hr acclimation to 5 o/oo, the $Na^+ + K^+$-ATPase should be almost completely activated. A comparison of in vivo sodium ion changes with time indicates that a reduction in hemolymph sodium ion dilution occurs at 2-3 hr in adaption to low salinity. Serum sodium concentrations continue to fall slowly until stabilization is reached at 270-310 meq/l in approximately 24 hr.

The activation of the ionic regulatory mechanism may be initiated by a certain percent decrease in the hemolymph sodium ion concentration upon entering brackish and fresh water

environments. Perhaps when a critical hemolymph sodium concentration is approached the Na^++K^+-ATPase enzyme system is activated fully. It has been demonstrated that a 5% decrease in hemolymph sodium concentration of Carcinus maenus is sufficient to activate the sodium transport system (Potts and Parry, 1964). The results of this study indicate that a 8.8-11.1% decrease in sodium concentration to about 355-360 meq/1 in approximately 2-3 hr is sufficient to activate the ATPase cation transport system in the gills of C. sapidus. Following the enzymatic activation, hemolymph sodium concentration gradually approaches stabilization at 270-310 meq/1 within 24 hr in 5 o/oo salinity.

Acclimation to high salinity (30 o/oo) occurs within approximately 2 hr after transfer from 5 o/oo and the serum sodium concentration stabilizes at approximately 380 meq/1. The time course of Na^++K^+-ATPase activity would be expected to show a major decrease in activity at approximately 2 hr, indicating that the hemolymph sodium concentration is isionic with the environment. Active uptake of ions has been shown to decline sharply when hemolymph sodium ion concentration approaches 400 meq/1 in C. maenus (Potts and Parry, 1964). The fact that the sodium concentration in the present study stabilized at 380 meq/1 and not at 405 meq/1 (the original hemolymph sodium concentration in 30 o/oo salinity) is not uncommon. Ballard and Abbott (1969) reported a hysteresis-like effect in the osmotic accomodation of the blue crab to different salinities. The hemolymph concentration after acclimation is higher when a given salinity is

approached from a higher salinity than when approached from a lower one.

It is possible that a complex neurohormonal system is involved in the acclimation process (Kamenoto and Ono, 1969; Kato and Kamemoto, 1969). Several investigations have revealed that a hormone present in the eyestalks of euryhaline Crustaceans may stimulate the $Na^+ + K^+$-ATPase in the gills (Prosser, 1973; Heit and Fingerman, 1975), or it may cause a reduced permeability of the epithelial cells (Whitney, 1974).

Further studies of ATPase activity and ionic regulation in the blue crab may provide clues to the many factors and complex mechanisms involved in active membrane transport.

LITERATURE CITED

Ballard, B. S. and W. Abott. 1969. Osmotic accomodation in Callinectes sapidus Rathbun. Comp. Biochem. Physiol., 29:671-687.

Barnes, H. J. 1954. Ionic composition of sea water, J. Exp. Biol., 31:582-588.

Colvocoresses, J. A., M. P. Lynch and K. L. Webb. 1974. Variations in serum constituents of the blue crab, Callinectes sapidus: Major cations. Comp. Biochem. Physiol., 49A:787-803.

Copeland, D. E. and A. T. Fitzjarrell. 1968. The salt absorbing cells of the blue crab. Z. Zellforschung, 92:1-22.

Costlow, J. D. and C. G. Bookout. 1954. The larval development of Callinectes sapidus Rathbun reared in the laboratory. Biol. Bull., 116:373-396.

Engel, D. W., E. M. Davis, D. E. Smith and J. W. Angelovic 1974. The effect of salinity and temperature on the ion levels in the hemolymph of the blue crab, Callinectes sapidus, Rathbun. Comp. Biochem. Physiol., 49A:259-266.

Engel, D. W. and L. D. Eggert. 1974. Effect of salinity and sex on respiration rates of excised gills of the blue crab. Comp. Biochem. Physiol., 47A:1005-1011.

Fiske, C. H. and Y. SubbaRow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem., 66:375-400.

Florkin, M. 1960. Blood chemistry. In: The physiology of Crustacea. T. H. Waterman, ed. Academic Press, New York, pp 670.

Florkin, M. and E. Schoffeniels. 1969. An osmotic extracellular and isosmotic intracellular regulations as examples of physiological radiations of biochemical systems. In: Molecular approaches to ecology. Academic Press, New York, pp 112-163.

Gerard, J. F. and R. Gilles. 1972. The free amino-acid pool in Callinectes sapidus (Rathbun)tissues and its role in the osmotic intracellular regulation. J. Exp. Mar. Biol. Ecol., 10:125-136.

Gifford, C. A. 1962. Some aspects of osmotic and ionic regulation in the blue crab, Callinectes sapidus, and the ghost crab, Ocypode albicans. Pub. Inst. Mar. Sci., Texas, 8:97-125.

Glynn, I. M. 1957. The action of cardiac glycosides on ion movements. Pharmacol. Rev., 16:381-407.

Glynn, I. M. 1968. Membrane ATPase and cation transport. Brit. Med. Bull., 14:165-169.

Glynn, I. M. and S. D. J. Karlish. 1975. The sodium pump. Ann. Rev. Physiol., 37:13-55.

Gunter, G. 1938. The common blue crab in fresh water (Callinectes sapidus). Sci., 87:87-88.

Haefner, P. A. 1971. The use of elastrator rings for binding crab claws. Chesap. Sci., 12:183-184.

Hedgepeth, J. 1967. Ecological aspects of the Laguna Madre, a hypersaline estuary. In: Estuaries. G. H. Lauff, ed. Amer. Assoc. Adv. Sci., Washington, D. C., pp 408-419.

Heit, M. and M. Fingerman. 1975. The role of an eyestalk hormone in the regulation of sodium concentration of the blood of the fiddler crab, Uca pugilator. Comp. Biochem. Physiol., 50A:277-280.

Hendler, E. D., J. Torretti, L. Kuper and F. H. Epstein. 1972. Effects of adrenalectomy and hormone replacement on Na-K-ATPase in renal tissue. Amer. J. Physiol., 222:754-760.

Kamemoto, F. I. and J. K. Ono. 1969. Endocrine control of osmoregulation in Crustaceans. Comp. Biochem. Physiol., 29:393-401.

Kato, K. N. and F. I. Kamemoto. 1969. Neuro-endocrine involvement in osmoregulation in the grapsid crab, Metopograpsus messor. Comp. Biochem. Physiol., 28:665-674.

King, E. N. 1965. The oxygen consumption of intact crabs and excised gills as a function of decreased salinity. Comp. Biochem. Physiol., 15:93-102.

King, E. N. and E. Schoffeniels. 1969. Gill potentials in crabs. Arch. Int. Physiol. Biochem., 77:105-111.

Krogh, A. 1939. Osmotic regulation in aquatic animals. Cambridge University Press, London, pp 65-98.

Lockwood, A. P. M. 1962. Osmoregulation of Crustaceans. Biol. Rev., 37:257-305.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randell. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193:265-275.

Lynch, M. P., K. L. Webb and W. A. Van Engel. 1973. Variations in serum constituents of the blue crab, Callinectes sapidus: Chloride and osmotic concentration. Comp. Biochem. Physiol. 44A:719-734.

Mangum, C. P. and L. M. Amende. 1972. Blood osmotic concentration of the blue crab, Callinectes sapidus Rathbun, found in fresh water. Chesap. Sci., 13:318-320.

Mangum, C. P., S. U. Silverthorn, J. L. Harris III, D. W. Towle and A. R. Krall. 1975. The relationship between blood pH, ammonia excretion and adaption to low salinity in the blue carb, Callinectes sapidus. J. Exp. Zool., submitted.

Mantel, L. H. 1967. Gill potentials and ion regulation in Callinectes sapidus. Comp. Biochem. Physiol., 20:743-753.

Nagel, H. 1934. Die Aufgeben der Exkretionsorgne und der Kiemen bei der Osmoregulation von Carcinus maenus. Z. Vergl. Physiol., 21:468-491.

Odum, H. T. 1953. Factors controlling marine invasion into Florida fresh waters. Bull. Mar. Sci. Gulf and Carib., 3:134-156.

Potts, W. T. W., and G. Parry. 1964. Osmotic and ionic regulation in animals. Pergamon Press, Oxford, pp 119-143.

Prosser, C. L. 1973. Comparative animal physiology. W. B. Sanders Co., Philadelphia.

Quinn, D. J., and C. E. Lane. 1966. Ionic regulation and Na^+-K^+-stimulated ATPase activity in the land crab, Cardisoma guanhumi. Comp. Biochem. Physiol., 19:533-543.

Sandoz, M. and R. Rogers. 1944. The effect of environmental factors on hatching, molting, and survival of zoeal larvae of the blue crab, Callinectes sapidus Rathbun. Ecol., 25:216-228.

Schoffeniels, E. and R. Gilles. 1970. Osmoregulation in aquatic arthropods. In: Chemical zoology, Vol 5. M. Florkin and B. T. Scheer, eds. Academic Press, New York, pp 255-286.

Skou, J. C. 1965. Enzymatic basis for active transport of Na^+ and K^+ across cell membranes. Physiol. Rev., 45:596-617.

Smith, D. S. and J. R. Linton. 1971. Potentiometric evidence for the active transport of sodium and chloride across excised gills of Callinectes sapidus. Comp. Biochem. Physiol., 39A:367-378.

Tagatz, M. E. 1971. Osmoregulatory ability of blue crabs in different temperature-salinity combinations. Chesap. Sci., 12:14-17.

Tan, E. C. and W. A. Van Engel. 1966. Osmoregulation in the blue crab, Callinectes sapidus Rathbun. Chesap. Sci., 7:30-35.

Towle, D. W. 1974. Equivalence of gill Na^++K^+-ATPase from blue crabs acclimated to high and low salinity. Amer. Zool., 14:1259.

Towle, D. W. and J. L. Harris III. 1976. Role of gill $Na^+ + K^+$-dependent ATPase in acclimation of blue crabs (Callinectes sapidus) to low salinity. J. Exp. Zool., submitted.

Van Engel, W. A. 1958. The blue crab and its fishery in the Chesapeake Bay - I. Reproduction, early development, growth and migration. Comml. Fish Rev., 20:6-17.

Weiland, A. L. and C. P. Mangum. 1975. The influence of environmental salinity on hemocyanin function in the blue crab, Callinectes sapidus. J. Exp. Zool., 193:265-274.

Whitney, J. O. 1974. The effect of external salinity upon lipid synthesis in the blue crab, Callinectes sapidus Rathbun, and the spider crab, Libinia emarginata Leech. Comp. Biochem. Physiol., 49A:433-440.

Figure 1. The sodium concentration of synthetic sea water.

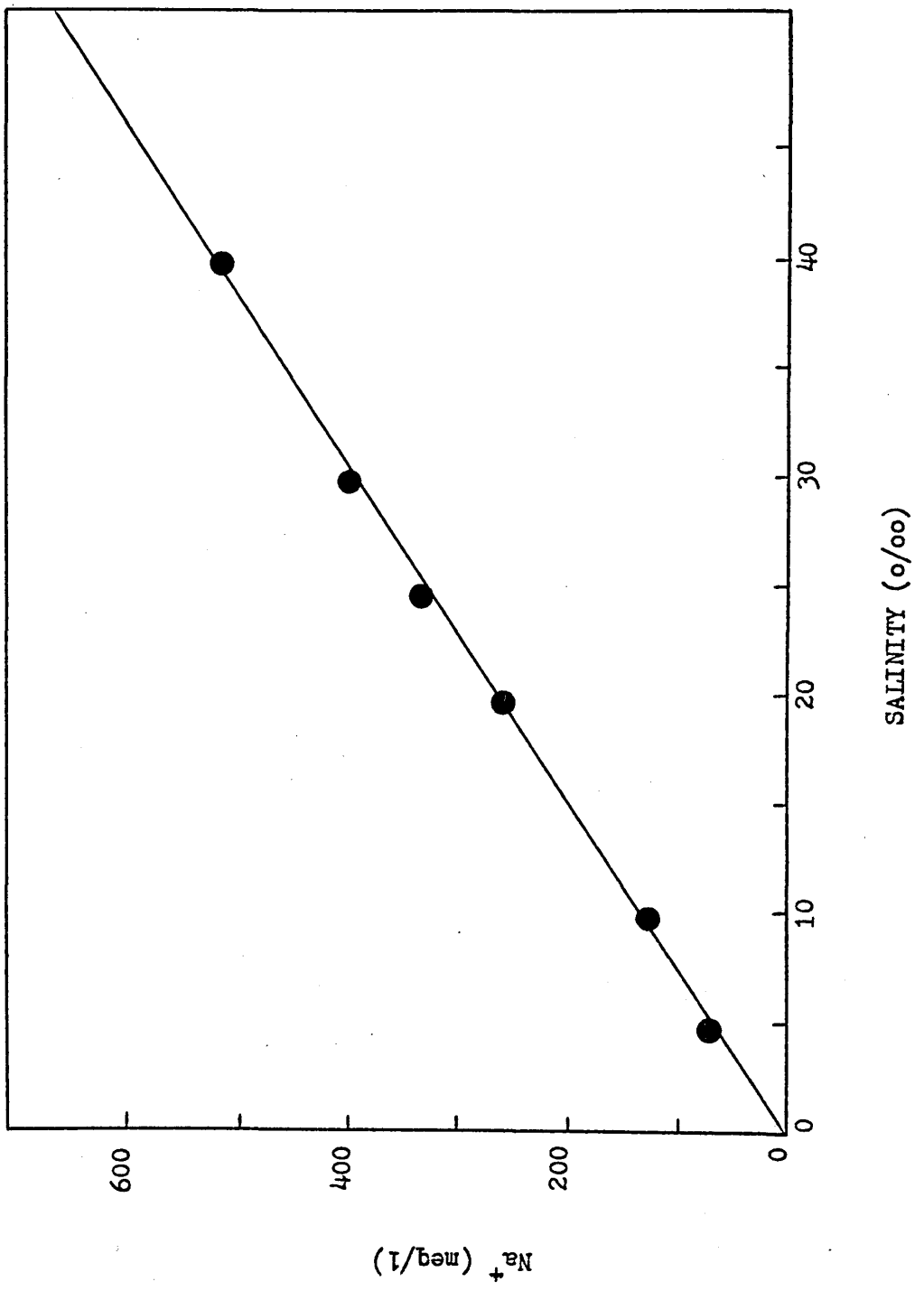

Na^+ (meq/l)
600
400
200
0
0
10
20
30
40
SALINITY (o/oo)

Figure 2. Changes in mean serum sodium concentration with changes in environmental salinity (N=5).

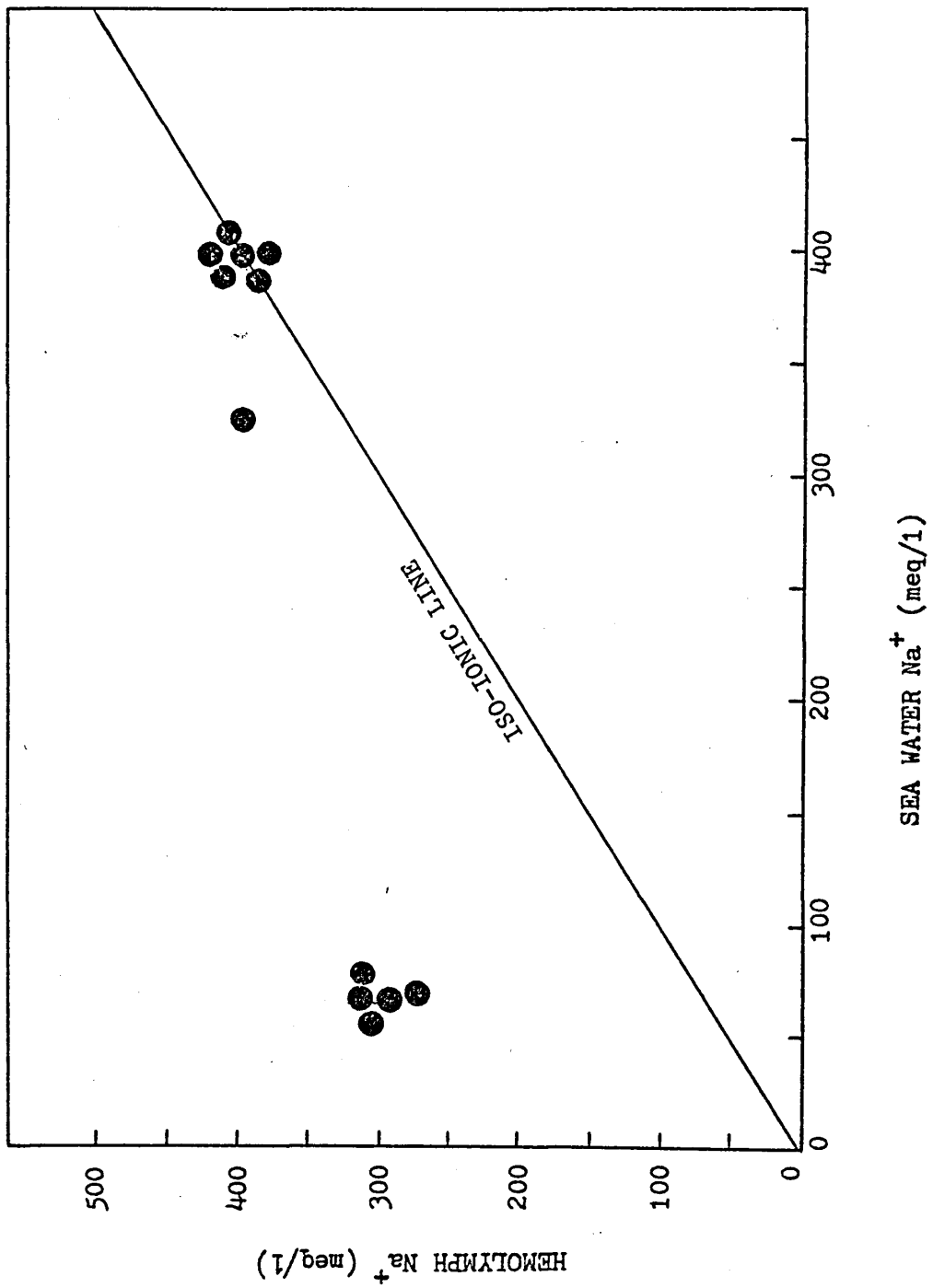

HEMOLYMPH Na^+ (meq/l)
SEA WATER Na^+ (meq/l)
ISO-IONIC LINE
0
100
200
300
400
500

Figure 3. Change in mean serum sodium concentration following transfer from 30 o/oo to 5 o/oo salinity (N=5).

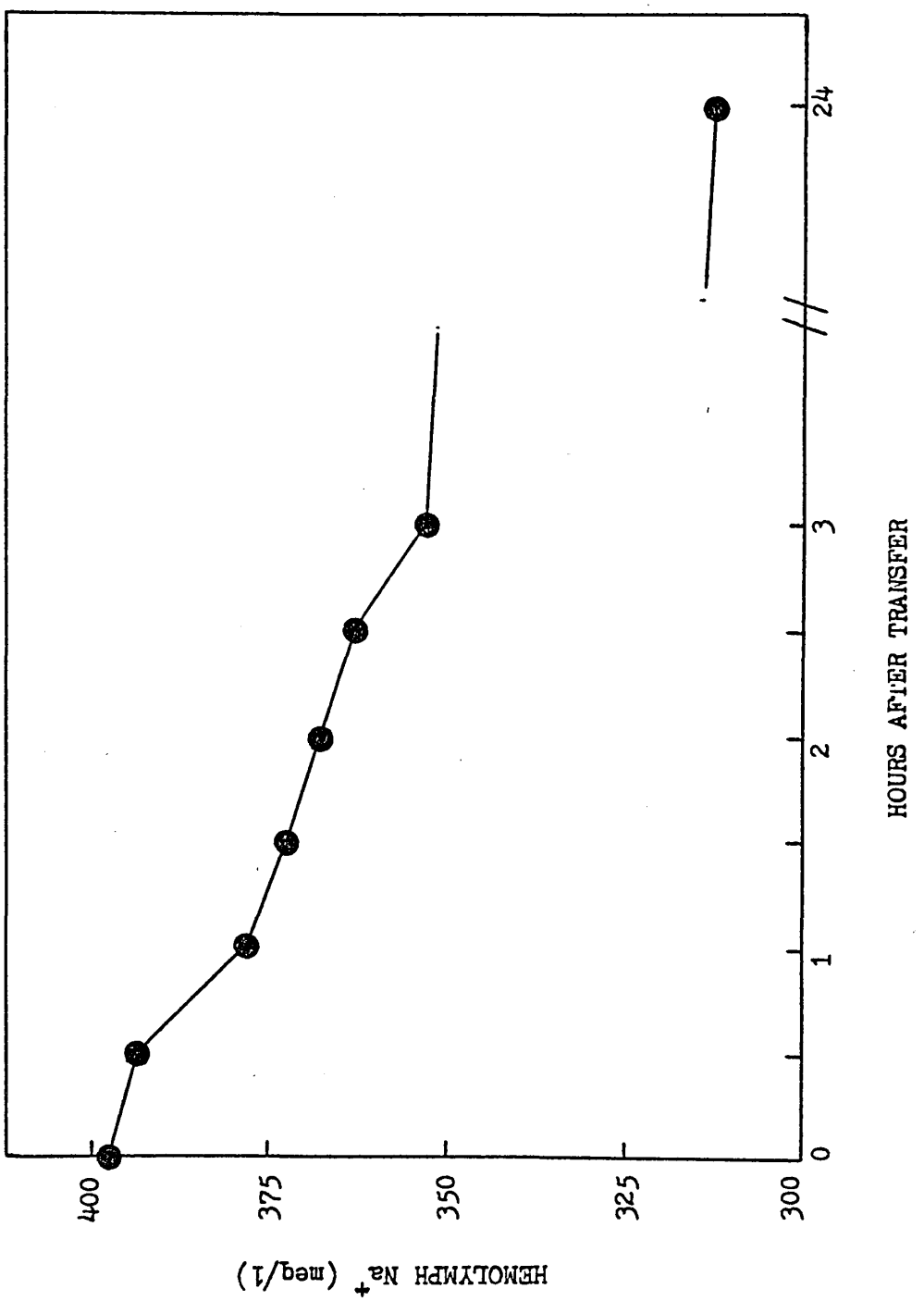

HEMOLYMPH Na^+ (meq/l)
400
375
350
325
300
0
1
2
3
24
HOURS AFTER TRANSFER

Figure 4. Change in mean serum sodium concentration following transfer from 30 o/oo to 5 o/oo salinity (N=3).

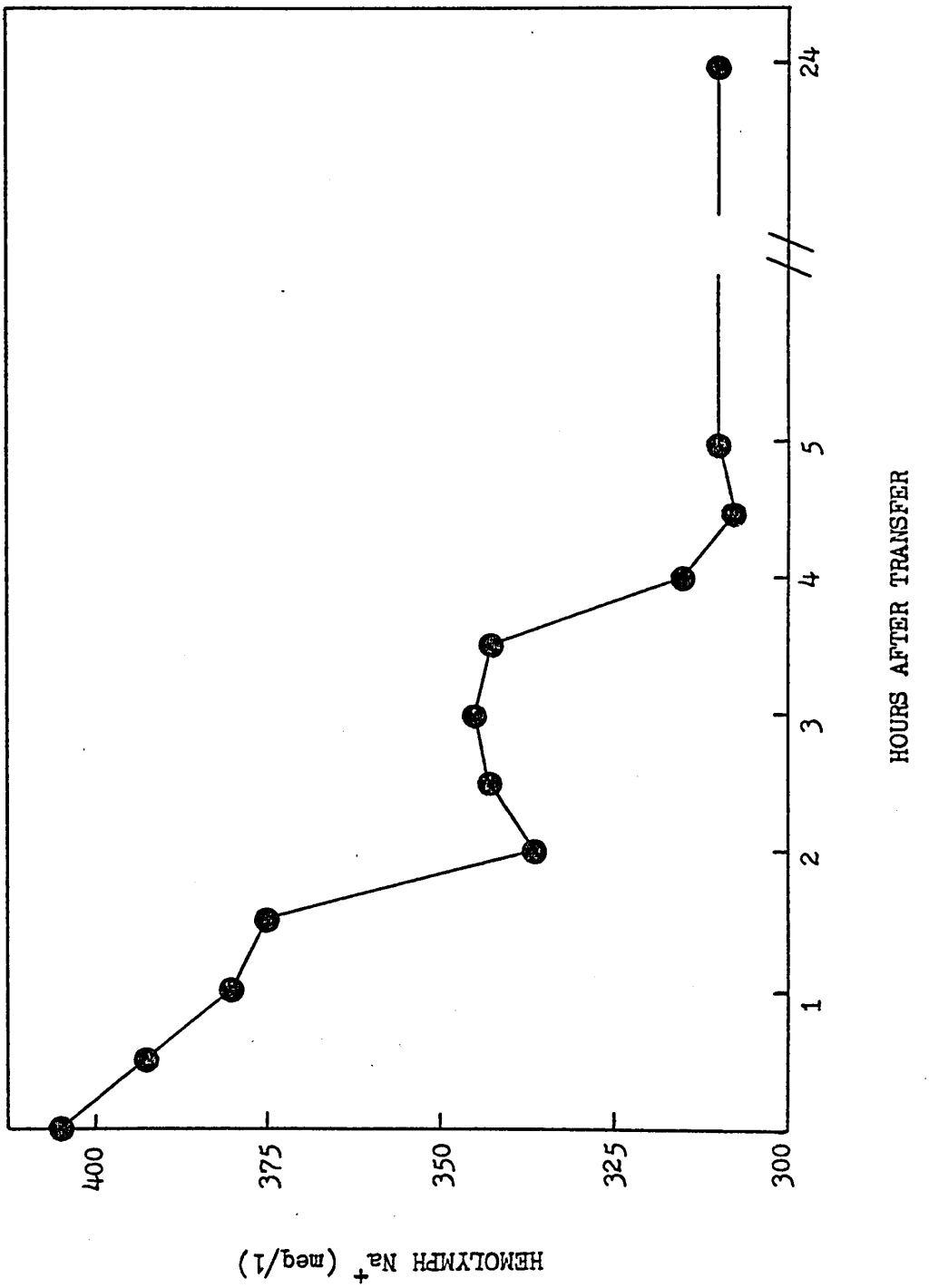

HEMOLYMPH Na^+ (meq/l)
400
375
350
325
300
1
2
3
4
5
24
HOURS AFTER TRANSFER

Figure 5. Change in mean serum sodium concentration following transfer from 5 o/oo to 30 o/oo salinity (N=3).

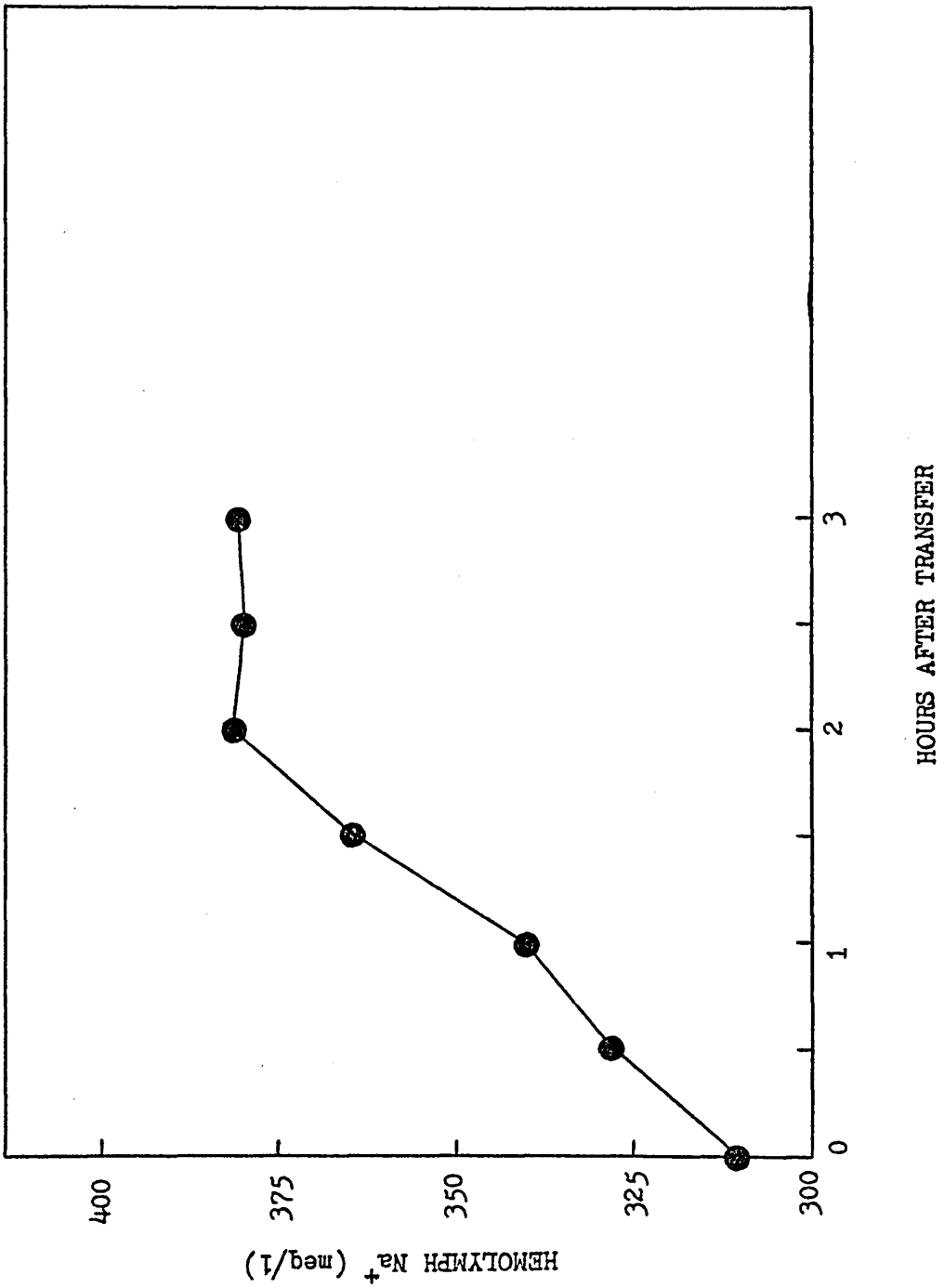

HEMOLYMPH Na^+ (meq/l)
400
375
350
325
300
0
1
2
3
HOURS AFTER TRANSFER

Figure 6. Change in mean serum sodium concentration following transfer from 25 o/oo to 5 o/oo salinity (N=5).

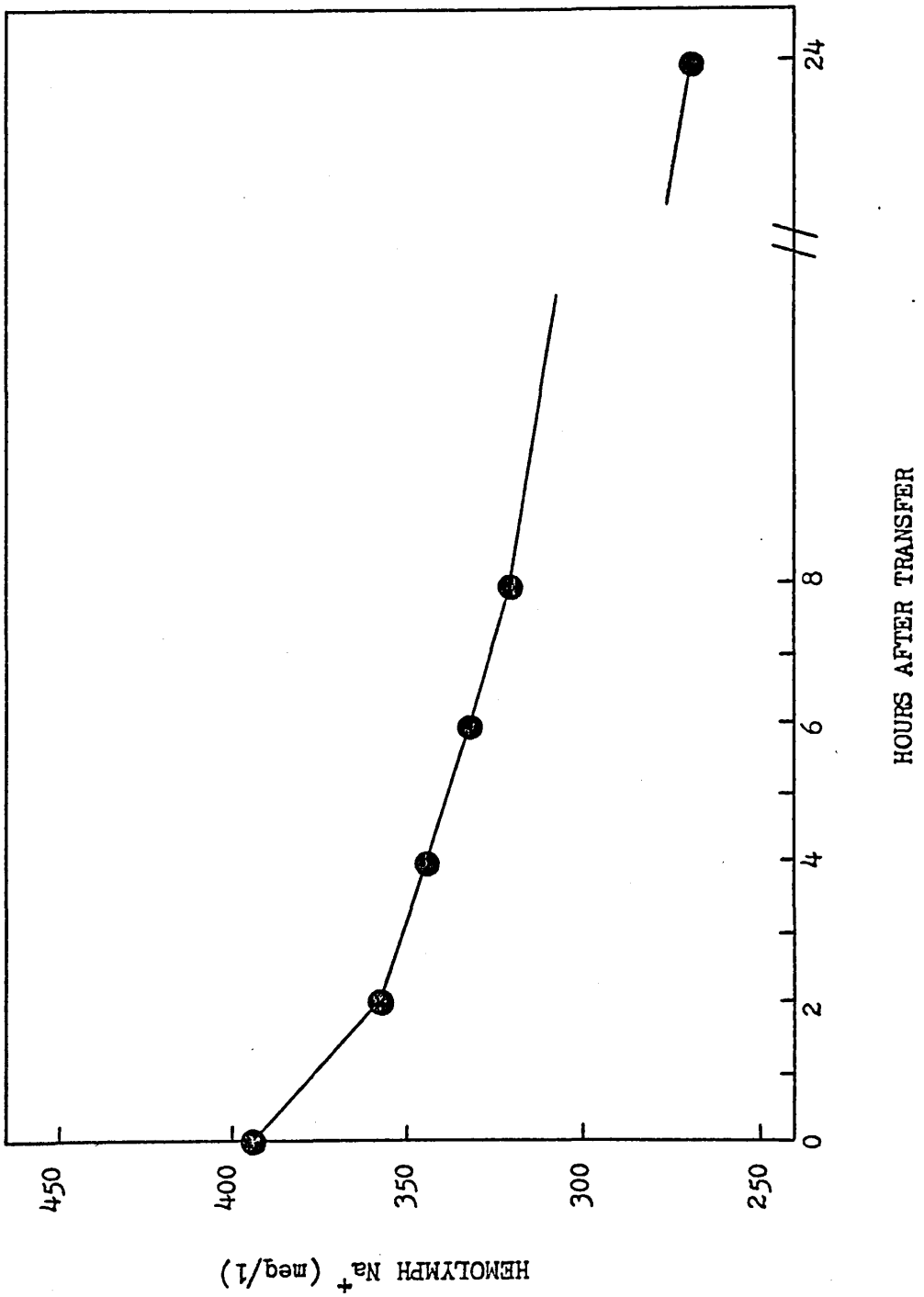
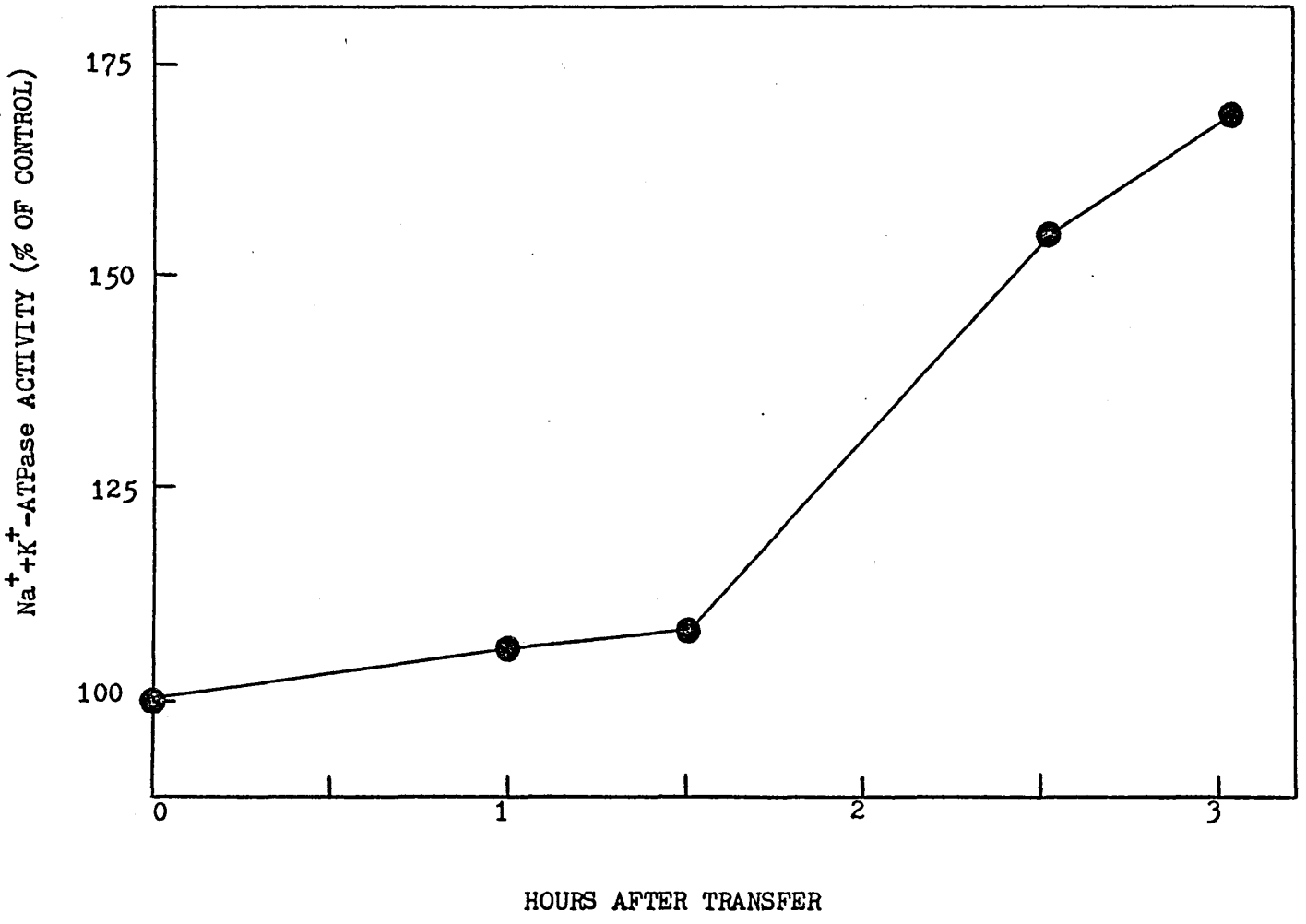

HEMOLYMPH Na^+ (meq/l)
450
400
350
300
250
0
2
4
6
8
24
HOURS AFTER TRANSFER

Figure 7. Responses of gill microsomal Na^++K^+-ATPase activity following transfer from 30 o/oo to 5 o/oo salinity (N=3).


175
150
125
100
0
1
2
3
$Na^+ + K^+$-ATPase ACTIVITY (% OF CONTROL)
HOURS AFTER TRANSFER

VITA

John Louis Harris III was born on February 14, 1948, in Augusta, Georgia. He attended Public Schools in Roanoke, Virginia, and was graduated from Patrick Henry High School in June 1966. In May 1970, he was graduated from Emory and Henry College with a Bachelor of Arts degree in biology. He completed the requirements for Master of Science in biology at the University of Richmond in May 1976. He was a member of Beta Beta Beta Biological Society at both Emory and Henry and the University of Richmond. In May 1973, he was married to Judy Elizabeth Burcham of Richmond, Virginia. Although a career in the biological sciences is sought, future plans are uncertain.