STUDIES ON SALINITY INDUCED STRESS ON NEUROSECRETORY CELLS, PROTEIN, FREE AMINO ACID CONTENT AND AMMONIA EXCRETION RATE OF PENAEID PRAWN *METAPENAEUS MONOCEROS* (FABRICIUS)

DISSERTATION SUBMITTED BY
Kum. R. KAMALA
IN PARTIAL FULFILMENT FOR THE DEGREE OF
MASTER OF SCIENCE (MARICULTURE)
OF THE
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

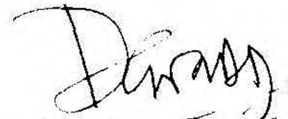
Library of the Central Marine Fisheries
Research Institute, Cochin
Date of receipt ... 5.4.90
Accession No D-96
Class No a494 KAM

POST-GRADUATE EDUCATION & RESEARCH PROGRAMME IN MARICULTURE
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
COCHIN - 682 031

OCTOBER 1989

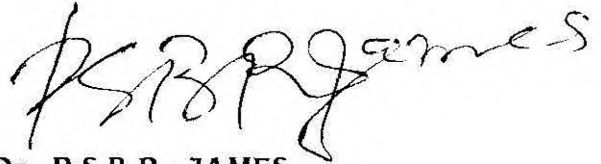
CERTIFICATE

This is to certify that this Dissertation is a bonafide record of work carried out by Kum. **R. KAMALA** under my Supervision and that no part thereof has been presented before for any other degree.



Dr. A.D. DIWAN,
SCIENTIST S-3,
CENTRAL MARINE FISHERIES
RESEARCH INSTITUTE,
COCHIN - 682 031.

Countersigned by:



Dr. P.S.B.R. JAMES,
DIRECTOR,
CENTRAL MARINE FISHERIES
RESEARCH INSTITUTE,
COCHIN - 682 031.

C O N T E N T S

	PAGE
PREFACE	1 - 4
INTRODUCTION	5 - 9
MATERIALS AND METHODS	10 - 14
RESULTS	15 - 47
DISCUSSION	48 - 60
SUMMARY	61 - 64
REFERENCES	65 - 75

....

PREFACE

An important adaptation in organisms to withstand osmotic variations in their external medium, is their efficiency of regulating intracellular concentration. In this context, cell volume regulation appears as a fundamental mechanism. The problem of cell volume regulation becomes crucial in the establishment of organisms in aquatic environment with fluctuating osmolarities.

The ability to osmoregulate may vary with developmental stages of organisms. Sexual differences in osmoregulatory ability have also been reported. It is suggested that as marine organisms invaded low saline waters, mechanism of osmoregulation evolved so that energy becomes expended by these organisms for osmoregulation (Croghan, 1961). Those organisms that are better adapted to osmoregulate would have a competitive advantage over other estuarine species which is reflected in their wide distribution in estuarine waters.

The brackishwater and marine environment are diverse with respect to environmental factors, which may probably have significant influence on the physiology, reproduction and growth of the animal. The euryhaline crustacean Metapenaeus monoceros, completes its life history in two distinct environment namely offshore and inshore waters. During its course of migration, the dual problem of internal dilution at lower salinities and internal concentration at higher salinities is also faced by this shrimp.

Crustaceans being with chitinous exoskeleton and open circulatory system, have limited ability to swell or shrink in response to changes in osmotic movement of water. A review of the literature reveals that considerable amount of work has been done dealing with osmoregulation in penaeid prawns. This is mainly because of the wide commercial interest in them in tropical and sub-tropical waters. Moreover, life history of these prawns has evinced special interest among many workers.

The compounds which were first recognised as potent osmotic effectors are the inorganic ions mainly that of Na and K. It is only very recently that some low molecular weight organic compounds like free amino acids have been studied in relation to their possible role as intracellular osmotic effectors. Though much work has been done on the osmotic and ionic regulation in crustaceans, much of the current understanding of the mechanisms is based on the study of a limited number of decapods and a few highly specialised examples of other forms. In spite of the common occurrence and osmotic problems faced by M. monoceros, little work has been done on its physiological adaptations to varying environment except by Panikkar and Vishwanathan (1948).

The present investigation was taken up with a perview to understand the modifications of organic compounds and excretory responses to different osmotic conditions in the prawn M. monoceros. Two sets of experiments were conducted with required repetitions in the present study. In the first set, low saline acclimatized prawns were transferred to high saline water

and in the second set, high saline acclimatized prawns were transferred to low saline water. The effect of osmotic stress was then monitored by determining the ammonia excretion rate and organic constituents like protein and total free amino acid in different tissues viz, muscle, hepatopancreas and haemolymph. Similar experiments were also conducted on isolated muscle tissue. The second aspect studied was on the responses of neurosecretory cells in different neuroendocrine masses under different osmotic environment. Though it has been established beyond doubt that in crustaceans hormones influence life processes like growth, molting and reproduction, very little work has been done on the hormonal impact on osmoregulation. Moreover, several of the earlier investigators did not consider the possible role of neuroendocrine factors in osmoregulation. Till recent times, very few studies have been made on the changes occurring in neurosecretory cells in response to osmotic stress. The observations made at present are promising and they show that different neuroendocrine masses of the neurosecretory system may have some role in the control of osmoregulation, which is yet to be clearly understood. Since the present work was time bound, other details of endocrine control on osmoregulation could not be investigated. The field is wide open for further detailed research.

I have great pleasure in acknowledging my deep sense of gratitude to my guide Dr. A.D. Diwan, Scientist-3 whose scholarly advice and encouragement made this work to materialize. I express my gratitude to Dr. P.S.B.R. James, Director, CMFRI, for providing all the facilities for the present study. I am also grateful to Dr. A. Noble, Principal Scientist

for his suggestions and encouragement. I thank Sri. M. Karthikeyan and Shri. M. Srinath for their help in statistical analysis. The help given by Shri. A. Nandakumar all through the period of dissertation is greatly appreciated. I also wish to thank Shri. V. Bhaskar, Senior Research Fellow and my classmates for their timely help. The fellowship received from the Indian Council of Agricultural Research is gratefully acknowledged.

INTRODUCTION

Estuarine crustaceans living in an environment of varying salinities, face great osmoregulatory problems. Presumably, such animals could either possess well developed osmoregulatory powers or lacking these they move on to favourable environment. Those organisms surviving in such demanding conditions have evolved adaptations to meet the variability (Kinne, 1967). Such organisms maintain stable osmotic pressure of blood and cells with changing external salinities either by extracellular anisosmotic or intracellular isosmotic regulation (Florkin, 1962). Considerable work has been done to investigate the ability to regulate the intracellular and extracellular osmolarity in marine invertebrates as reviewed by Potts and Parry (1963); Florkin and Scheer (1970) and Gilles (1979).

It is only very recently that some organic compounds have been studied in relation to their possible role as intracellular osmotic effectors. The basic concept behind this is that there is a close link between deamination activity and amino acid modification in isosmotic regulation (Gilles, 1977). The role of protein molecules as a storage system for amino acids has also been implicated (Wieser, 1965; Gilles, 1977).

Many studies in recent years have dealt with the free amino acid content of animals, isolated tissues and cells exposed to osmotic stresses. Duchateau and Florkin (1955) were the first to analyse the free amino compounds present in the tissue of Eriocheir sinensis subjected to osmotic

shock. The volume control process as well as related adjustments of intracellular amino acid content have been studied in protozoans like *Miamiensis avidus* (Kaneshiro *et al.*,1969)and in a variety of invertebrates and vertebrates as reviewed by Gilles (1974) and recently in non-halophilic bacteria (Measures, 1975). The role of amino acids and their modifications under hyposmotic stress has been studied in the shore crab, *Carcinus maenas* (Binns, 1969) and the mud crab, *Panopeus herbstii* (Boone and Claybrook,1977). Baginski and Pierce (1977) studied the time course of intracellular free amino acid accumulation in the tissues of the mussel *Modiolus demissus*, during high salinity adaptation. Role of free amino acid pool in adjustments to the increase or decrease in external salinity was reported by Farmer and Reeve (1978) in the copepod, *Acartia tonsa*. Mc Coid *et al.*(1984) observed variations in the free amino acid content in the muscle of *Penaeus vannamei* when subjected to increasing salinities. Similar studies have also been carried out by Dalla Via (1986) in the juveniles of kuruma prawn, *Penaeus japonicus*.

Studies have revealed that isolated tissues also exhibit the phenomena of volume regulation, especially under hyposmotic conditions. Schoffeniels (1960) demonstrated the accumulation of ninhydrine positive substances following hyperosmotic shock in the isolated axons of the crab *E. sinensis*. Changes in the amino acid pool under hyposmotic conditions were also studied in the isolated axons of *Callinectes sapidus* (Lang and Gainer, 1969) and isolated heart muscle of *M. modiolus* (Pierce and Greenberg, 1970). Bedford (1971) reported on the variations in amino-nitrogen

in isolated foot muscle of the mollusc Melanopsis trifasciata, submitted to hyperosmotic shock. The fluxes of amino acids in relation to different osmotic conditions have been studied in the isolated axons of C. sapidus and E. sinensis (Gerard and Gilles, 1972; Gilles, 1973). While these studies suggest a possible osmoregulatory role for tissue free amino acids, the physiological and metabolic mechanisms controlling the variations in the concentration of amino acids are yet to be completely resolved.

The modifications of blood protein concentration following the application of hyposmotic stress have been reported in C. maenas (Siebers et al.,1972; Boone and Schoffeniels, 1979) and E. sinensis (Gilles, 1977). The effect of salinity on the total protein concentration in the haemolymph of Penaeus monodon was also investigated by Ferraris et al.(1986). Bedford (1971) conducted studies on the control of intracellular isomotic regulation in M. trifasciata and emphasised the role of protein as a reserve for amino acids.

Several studies were conducted to determine the changes in ammonia excretion in euryhaline species. Haberfield et al. (1975) investigated the excretion of ammonium ions and free amino acids after a decrease in salinity in the shore crab, C. maenas and polychaete, Nereis virens. The ammonia excretion during low saline acclimation was studied in C. sapidus (Mangum et al.,1976) and the euryhaline bivalve, M. demissus (Bartberger and Pierce, 1976). The uptake of exogenous ammonia and changes in endogenous nitrogen compounds during high salinity acclimation was investigated by

Armstrong et al.(1981) in the prawn Macrobrachium rosenbergii. Excretion of nitrogenous products by P. japonicus in relation to high saline and low saline condition was elaborated by Spaargaren et al. (1982). Study was also made in Crangon crangon (Regnault, 1984) on the salinity induced changes in ammonia excretion.

Extensive work has been done on the effect of ligation or removal of eyestalks on osmoregulation in a number of decapods. Heit and Fingerman (1975) elaborated the role of an eyestalk hormone in the sodium concentration of the blood of fiddler crab, Uca pugilator. Comparable study was also made by Davis (1978) in the same species. Hormonal control of osmoregulation was also demonstrated by Nagabhushanam and Jyothi(1977) in Caridina weberi and Diwan and Laxminarayana (1989) in Penaeus indicus. Raghavaiah et al. (1980) and Ramamurthi et al. (1982) reported on the neuroendocrine control of nitrogen metabolism in Indian field crab, Oziotelphusa senex senex. Raman et al. (1981) concluded that an eyestalk hormone regulates ammonia excretion in Macrobrachium lanchesteri.

Metapenaeus monoceros though a marine penaeid, lives in the estuaries. Life histories of many species belonging to the family Penaeidae have been investigated (Menon, 1933 and 1951; George and Vedavyasa Rao, 1968; Panikkar, 1968). It has been reported that the highly fecund females of this species liberate the eggs demersally in the offshore waters and post larvae migrate to the brackishwaters for growth. Panikkar and Vishwanathan(1948), pointed out that adults of M. monoceros have better osmoregulatory abilities than Penaeus setiferus and Penaeus aztecus. However,

sufficient data would be required to understand whether the euryhalinity of this species depends on the efficiency of their mechanism of intracellular fluid isosmotic regulation.

Though many studies have been made on the regulation of inorganic molecules in response to osmotic stress in crustaceans (Denne, 1968; Dall 1970; Castille and Lawrence, 1981), it is only very recent that variations of some organic molecules mostly free amino acids were studied under different osmotic conditions (Florkin and Schoffeniels 1969; Schoffeniels and Gilles, 1970; Lange, 1972; Gilles, 1974 and 1977). However, information is lacking with regard to protein variation and its relation to amino acid pool under osmotic stress. And also a review of literature pertaining to neurosecretory studies till to day reveals that very few studies have been made on the changes occurring in neurosecretory cells in response to osmotic stress in crustaceans.

The present study was initiated to understand the physiological mechanisms involving the amino acid and protein regulation, that might account for the success of the species in salinity acclimation. Therefore in the present investigation, an attempt was made to study the sudden effect of osmotic stress on the neurosecretory cells of different neuro-endocrine masses, protein and free amino acid content of haemolymph, muscle and hepatopancreas and the ammonia excretion rate of the prawn *M. monoceros.*

MATERIALS AND METHODS

For the study, seventy two adult prawns Metapenaeus monoceros of length ranging between 90-100 mm were collected from wild. Normal and healthy animals were chosen and transported to the laboratory in live condition. Only intermolt animals were selected for experimental purpose, without regard to sex. The salinity of the water at the time of collection of animals was around 3.8‰. The animals were then divided into two groups I and II, each comprising of 36 animals and transferred to two large 1 tonne fibre glass tanks filled with filtered and well aerated seawater of strength 5‰ and 35‰ respectively. The desired salinities were prepared either by diluting seawater with tapwater or by partial freezing of seawater. The animals were acclimatized to the above salinities for a period of ten days before using them for the experiment. During this period they were fed on clams. After acclimatization, animals of Group I were further divided into two batches A and B, each comprising of 18 animals which were again divided into 6 sub-groups of 3 animals each. Likewise, group II was also divided into two batches C and D, which were further divided into 6 sub-groups.

In order to study the effect of osmotic stress, animals of batch B which were acclimatized to low saline water were transferred to high saline water of strength 35‰ and kept in 6 plastic tubs. Each of these aerated tubs contained 3 animals. Similarly, the animals of batch D

acclimatized to high saline water were transferred to low saline water of strength 5‰. Batches A and C were used as control and werre maintained in two sets of 6 corresponding tubs with the experimental groups. The experiment was set up for a period of 48 hours and the prawns were not fed then.

Before initiating the experiment, the first water sample from all the tubs were taken and the initial concentration of ammonia recorded. After the start of experiment, animals of the first tub from all the four batches A, B, C and D were sacrificed at 'zero hours' and thereafter 3, 6, 12, 24 and 48 hours respectively. Before sacrificing, the animals were weighed individually and their haemolymph collected through the pericardial cavity with the help of hypodermic syringe. Every time before the extraction of haemolymph, the syringe was rinsed with an anticoagulant (10% sodium citrate). Haemolymph thus collected was transferred to glass vials and maintained in frozen condition until use. Simultaneously, water samples from all the tubs were collected at every corresponding hour to determine the ammonia concentration. After extracting the haemolymph, animals were quickly dissected to remove the muscle and hepatopancreatic tissue and analysed for protein and total free amino acid content. In order to study and identify changes occurring in the neursecretory cells, the optic, cerebral, thoracic and abdominal ganglia were removed and fixed in Bouin's fluid.

After 48 hours of fixation, the tissues were washed in running tapwater for atleast 4 hours. The tissues were then dehydrated in ascending

grades of alcohol (70-100%), keeping in each grade for 10-15 minutes and later cleared in xylene. Before embedding the tissues in paraffin wax, hot impregnation was done (in molten wax) for half an hour. After hot impregnation, blocks were prepared and trimmed and serial sections were then cut at 4-6 μ, using a manual rotary microtome. Sections were then deparaffined in xylene, stained with Mallory's triple stain and mounted in DPX. Photomicrographs of the histological preparations were taken using Olympus Universal Research Microscope.

Isolated muscle tissue

For conducting experiments with isolated muscle tissue, 84 adult prawns were collected from wild and divided into two groups I and II, each comprising of 42 animals and acclimatized as mentioned earlier in saline waters of strength 5‰ and 35‰ respectively. After acclimatization, animals of group I were divided into two batches A and B, which were further divided into 7 sub-groups of 3 animals each. Likewise, group II was also divided into two batches C and D, which were further divided into 7 sub-groups. Muscle tissue was isolated from each animal of all the sub-groups and weighed individually. For the present experiment 500 mg of muscle tissue from each animal was used.

Muscle tissue individually isolated from all the three animals of each sub-group of batch B which were acclimatized to low saline water were placed in three separate 1 litre beakers, each containing 500 ml of

high saline water of strength 35‰. The water was well aerated. Likewise, muscle tissue isolated from all the three animals of each sub-group of batch D which were acclimatized to high saline water were also placed in three separate 1 litre beakers, each containing 500 ml of low saline water of strength 5‰. Isolated muscle tissue from animals of batches A and C were used as control and maintained in 2 sets of 21 corresponding beakers with the experimental groups. The experiment was set up for a period of 12 hours.

Before initiating the experiment, the first water sample from all the beakers were sampled and the initial concentration of ammonia recorded. After the start of experiment, isolated muscle tissue from 3 beakers of the first sub-group of all the four batches A, B, C and D were removed at 'zero hours' and subsequently after 2, 4, 6, 8, 10 and 12 hours. The muscle tissue was analysed for protein and total free amino acid content.

Chemical analysis

Protein content of the muscle, hepatopancreas and haemolymph was determined by Biuret method (Gornall et al., 1949). Bovine serum albumin was used to prepare the standard curve.

Total free amino acid content was determined as per the method described by Yemm and Cocking (1955). A mixture of glycine and glutamic acid was used as the standard. The optical density was measured at 540 nm

for protein and 570 nm for total free amino acid using a UV-VIS Spectrophotometer.

Ammonia in the water sample was determined by phenol hypochlorite method (Solarzano, 1969). Analysis was carried out immediately and optical density read at 640 nm. The hourly excretion per gram of body tissue was calculated as per the method elaborated by Regnault (1984).

Statistical comparison of means between control and experimental groups were carried out according to the method described by Snedecor and Cochran (1967). Students 't' test was applied for testing the hourly significance of experimental values in those which showed no overall significance.

Adult specimens of *M. monoceros* acclimatized to low saline (right) and high saline water (left).

R E S U L T S

Whole animal

Ammonia

The ammonia excretion rate of prawns transferred from low to high saline water ranged from 62.5±0.24 to 46.18±0.14 μgNg^{-1}wet wt.h^{-1} (Table 1, Fig. 1). The values showed a decreasing trend throughout the experimental period. Significant variations ($P < 0.05$) could be seen when comparison is done with the corresponding control values. The ammonia excretion rate of prawns transferred from high to low saline water, on the other hand, showed an increasing trend from 48.22±0.41 to 145.75±0.36 μgNg^{-1}wet wt. h^{-1} at the end of 12th hr after which recouping effect was noticed. However, the value remained high (111.93±0.47 μgNg^{-1}wet wt. h^{-1}) when compared to control, even at the end of 48th hr (Table 2, Fig. 1). Anova showed that values were statistically significant ($P < 0.05$) when compared to control values.

Total Free Amino Acid (TFAA)

TFAA content in the haemolymph of prawns transferred from low to high saline water ranged from 1.0±0.02 to 1.53±0.04 mg% (Table 3, Fig. 2). The values showed an increasing trend throughout the experimental period. Significant increase ($P < 0.05$) could be seen when comparison is made with the control values. The TFAA content of the haemolymph of prawns transferred from high to low saline water ranged between 0.88±3.9

Table 1. Ammonia excretion rate of the prawn M. monoceros, transferred from low saline (5‰) to high saline water (35‰)

Hours	Ammonia excretion rate ($\mu gN g^{-1}$ wet wt. $hour^{-1}$) Control	Experimental
0	4.92 ±0.02	4.60 ±0.01
3	64.47 ±0.16	62.50 ±0.24
6	70.99 ±0.28	58.16 ±0.24
12	84.90 ±0.36	59.12 ±0.31
24	73.20 ±0.06	58.20 ±0.28
48	77.24 ±0.71	46.18 ±0.14

All values are mean of 3 determinations and figures represent $\bar{X} \pm$ S D

ANOVA TABLE

Source	d.f.	Sum.Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	630.133	630.133	8.26	SIG (5%)
Error	5	381.496	76.299		

Table 2. Ammonia excretion rate of the prawn M. monoceros, transferred from high saline (35‰) to low saline water (5‰)

Hours	Ammonia excretion rate (μgNg^{-1} wet wt. $hour^{-1}$) Control	Experimental
0	8.09 ±0.30	8.10 ±0.007
3	54.97 ±1.15	48.22 ±0.41
6	71.10 ±0.17	121.14 ±0.41
12	60.98 ±0.18	145.75 ±0.36
24	63.48 ±0.39	119.40 ±0.45
48	55.88 ±0.41	111.93 ±0.47

All values are mean of 3 determinations and figures represent $\overline{X} \pm SD$

ANOVA TABLE

Source	d.f	Sum. sqr.	Mean. sqr.	F-Value	Remarks
Treat	1	4801.364	4801.364	7.50	SIG (5%)
Error	5	3200.496	640.099		

Fig. 1. Changes in the ammonia excretion rate of experimental animals, M. monoceros transferred from their previous low saline acclimatization to high saline water (35‰)and high saline acclimatization to low saline water (5‰), in comparison with control animals.

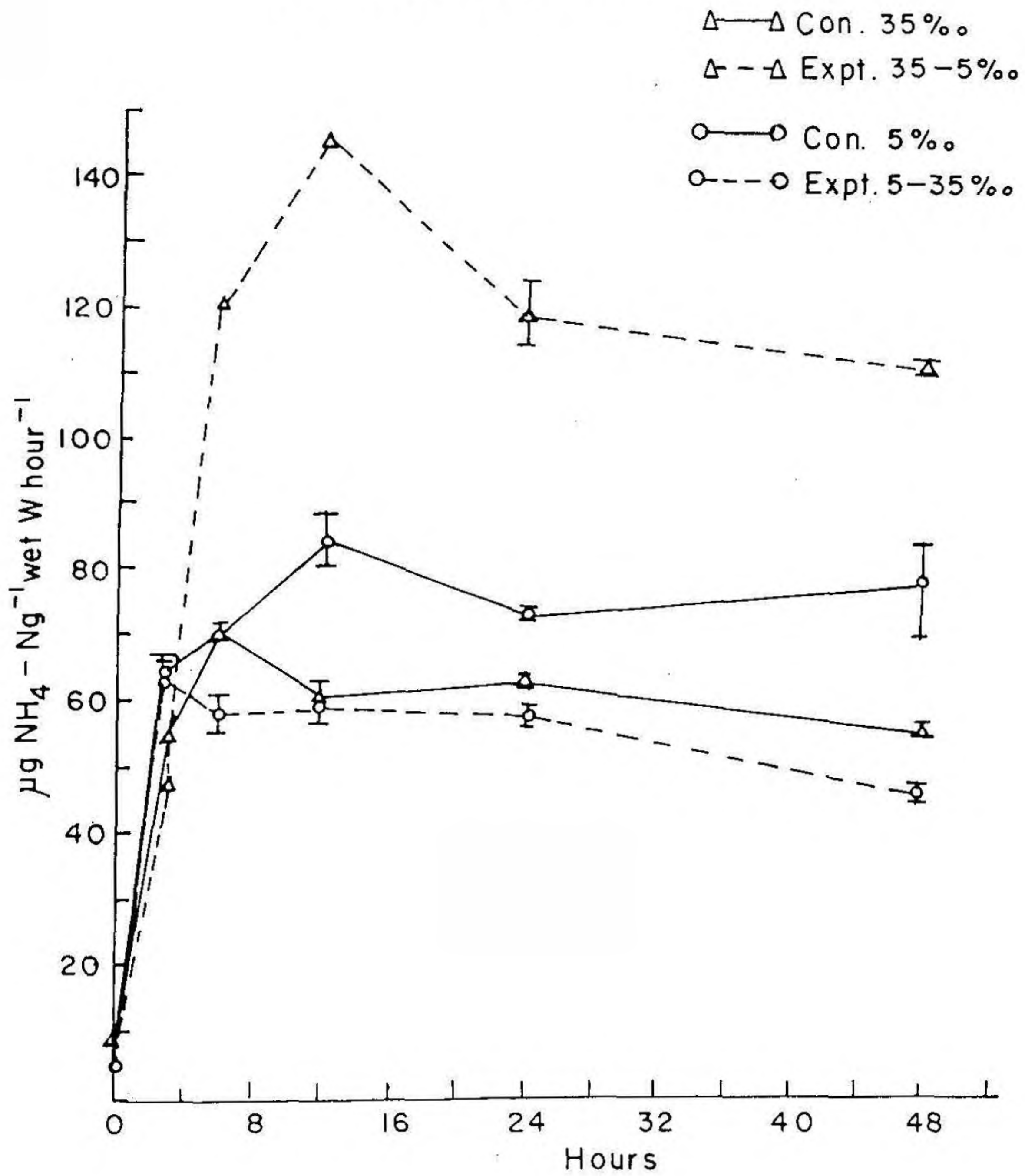

AMMONIA EXCRETION RATE
Con. 35‰
Expt. 35–5‰
Con. 5‰
Expt. 5–35‰
140
120
100
80
60
40
20
μg NH4 – Ng-1 wet W hour-1
0
8
16
24
32
40
48
Hours

Table 3. Total free amino acid content of haemolymph of the prawn M. monoceros, transferred from low saline (5‰) to high saline water (35‰)

Hours	Total free amino acid (mg%) Control	Experimental
0	1.09 ±0.04	1.00 ±0.02
3	1.10 ±0.04	1.24 ±0.05
6	0.97 $\pm 6.8 \times 10^{-3}$	1.34 ±0.04
12	0.91 $\pm 8.6 \times 10^{-3}$	1.36 $\pm 6.5 \times 10^{-3}$
24	0.97 ±0.01	1.51 ±0.04
48	0.97 ±0.01	1.53 ±0.04

All values are mean of 3 determinations and figures represent $\overline{X} \pm SD$

ANOVA TABLE

Source	d.f.	Sum. Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	0.325	0.325	9.83	SIG (5%)
Error	5	0.165	0.033		

x 10^{-3} to 0.95±2.05 x 10^{-3} mg% (Table 4, Fig. 2). The values showed a decreasing trend during the experimental period. Anova showed significant decrease ($P < 0.01$) when compared to control values.

TFAA content in the muscle of prawns transferred from low to high saline water showed a transitory increase during the experimental period, when comparison was made with the control values. The level fluctuated between 1.37±8.5 x 10^{-3} to 2.33±0.06 mg% at the end of 12th hr after which there was a decrease (1.9±5.76 x 10^{-3} mg%) at the end of 48th hr, showing recouping effect of stress (Table 5, Fig. 3). The values were found to be statistically significant ($P < 0.01$). TFAA in the muscle of prawns transferred from high to low saline water showed a transitory decrease from 2.54±0.07 to 0.99±5.4 x 10^{-3} mg% (Table 6, Fig. 3) at the end of 12th hr after which there was an increase (2.21±0.03 mg%) at the end of 48th hr showing recouping effect. Though anova did not show overall significance ($P > 0.05$), significant decrease could be seen during 12 and 24 hrs when comparison is made between the experimental and corresponding control values.

TFAA content in the hepatopancreas of prawns transferred from low to high saline water showed a transitory increase from 2.32±0.03 to 4.95±0.13 mg% (Table 7, Fig. 4) at the end of 12th hr after which there was a decrease (3.24±0.07 mg%) at the end of 48th hr. However, the level remained high when compared to control. Anova showed significant increase ($P < 0.05$) throughout the experimental period. The level of TFAA

Table 4. Total free amino acid content of haemolymph of the prawn *M. monoceros*, transferred from high saline (35‰) to low saline water (5‰)

Hours	Total free amino acid (mg%) Control	Experimental
0	1.12 ±5.5 x 10^{-3}	0.95 ±2.05 x 10^{-3}
3	1.04 ±0.32 x 10^{-3}	0.88 ±3.9 x 10^{-3}
6	1.10 ±0.04	0.88 ±4.6 x 10^{-3}
12	1.33 ±0.03	0.92 ±4.8 x 10^{-3}
24	1.36 ±0.02	0.88 ±9.8 x 10^{-3}
48	1.35 ±0.07	0.94 ±5.26 x 10^{-3}

All values are mean of 3 determinations and figures represent $\overline{X} \pm$ S D

ANOVA TABLE

Source	d.f.	Sum. Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	0.290	0.290	30.12	HI.SIG (1%)
Error	5	0.018	0.010		

Fig.2. Changes in the total free amino acid content of haemolymph of experimental animals transferred from their previous low saline acclimatization to high saline water (35‰) and high saline acclimatization to low saline water (5‰), in comparison with control animals.

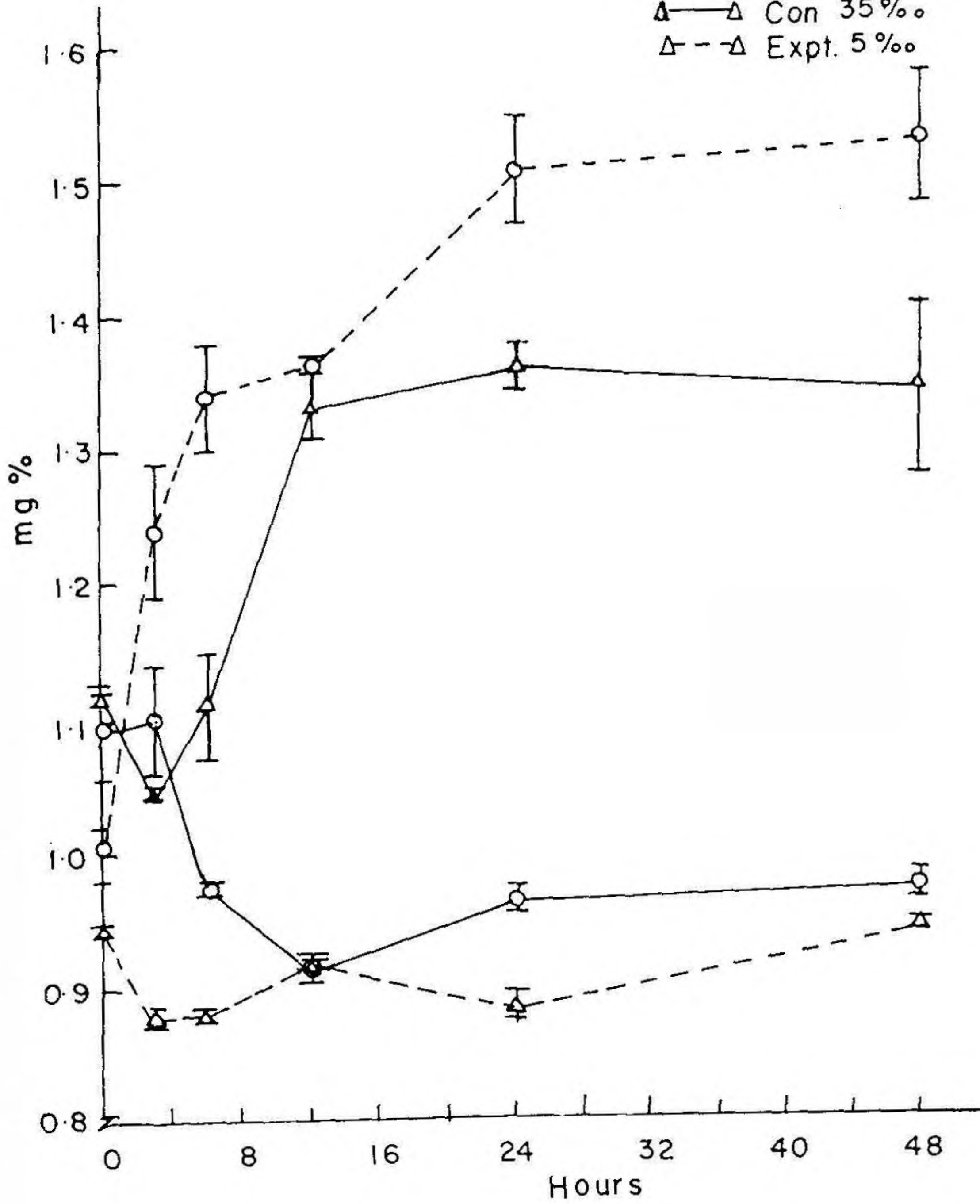

TOTAL FREE AMINO ACID
HAEMOLYMPH
Con. 5‰
Expt. 35‰
Con 35‰
Expt. 5‰
mg %
1·6
1·5
1·4
1·3
1·2
1·1
1·0
0·9
0·8
0
8
16
24
32
40
48
Hours

Table 5. Total free amino acid content of muscle of the prawn M. monoceros, transferred from low saline (5‰) to high saline water (35‰)

Hours	Total free amino acid (mg%) Control	Experimental
0	1.27 ±0.06	1.37 ±8.5 x 10^{-3}
3	1.34 ±0.01	1.83 ±0.03
6	1.52 ±0.03	2.23 ±0.09
12	1.31 ±0.01	2.33 ±0.06
24	1.41 ±0.04	2.13 ±0.02
48	1.51 ±0.02	1.90 ±5.76 x 10^{-3}

All values are mean of 3 determinations and figures represent $\overline{X} \pm$ S D

ANOVA TABLE

Source	d.f.	Sum.Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	0.981	0.981	19.43	HI.SIG(1%)
Error	5	0.252	0.050		

Table 6. Total free amino acid content of muscle of the prawn M. monoceros, transferred from high saline (35‰) to low saline water (5‰)

Hours	Total free amino acid (mg%) Control	Experimental
0	2.65 ±0.06	2.54 ±0.07
3	2.33 ±0.06	2.55 ±0.05
6	2.14 ±0.04	2.08 ±0.05
12	2.20 ±0.03	0.99* $\pm 5.4 \times 10^{-3}$
24	2.15 ±0.04	1.46* ±0.05
48	2.20 ±0.49	2.21 ±0.03

All values are mean of 3 determinations and figures represent $\overline{X} \pm$ S D

*Values significant at $P < 0.05$ level.

ANOVA TABLE

Source	d.f.	Sum.Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	0.283	0.283	1.93	N.S.
Error	5	0.731	0.146		

Fig. 3. Changes in the total free amino acid content of muscle of experimental animals transferred from their previous low saline acclimatization to high saline water (35‰) and high saline acclimatization to low saline water(5‰) in comparison with control animals.

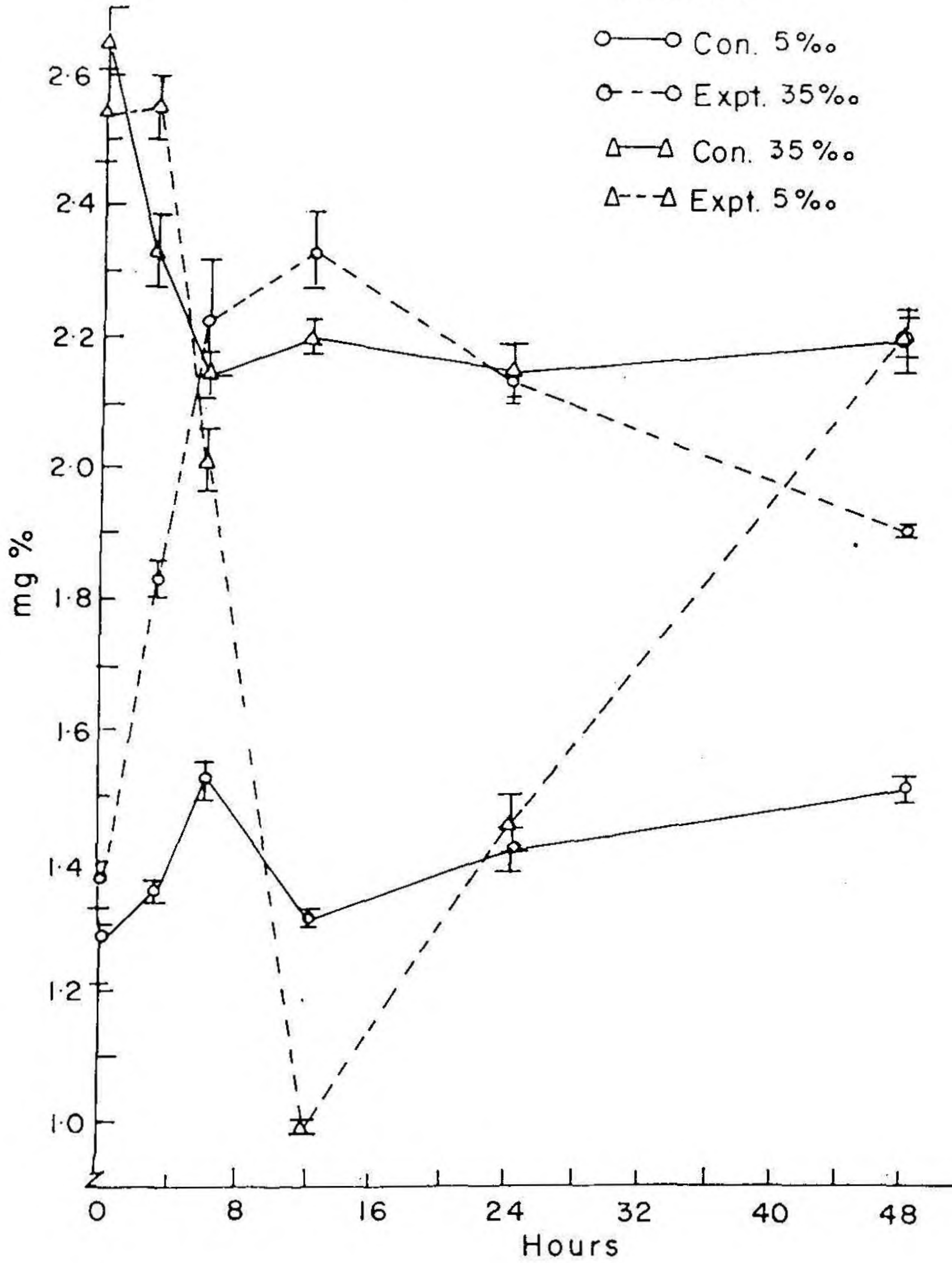

TOTAL FREE AMINO ACID
MUSCLE
Con. 5‰
Expt. 35‰
Con. 35‰
Expt. 5‰
mg %
2·6
2·4
2·2
2·0
1·8
1·6
1·4
1·2
1·0
0
8
16
24
32
40
48
Hours

Table 7. Total free amino acid content of hepatopancreas of the prawn <u>M. monoceros</u>, transferred from low saline (5‰) to high saline water (35‰)

Hours	Total free amino acid (mg%) Control	Experimental
0	2.18 ±0.04	2.32 ±0.03
3	2.23 ±0.06	3.28 ±0.02
6	2.61 ±0.02	4.49 ±0.06
12	2.21 ±0.03	4.95 ±0.13
24	2.27 ±0.09	4.48 ±0.08
48	2.27 ±0.03	3.24 ±0.07

All values are mean of 3 determinations and figures represent $\overline{X} \pm$ S D

ANOVA TABLE

Source	d.f.	Sum.Sqr.	Mean.Sqr.	F-Value	Remarks
Treat	1	6.728	6.728	14.88	SIG (5%)
Error	5	2.261	0.452		

content in the hepatopancreas of prawns transferred from high to low saline water ranged between 1.32±0.03 to 3.61±0.03 mg% (Table 8, Fig. 4). The values showed a decreasing trend throughout the experimental period. Though anova did not show overall significance ($P > 0.05$) between the control and experimental values, significant decrease could be seen after 12 hrs when comparison is made with the corresponding control values.

Protein

Protein content of haemolymph in prawns transferred from low to high saline water ranged between 81.78±0.52 to 175.16±0.13 mg% (Table 9, Fig. 5). The values showed a sustained decrease throughout the experimental period. Statistically significant variations ($P < 0.05$) could be seen between the experimental and control values. Haemolymph of prawns transferred from high to low saline water, showed high levels of protein throughout the experimental period. The values ranged between 70.86±1.40 to 204.74±0.26 mg% (Table 10, Fig. 5). Anova showed significant increase ($P < 0.01$) throughout the experimental period.

Protein content of the muscle in prawns transferred from low to high saline water fluctuated between 16.46±0.01 to 24.81±0.63 mg% (Table 11, Fig. 6). When comparison is done between the experimental and corresponding control values, significant increase could be seen during 24 and 48 hrs though anova did not show overall significance. In the prawns transferred from high to low saline water, the protein content of the

Table 8. Total free amino acid content of hepatopancreas of the prawn M. monoceros, transferred from high saline (35‰) to low saline water (5‰)

Hours	Total free amino acid (mg%) Control	Experimental
0	3.22 ±0.06	3.44 ±0.03
3	3.23 ±0.03	3.61 ±0.03
6	3.30 ±0.02	3.37 ±0.03
12	3.36 ±0.01	2.17 ±0.06
24	3.43 ±0.02	1.60* ±0.04
48	3.60 ±0.06	1.32* ±0.03

All values are mean of 3 determinations and figures represent $\overline{X} \pm$ S D

*Values significant at $P < 0.05$ level.

ANOVA TABLE

Source	d f.	Sum Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	1.786	1.786	2.72	N.S.
Error	5	3.289	0.658		

Fig. 4. Changes in the total free amino acid content of hepatopancreas of experimental animals transferred from their previous low saline acclimatization to high saline water (35‰) and high saline acclimatization to low saline water(5‰), in comparison with control animals.

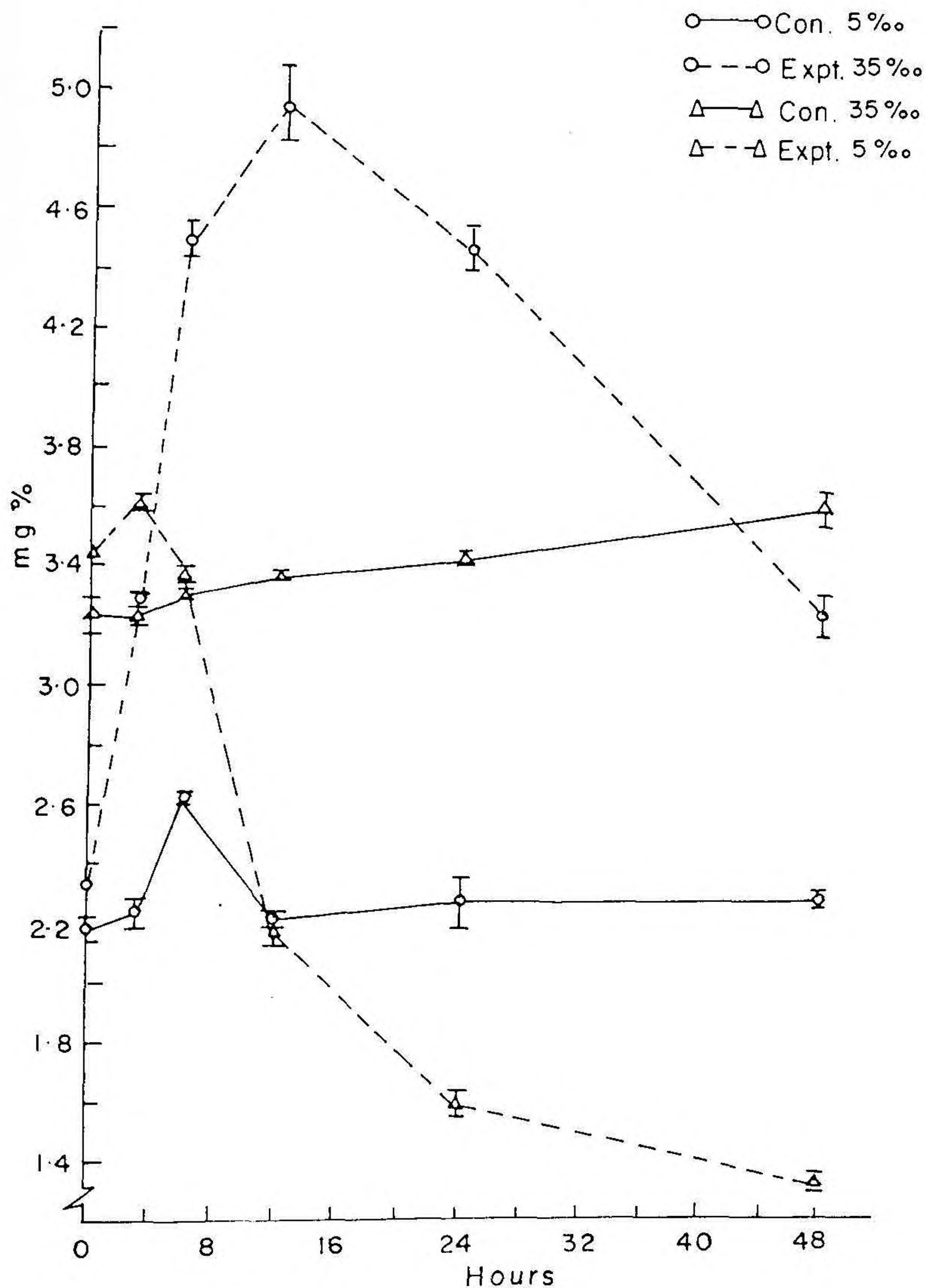

TOTAL FREE AMINO ACID
HEPATOPANCREAS
Con. 5‰
Expt. 35‰
Con. 35‰
Expt. 5‰
mg %
5·0
4·6
4·2
3·8
3·4
3·0
2·6
2·2
1·8
1·4
0
8
16
24
32
40
48
Hours

Table 9. **Protein content of haemolymph of the prawn *M. monoceros*, transferred from low saline (5‰) to high saline water (35‰)**

Hours	Protein (mg%) Control	Experimental
0	171.35 ±0.27	175.16 ±0.13
3	183.56 ±1.37	162.55 ±2.29
6	181.12 ±0.10	146.03 ±0.42
12	185.13 ±0.35	145.89 ±0.26
24	186.49 ±0.58	122.15 ±0.60
48	185.45 ±0.16	81.78 ±0.52

All values are mean of 3 determinations and figures represent $\overline{X} \pm$ S D

ANOVA TABLE

Source	d.f.	Sum. Sqr.	Mean Sqr.	F- Value	Remarks
Treat	1	5613.157	5613.157	8.15	SIG (5%)
Error	5	3443.625	688.725		

Table 10. Protein content of haemolymph of the prawn M. monoceros, transferred from high saline (35‰) to low saline water (5‰)

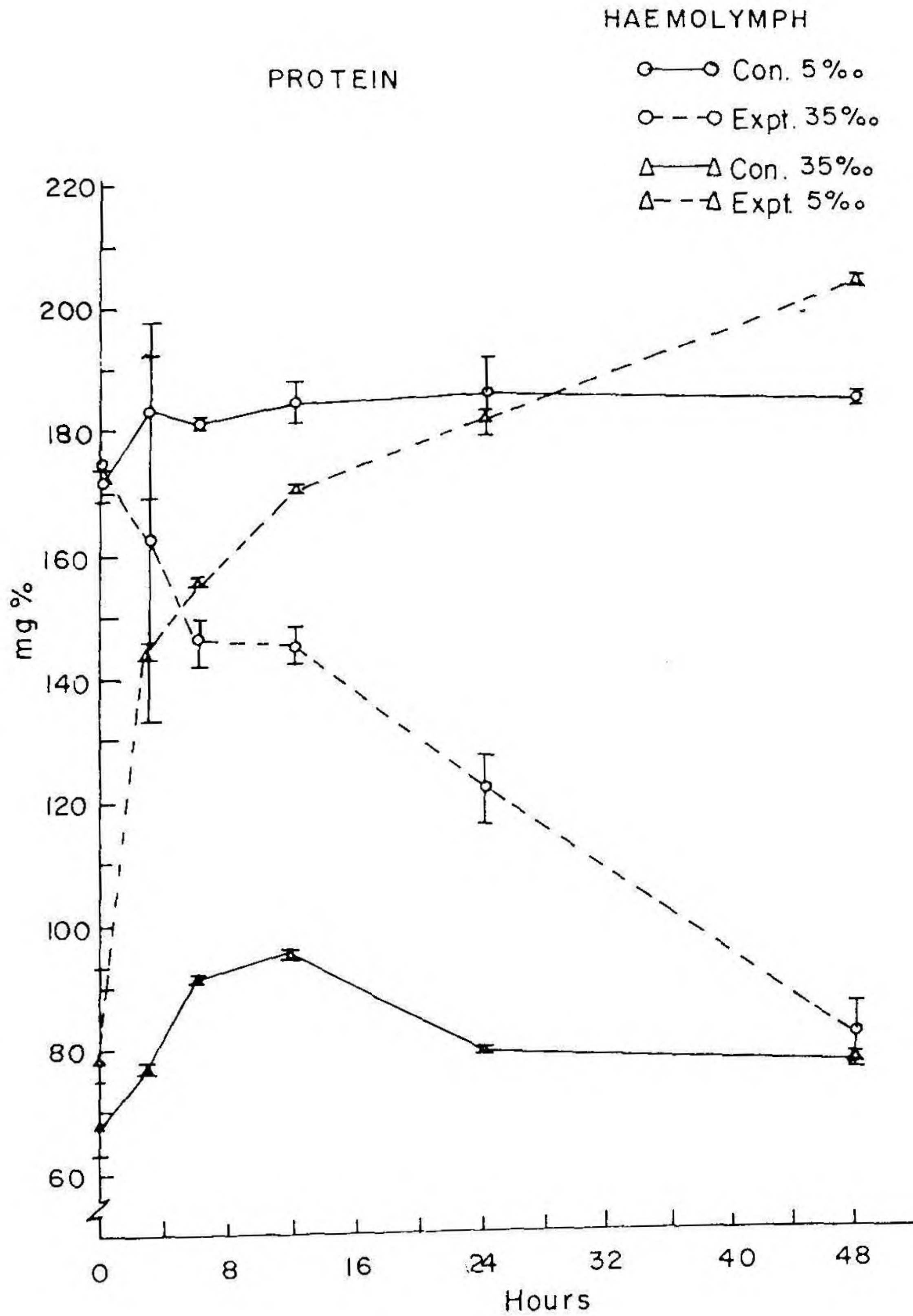
Hours	Protein (mg%) Control	Experimental
0	67.79 ±0.33	70.86 ±1.40
3	77.15 ±0.68	144.41 ±1.41
6	91.25 ±0.29	155.79 ±0.64
12	95.47 ±0.44	171.20 ±0.33
24	79.39 ±0.14	183.21 ±0.33
48	77.74 ±0.40	204.74 ±0.26

All values are mean of 3 determinations and figures represent $\overline{X} \pm$ S D

ANOVA TABLE

Source	d.f.	Sum.Sqr.	Mean sqr	F-Value	Remarks
Treat	1	16242.990	16242.990	18.32	HI-SIG(1%)
Error	5	4433.078	886.616		

Fig. 5. Changes in the protein content of haemolymph of experimental animals transferred from their previous low saline acclimatization to high saline water (35‰) and high saline acclimatization to low saline water (5‰), in comparison with control animals.



HAEMOLYMPH
PROTEIN
Con. 5‰
Expt. 35‰
Con. 35‰
Expt. 5‰
220
200
180
160
140
120
100
80
60
mg %
0
8
16
24
32
40
48
Hours

Table 11. Protein content of muscle of the prawn M. monoceros, transferred from low saline (5‰) to high saline water (35‰)

Hours	Protein (mg%) Control	Experimental
0	15.35 ±0.09	16.46 ±0.10
3	16.21 ±0.23	17.13 ±0.09
6	15.41 ±0.16	17.67 ±0.17
12	16.65 ±0.19	18.83 ±0.79
24	16.23 ±0.45	21.23* ±0.52
48	14.14 ±0.36	24.81* ±0.63

All values are mean of 3 determinations and figures represent $\overline{X} \pm SD$

*Values significant at $P < 0.05$ level.

ANOVA TABLE

Source	d.f.	Sum. Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	40.806	40.806	5.91	N.S.
Error	5	34.547	6.909		

muscle ranged between 20.44±0.04 to 24.08±0.39 mg% (Table 12, Fig. 6). The values showed a decreasing trend throughout the experimental period. Though anova showed no overall significance, significant decrease could be seen during 24 and 48 hrs.

Protein content of the hepatopancreas in prawns transferred from low to high saline water ranged from 19.24±0.46 to 28.01±0.36 mg% (Table 13, Fig. 7). Significant increase ($P < 0.05$) could be seen between the experimental and control values throughout the experimental period. In prawns transferred from high to low saline water, protein content of hepatopancreas fluctuated between 17.56±0.26 to 24.56±0.15 mg%(Table 14, Fig. 7). The values throughout the experimental period showed a decreasing trend. Though anova showed no overall significance ($P > 0.05$), significant decrease could be seen during 12, 24 and 48 hrs.

Isolated Muscle Tissue

Ammonia

The rate of ammonia excretion of isolated muscle tissue transferred from low to high saline water showed initially a transitory increase from 43.43±0.67 to 62.18±0.12 μNg^{-1}wet wt.h^{-1} at the end of 6th hr though the value remained low when compared to control. After 6th hr, the ammonia excretion rate started decreasing and reached 45.72±0.42 μgNg^{-1} wet wt.h^{-1}at the end of 48th hr(Table 15,Fig.8). Significant decrease($P < 0.01$) could be observed throughout the experimental period. Ammonia excretion

Table 12. Protein content of muscle of the prawn M. monoceros, transferred from high saline (35‰) to low saline water (5‰)

Hours	Protein (mg%) Control	Experimental
0	23.67 ±0.39	24.08 ±0.39
3	21.14 ±0.31	23.38 ±0.52
6	22.11 ±0.34	24.05 ±0.11
12	23.35 ±0.33	22.12 ±0.22
24	23.88 ±0.42	20.31* ±0.18
48	24.90 ±0.13	20.44* ±0.04

All values are mean of 3 determinations and figures represent $\overline{X} \pm$ S D

*Values significant at $P < 0.05$ level.

ANOVA TABLE

Source	d.f.	Sum Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	1.458	1.458	0.39	N.S.
Error	5	18.477	3.695		

Fig. 6. Changes in the protein content of muscle of experimental animals transferred from their previous low saline acclimatization to high saline water (35 $\%_0$) and from high saline acclimatization to low saline water (5 $\%_0$), in comparison with control animals.

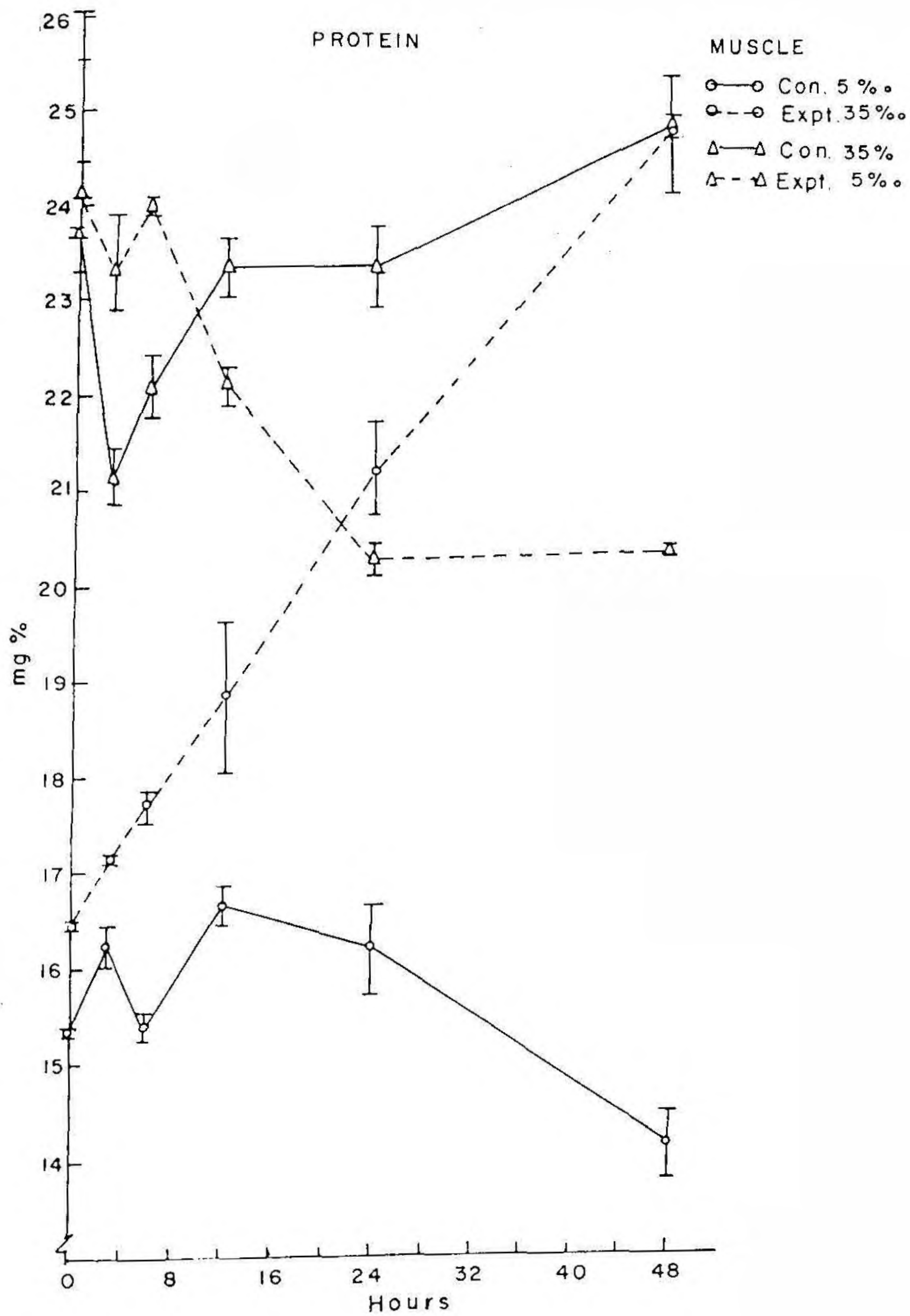

PROTEIN
MUSCLE
Con. 5 ‰
Expt. 35 ‰
Con. 35 %
Expt. 5 ‰
mg %
Hours
26
25
24
23
22
21
20
19
18
17
16
15
14
0
8
16
24
32
40
48

Table 13 **Protein content of hepatopancreas of the prawn M. monoceros, transferred from low saline (5‰) to high saline water (35‰)**

Hours	Protein (mg%) Control	Experimental
0	18.92 ±0.29	19.24 ±0.46
3	18.26 ±0.59	21.57 ±0.03
6	18.32 ±0.43	21.78 ±0.51
12	18.12 ±0.68	24.00 ±0.38
24	18.92 0.42	26.13 ±0.53
48	19.13 ±0.35	28.01 ±0.36

All values are mean of 3 determinations and figures represent $\overline{X} \pm$ S D

ANOVA TABLE

Source	d.f.	Sum.Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	70.42	70.421	14.73	SIG (5%)
Error	5	23.901	4.780		

Table 14. Protein content of hepatopancreas of the prawn M. monoceros, transferred from high saline (35‰) to low saline water (5‰)

Hours	Protein (mg%) Control	Experimental
0	21.44 ±0.33	21.88 ±0.31
3	26.13 ±0.29	24.11 ±0.03
6	24.32 ±0.14	24.56 ±0.15
12	26.35 ±0.16	21.72* ±0.12
24	25.29 ±0.38	19.76* ±0.18
48	25.73 ±0.06	17.56* ±0.26

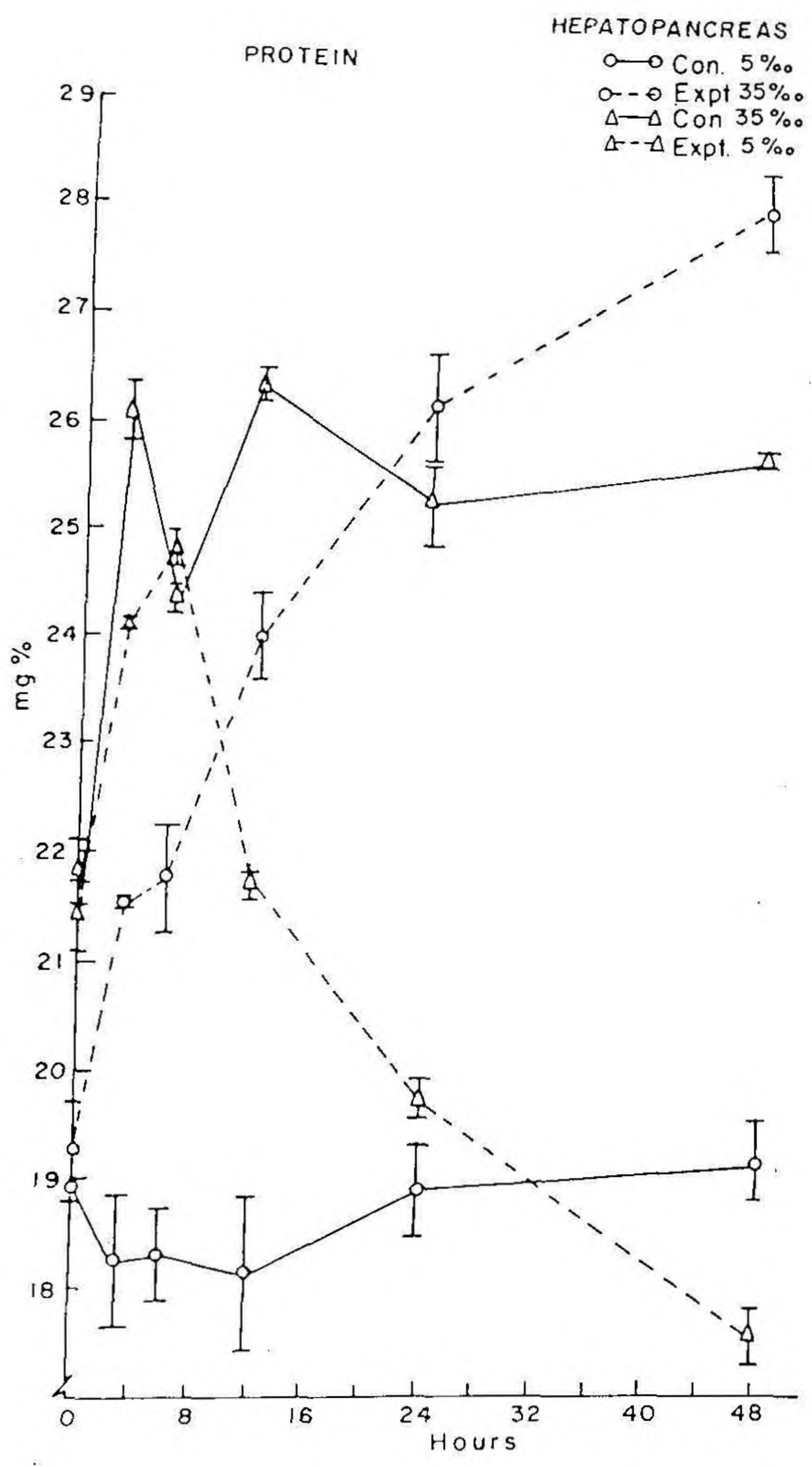
All values are mean of 3 determinations and figures represent $\overline{X} \pm$ S D

*Values significant at $P < 0.05$ level.

ANOVA TABLE

Source	d.f.	Sum. Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	32.292	32.292	5.51	N.S.
Error	5	29.328	5.866		

Fig. 7. Changes in the protein content of hepatopancreas of experimental animals transferred from their previous low saline acclimatization to high saline water (35‰) and from high saline acclimatization to low saline water(5‰) in comparison with control animals.



PROTEIN
HEPATOPANCREAS
Con. 5‰
Expt 35‰
Con 35‰
Expt. 5‰
mg %
29
28
27
26
25
24
23
22
21
20
19
18
0
8
16
24
32
40
48
Hours

Table 15. Ammonia excretion rate of isolated muscle tissue of the prawn M. monoceros, transferred from low saline (5‰) to high saline water (35‰)

Hours	Ammonia excretion rate (μgNg^{-1} wet wt. $hour^{-1}$) Control	Experimental
0	7.28 ±0.36	5.30 ±0.11
2	55.85 ±1.36	43.43 ±0.67
4	74.27 ±1.27	55.84 ±1.27
6	90.81 ±0.55	62.18 ±0.12
8	86.44 ±1.54	52.95 ±0.52
10	82.66 ±0.84	48.35 ±0.61
12	93.19 ±0.47	45.72 ±0.42

All values are mean of 3 determinations and figures represent $\overline{X} \pm$ S D

ANOVA TABLE

Source	d.f.	Sum. Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	2231.051	2231.051	19.02	HI .SIG(1%)
Error	6	703.871	117.312		

rate of isolated muscle tissue transferred from high to low saline water ranged from 66.36±0.11 to 98.41±0.40 μgNg^{-1}wet wt.h^{-1}(Table 16, Fig. 8). The values showed an increasing trend and overall excretion rate was significantly high ($P < 0.01$), when compared to control throughout the experimental period.

Total Free Amino Acid

TFAA content in isolated muscle tissue transferred from low to high saline water increased from 1.62±0.05 to 2.2±0.03 mg% (Table 17, Fig. 9). When compared to the control, significant increase ($P < 0.01$) could be seen throughout the experimental period. Isolated muscle tissue transferred from high to low saline water showed a decreasing trend in the TFAA content. The values ranged between 1.11±0.02 to 2.1±0.01 mg% (Table 18, Fig. 9). Anova showed that values were statistically significant ($P < 0.01$) when compared to the control.

Protein

Protein content in isolated muscle tissue transferred from low to high saline water ranged between 17.68±0.30 to 24.01±0.38 mg% (Table 19, Fig. 10). The values showed an increasing trend up to 8th hr, after which there was a decrease (23.37±0.29) at the end of 12th hr. However, the value remained high when compared to control. In isolated muscle tissue transferred from high to low saline water, the protein content ranged between 18.15±0.27 to 22.18±0.41 mg% (Table 20, Fig. 10). The values showed a decreasing trend throughout the experimental period. Anova

Table 16. Ammonia excretion rate of isolated muscle tissue of the prawn <u>M. monoceros</u>, transferred from high saline (35‰) to low saline water (5‰)

Hours	Ammonia excretion rate (μgNg^{-1} wet wt. $hour^{-1}$) Control	Experimental
0	4.46 ±0.17	8.62 ±0.28
2	58.10 ±0.52	66.36 ±0.11
4	64.93 ±0.49	78.46 ±0.43
6	47.99 ±0.36	88.36 ±1.05
8	42.80 ±0.60	95.16 ±0.32
10	47.36 ±0.27	98.32 ±0.35
12	48.11 ±0.93	98.41 ±0.40

All values are mean of 3 determinations and figures represent $\bar{X} \pm$ S D

<u>ANOVA TABLE</u>

Source	d.f.	Sum.Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	3454.949	3454.949	14.52	HI.SIG(1%)
Error	6	1428.133	238.022		

Fig. 8. Changes in the ammonia excretion rate of isolated muscle tissue taken from prawns of low saline acclimatization and transferred to high saline condition (35‰) and high saline acclimatization to low saline condition (5‰) in comparison with control group.

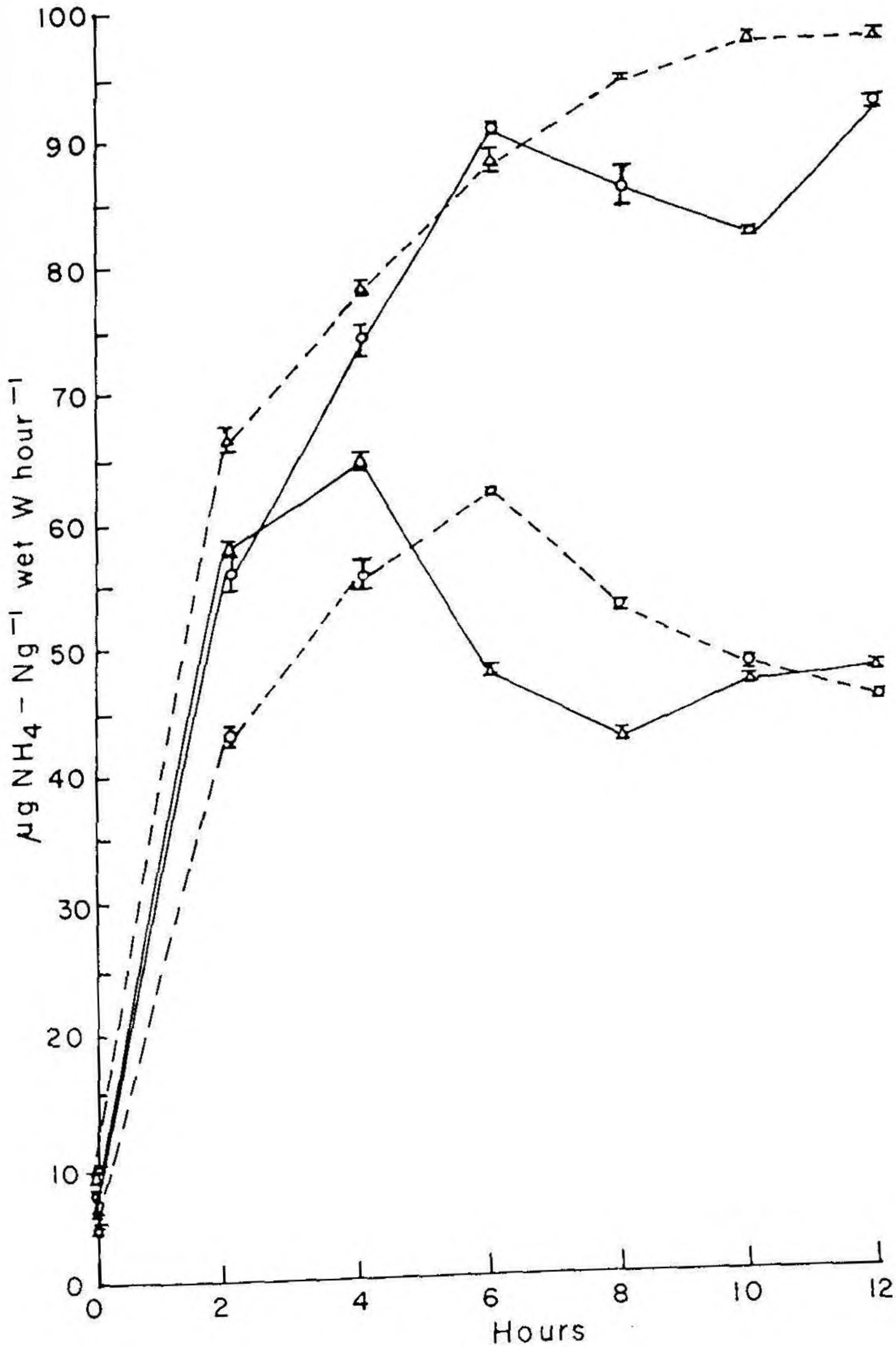

ISOLATED MUSCLE TISSUE
AMMONIA EXCRETION RATE
Con 35‰
Expt. 5‰
Con. 5‰
Expt. 35‰
μg NH4 - Ng⁻¹ wet W hour⁻¹
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
Hours

Table 17. Total free amino acid content of isolated muscle tissue of the prawn M. monoceros, transferred from low saline (5‰) to high saline water (35‰)

Hours	Total free amino acid (mg%) Control	Experimental
0	1.48 ±0.04	1.62 ±0.05
2	1.86 ±0.08	1.90 ±0.11
4	1.38 ±0.04	1.85 ±0.02
6	1.46 ±0.10	1.70 ±0.03
8	1.40 ±0.01	2.00 ±0.07
10	1.37 ±0.13	2.15 ±0.02
12	1.49 ±0.05	2.20 ±0.03

All values are mean of 3 determinations and figures represent $\bar{X} \pm$ S D

ANOVA TABLE

Source	d.f.	Sum. Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	0.628	0.628	14.95	HI.SIG(1%)
Error	6	0.252	0.042		

Table 18. Total free amino acid content of isolated muscle tissue of the prawn M. monoceros, transferred from high saline (35‰) to low saline water (5‰)

Hours	Total free amino acid (mg%) Control	Experimental
0	2.20 ±0.08	2.10 ±0.01
2	2.24 ±0.07	2.03 ±0.02
4	2.35 ±0.16	1.82 ±0.05
6	2.44 ±0.17	1.64 $\pm 8.4 \times 10^{-3}$
8	2.22 ±0.07	1.33 ±0.01
10	2.28 ±0.05	1.22 ±0.02
12	2.21 ±0.09	1.11 ±0.02

All values are mean of 3 determinations and figures represent $\bar{X} \pm$ S D

ANOVA TABLE

Source	d.f.	Sum Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	1.574	1.574	19.89	HI.SIG(1%)
Error	6	0.475	0.079		

Fig.9. Changes in the total free amino acid content of isolated muscle tissue taken from prawns of low saline acclimatization and transferred to high saline condition (35‰) and high saline acclimatization to low saline condition (5‰), in comparison with control group.

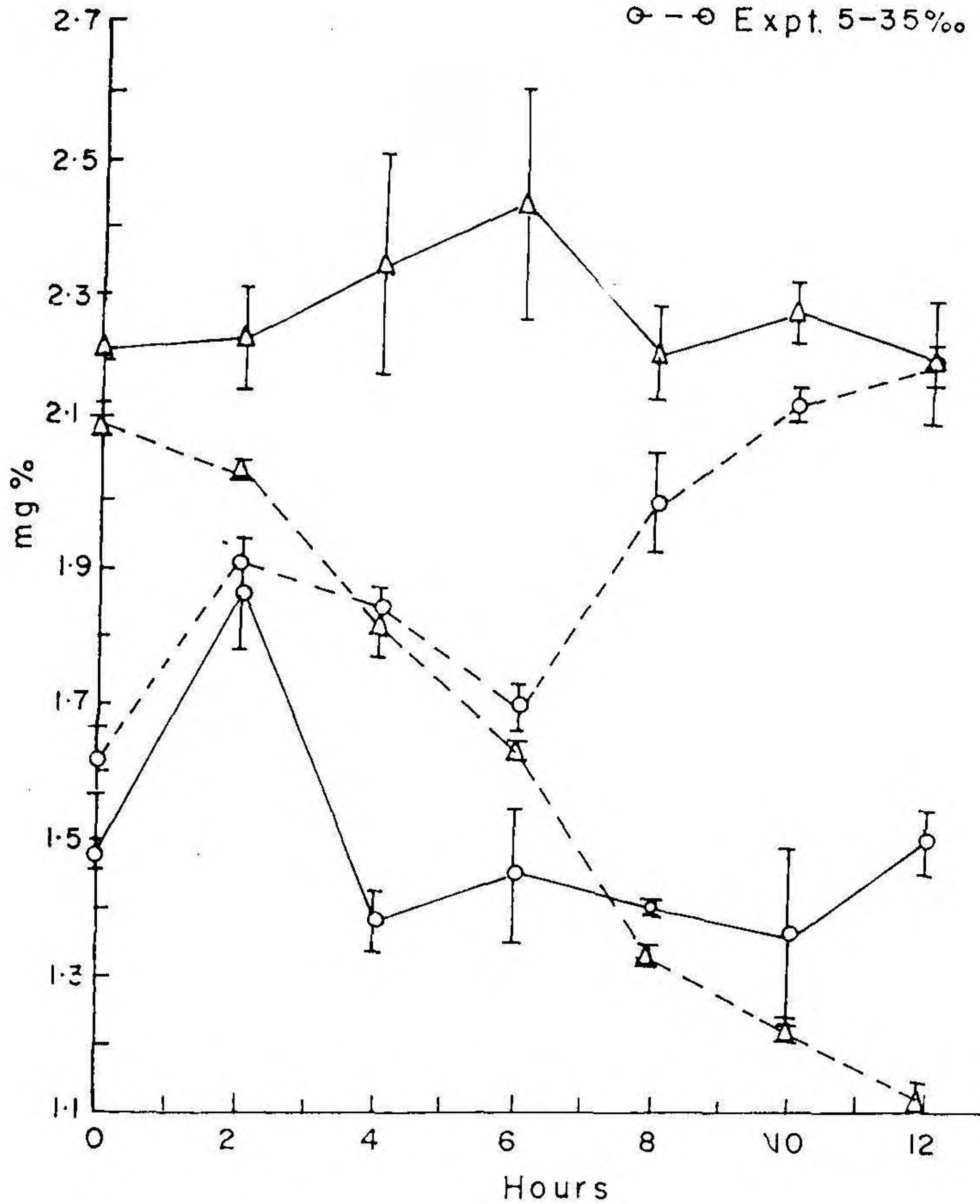

ISOLATED MUSCLE TISSUE
TOTAL FREE AMINO ACID
Con. 35‰
Expt. 35-5‰
Con. 5‰
Expt. 5-35‰
2·7
2·5
2·3
2·1
1·9
1·7
1·5
1·3
1·1
mg %
0
2
4
6
8
10
12
Hours

Table 19. Protein content of isolated muscle tissue of the prawn M. *monoceros*, transferred from low saline (5‰) to high saline (35‰) water

Hours	Protein (mg%) Control	Experimental
0	18.20 ±0.05	18.18 ±0.09
2	18.98 ±0.87	17.68 ±0.30
4	19.68 ±0.27	22.12 ±0.12
6	20.38 ±0.29	23.00 ±0.33
8	22.42 ±0.25	24.01 ±0.38
10	18.15 ±0.51	23.37 ±0.12
12	19.32 ±0.25	23.37 ±0.29

All values are mean of 3 determinations and figures represent $\bar{X} \pm$ S D

ANOVA TABLE

Source	d.f.	Sum Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	15.190	15.190	6.02	SIG (5%)
Error	6	15.151	2.525		

Table 20. Protein content of isolated muscle tissue of the prawn M. monoceros, transferred from high saline (35‰) to low saline water (5‰)

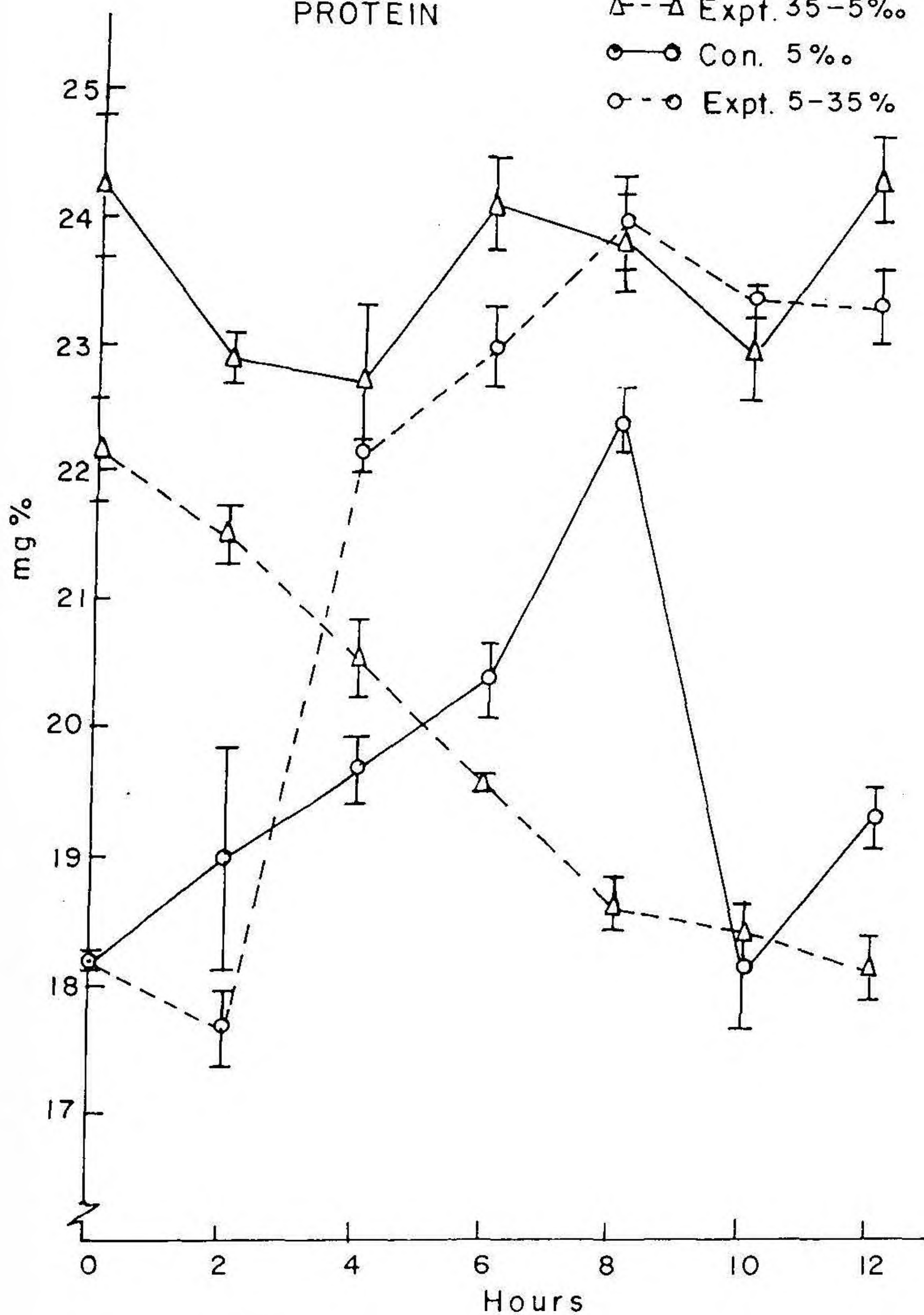
Hours	Protein (mg%) Control	Experimental
0	24.25 ±0.54	22.18 ±0.41
2	22.91 ±0.20	21.53 ±0.22
4	22.74 ±0.58	20.55 ±0.33
6	24.11 ±0.37	19.57 ±0.06
8	23.84 ±0.38	18.66 ±0.21
10	23.01 ±0.39	18.44 ±0.14
12	24.34 ±0.33	18.15 ±0.27

All values are mean of 3 determinations and figures represent $\bar{X} \pm$ S D

ANOVA TABLE

Source	d.f.	Sum Sqr.	Mean Sqr.	F-Value	Remarks
Treat	1	48.717	48.717	29.04	HI.SIG(1%)
Error	6	10.065	1.678		

Fig. 10 Changes in protein content of isolated muscle tissue taken from prawns of low saline acclimatization and transferred to high saline condition (35‰) and high saline acclimatization to low saline condition (5‰) in comparison with control group.


ISOLATED MUSCLE TISSUE
PROTEIN
Con. 35‰
Expt. 35-5‰
Con. 5‰
Expt. 5-35‰
mg %
25
24
23
22
21
20
19
18
17
0
2
4
6
8
10
12
Hours

showed significant variation ($P < 0.01$) on comparing with control values.

Protein and TFAA content of prawns in low and high saline waters

Protein and TFAA content of haemolymph, muscle and hepatopancreas of prawns of low saline water are presented in Tables 21a and b. It was found that protein content of muscle (15.67±0.82 mg%) and hepatopancreas (18.61±0.39 mg%) was low in prawns of low saline water, whereas in the haemolymph, the protein content recorded was high (Fig. 11). TFAA content in the haemolymph (1.00±0.06 mg%), muscle (1.39±0.09 mg%) and hepatopancreas (2.29±0.14 mg%) of prawns of low saline water was found to be low (Fig. 12). Protein and TFAA content of haemolymph, muscle and hepatopancreas of prawns of high saline water are given in the Tables 22 a and b. It was found that protein content of muscle (23.18±1.23 mg%) and hepatopancreas (24.87±1.67 mg%) was high in prawns of high saline water, whereas in the haemolymph,the protein content recorded was low (Fig. 11). TFAA content in the haemolymph (1.22±0.13 mg%), muscle (2.28±0.18 mg%) and hepatopancreas (2.58±0.93 mg%) of prawns of high saline water was found to be high (Fig. 12).

Neurosecretory cells

Depending on the size, shape and staining properties, the neurosecretory cells observed in different neuroendocrine masses of M. monoceros, were classified into five cell types viz, giant cells and type A, B, C and D cells. In the optic ganglion of the eyestalk, only C and D type cells

could be seen in the present study. In the cerebral ganglion all the five types of cells have been observed. Whereas in the thoracic and abdominal ganglion, giant cells along with A, B and C cell types have been noticed. The changes that appeared in the neurosecretory cells of different neuro-endocrine masses, after subjecting the prawns to low and high saline media are summarized in Table 23.

Prawns transferred from low to high saline water showed less changes in their neurosecretory cell activity when compared to prawns transferred from high to low saline water. Only the giant cells and type A cells exhibited some response. The cytoplasm of these cells, appeared granular with few vacuoles. Nucleus with nucleoli was prominent in most of these cells. Some of the giant cells and type A cells, showed irregularities in cellular profile (Plates L-P). There were no significant changes in the diameter of cells in comparison with those of control prawns (Plates A-E).

Prawns transferred from high to low saline water shown increased secretory activity in their neurosecretory cells. Here also, only the giant cells and type A cells exhibited response. The nucleus was large and prominent with many nucleoli in them. The granular material of the cytoplasm was less dense and showed extensive vacuolization in many of these cells (Plates Q-V). The cellular profile showed irregularities and some of the cells were highly enlarged when compared to those of control prawns (Plates F-K).

Table 21(a). **Protein content of the haemolymph, muscle and hepatopancreas of the prawn M. monoceros, of low saline water (5‰)**

	Haemolymph	Muscle	Hepatopancreas
Protein (mg%)	182.18 ±5.14	15.67 ±0.82	18.61 ±0.39

Table 21(b). **Total free amino acid content of the haemolymph, muscle and hepatopancreas of the prawn M. monoceros, of low saline water(5‰)**

	Haemolymph	Muscle	Hepatopancreas
Total free amino acid (mg%)	1.00 ±0.06	1.39 ±0.09	2.29 ±0.14

All values are mean of 6 determinations and figures represent $\bar{X} \pm$ SD

Table 22(a): **Protein content of the haemolymph, muscle and hepatopancreas of the prawn M. monoceros, of high saline water (35‰)**

	Haemolymph	Muscle	Hepatopancreas
Protein (mg%)	81.46 ±9.27	23.18 ±1.23	24.87 ±1.67

Table 22(b). **Total free amino acid content of the haemolymph, muscle and hepatopancreas of the prawn M. monoceros, of high saline water (35‰)**

	Haemolymph	Muscle	Hepatopancreas
Total free amino acid (mg%)	1.22 ±0.13	2.28 ±0.18	2.58 ±0.93

All values are mean of 6 determinations and figures represent $\overline{X} \pm$ S D

Fig. 11. Protein content of haemolymph, muscle and hepatopancreas of prawns of low and high saline waters.

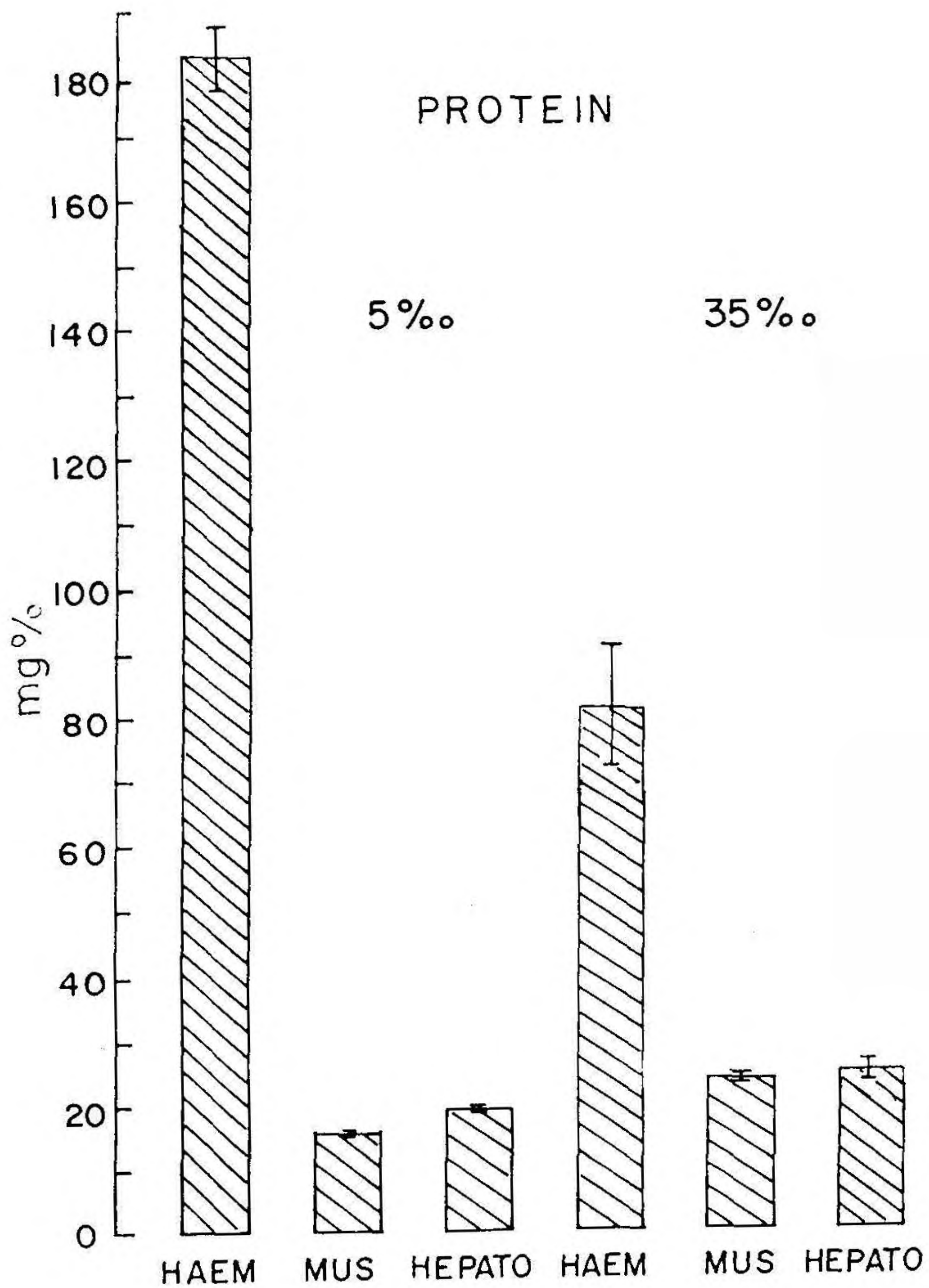

PROTEIN
5‰
35‰
mg%
180
160
140
120
100
80
60
40
20
0
HAEM
MUS
HEPATO
HAEM
MUS
HEPATO

Fig. 12. Total free amino acid content of haemolymph, muscle and hepatopancreas of prawns of low and high saline waters.

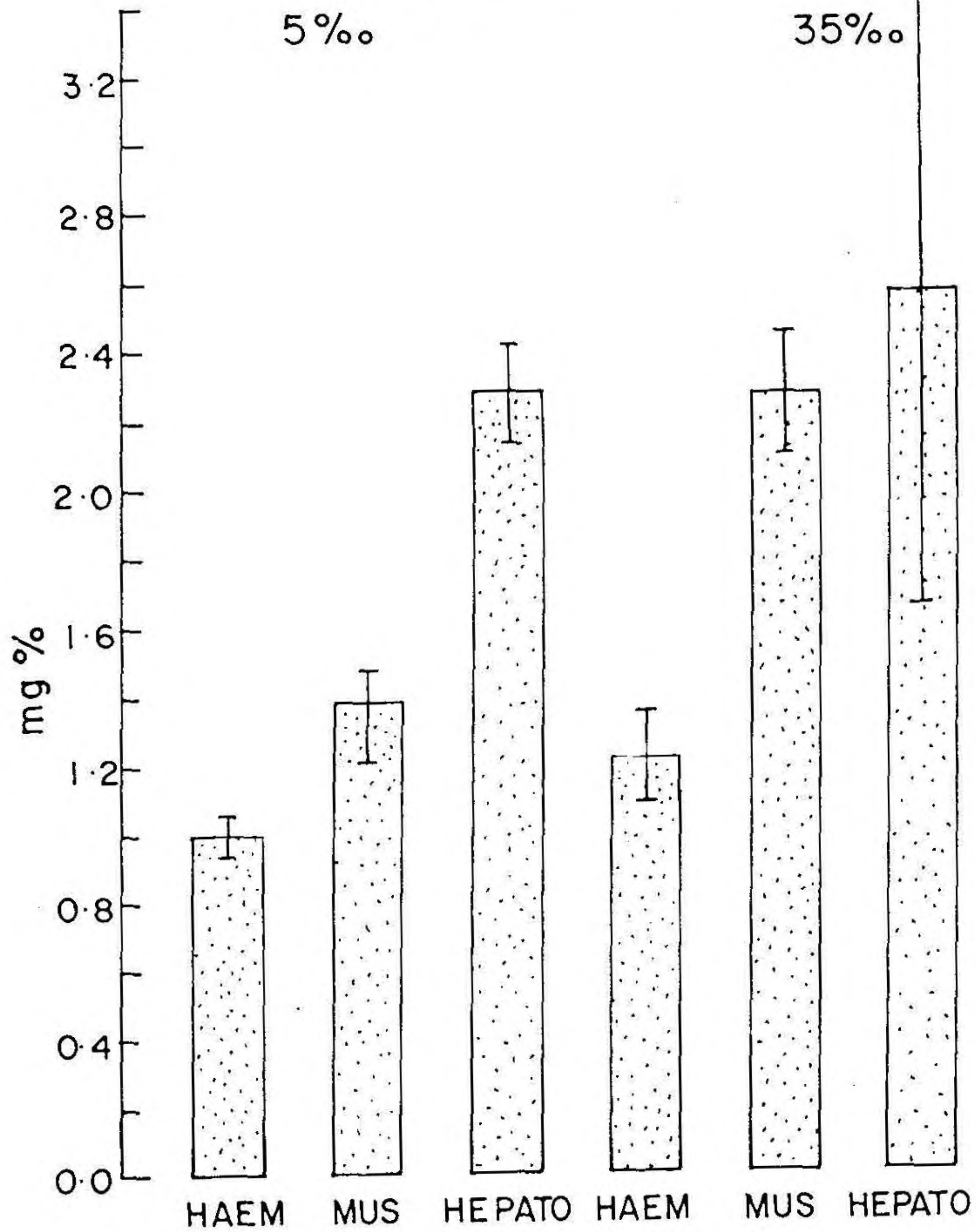

TOTAL FREE AMINO ACID
5‰
35‰
mg %
3·2
2·8
2·4
2·0
1·6
1·2
0·8
0·4
0·0
HAEM
MUS
HEPATO
HAEM
MUS
HEPATO

Table 23 Histological changes observed in the neurosecretory cells of M. monoceros in response to osmotic stress.

Neuro-endocrine tissue	Neuro-secretory cell type	SALINITY PRESSURE			
		CONTROL 5‰	EXPERIMENTAL 35‰	CONTROL 35‰	EXPERIMENTAL 5‰
1. Optic gangl-ion	Type C	Cells compact, cytoplasm with granular material. Cell diameter ranged b/w 14-18 μ.	Not much response.	Cells compact, cytoplasm with granular material. Cell diameter ranged b/w 14-18 μ.	Not much response.
	Type D	Small cells wih scanty granular material. Cell diameter ranged b/w 7-10 μ.	Not much response.	Small cells with scanty granular material. Cell diameter ranged b/w 7-10 μ.	Not much response.
2. Cerebr-al gan-glion	Giant cell type	Nucleus with or without nucleoli and granular dense cytoplasm. Cell diameter ranged b/w 53-61 μ.	Nucleus with many nucleoli prominent, granular cytoplasm less dense with few vacuoles. Cell diameter ranged b/w 53-61 μ.	Nucleus prominent with or without nucleoli, granular and dense cytoplasm with few vacuoles. Cells compact without irregularities. Cell diameter ranged b/w 53-61 μ.	Many nucleoli in nucleus prominent. Cells highly irregular with many vacuoles. Cell diameter ranged b/w 53-61 μ.

	CON 5‰	EXPT 35‰	CON 35‰	EXPT 5‰
Type A	Nucleus prominent with or without nucleoli, cytoplasm granular and dense. Cell diameter ranged b/w 32-39 μ.	Some cells without nucleoli, vacuoles seen here and there, less dense cytoplasm and cells slightly irregular. Cell diameter ranged b/w 35-39 μ.	Cells compact, prominent nucleus with or without nucleoli, with granulated and dense cytoplasm. Cell diameter ranged b/w 32-39 μ.	Many nucleoli in the nucleus, cells irregular in profile with extensive vacuolization. Cytoplasm was granular but less dense. Cell diameter ranged b/w 21-25 μ.
Type B	Nucleus prominent, cytoplasm granular and dense. Cell diameter ranged b/w 21-29 μ.	Not much response.	Nucleus prominent, cytoplasm granular and dense. Cell diameter ranged b/w 21-29 μ.	Not much response.
Type C	Nucleus prominent, cells with granular and dense cytoplasm. Cell diameter ranged b/w 14-18 μ.	Not much response.	Nucleus prominent, cells with granular and dense cytoplasm. Cell diameter ranged b/w 14-18 μ.	Not much response.
Type D	Cells with scanty cytoplasm. Cell diameter ranged b/w 7-10 μ.	Not much response.	Cells with scanty cytoplasm. Cell. diameter ranged b/w 7-10 μ.	Not much response.

		CON 5‰	EXPT 35‰	CON 35‰	EXPT 5‰
Thoracic ganglion	Giant cell type	Cells with prominent nucleus with or without nucleoli, granulated and dense cytoplasm. Cell diameter ranged b/w 53-75 μ.	Some cells without nucleus and others with many nucleoli in the nucleus, cytoplasm granulated and less dense with vacuoles here & there. Cell diameter around 75 μ.	Nucleus prominent with or without nucleoli and with dense granulated cytoplasm. Cell diameter ranged b/w 53-75 μ.	Some of the cells without nucleus while other cells with many nucleoli in the nucleus, cytoplasm less dense and highly vacuolated and cells enlarged. Cell diameter around 89 μ.
	Type A	Nucleus and nucleoli prominent, cytoplasm granulated and dense without vacuoles. Cells compact without irregularities. Cell diameter ranged b/w 28-36 μ.	Some cells without nucleus and nucleoli, with less dense vacuolated cytoplasm and slightly irregular cell margins. Cell diameter ranged b/w 21-25 μ.	Nucleus and nucleoli prominent with granular and dense cytoplasm. Cells are compact. Cell diameter ranged b/w 28-36 μ.	Large nucleus with many nucleoli. Cells slightly enlarged and irregular. Cell diameter ranged b/w 28-38 μ.
	Type B	Nucleus prominent with granular cytoplasm. Cell diameter ranged b/w 18-29 μ.	Not much response.	Nucleus prominent with granular cytoplasm. Cell diameter ranged b/w 18-29 μ.	Not much response.
	Type C	Cells with scanty granular material. Cell diameter ranged b/w 14-18 μ.	Not much response	Cells with scanty granular material. Cell diameter ranged b/w 14-18 μ.	Not much response

		CON 5‰	EXPT 35‰	CON 75‰	EXPT 35‰
Abdominal ganglion	Giant cell type	Nucleus prominent with or without nucleoli with granulated and dense cytoplasm. Cells are compact without irregularities. Cell diameter ranged b/w 51-69 μ.	Many nucleoli in the nucleus, cytoplasm granular but less dense with extensive vacuolization and cellular profile showed irregularities. Cell diameter around 75 μ.	Nucleus with nucleoli prominent, granulated and dense cytoplasm. Cell diameter ranged b/w 51-69 μ.	Many nucleoli in the nucleus prominent, granulated and less dense cytoplasm with extensive vacuolization and highly enlarged. cells. Cell diameter around 90 μ.
	Type A	Nucleus with nucleoli prominent, granulated and dense cytoplasm. Cells are compact without irregularities Cell diameter ranged b/w 21-36 μ	Some cells without nucleus and highly irregular while others with prominent nucleus and nucleoli and with less granulated cytoplasm. Cell diameter ranged b/w 21-25 μ.	Cells with prominent nucleus and nucleoli, granulated cytoplasm without vacuoles. Cells compact without irregularities. Cell diameter ranged b/w 21-36 μ.	Large nucleus prominent with many nucleoli, granulated and less dense cytoplasm. Cellular profile showed irregularities. Cell diameter ranged b/w 17-32 μ.
	Type B	Cells with prominent nucleus and granulated cytoplasm. Cell diameter ranged b/w 18-29 μ.	Not much response.	Cells with prominent nucleus and granulated cytoplasm. Cell diameter ranged b/w 18-29 μ.	Not much response.
	Type C	Small cells with scanty granular material. Cell diameter ranged b/w 17-29 μ.	Not much response	Small cells with scanty granular material. Cell diameter ranged b/w 17-20 μ.	Not much response

All the above observations were made from atleast 12 animals at each salinity concentration.

Pl. A T.S. of brain showing A, B and C type of cells of neurosecretory cell group in low saline acclimatized prawn. Note the dense granular cytoplasm, with few vacuoles in them. Mallory's triple stain X 200.

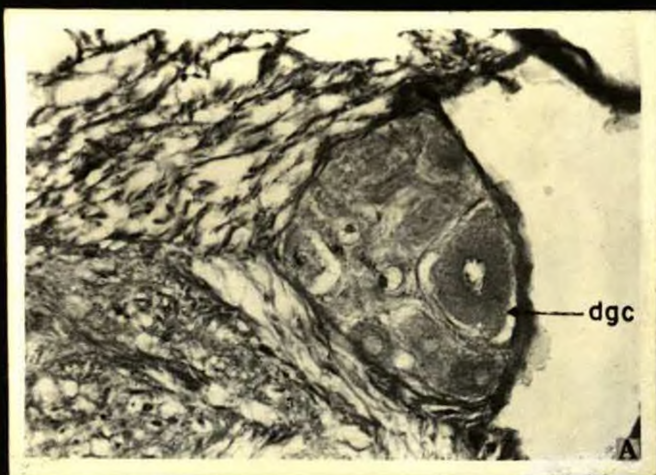
Pl. B. Longitudinal section of thoracic ganglion (T.G) showing A, B and C type of cells of low saline acclimatized prawn. Note the dense granular cytoplasm and compact cells of the neurosecretory cell group. Mallory's triple stain X 200.

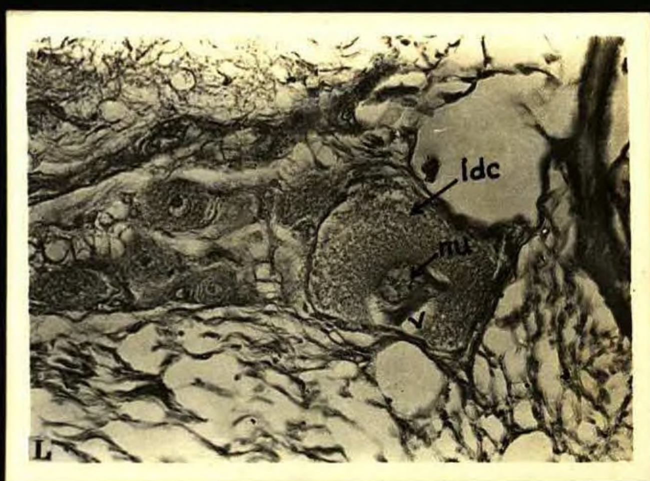
PL. C. Another view of L.S. of T.G. showing giant cell type in low saline acclimatized prawn. Note the dense granular cytoplasm with few vacuoles and prominent nucleus. Mallory's triple stain X 200.

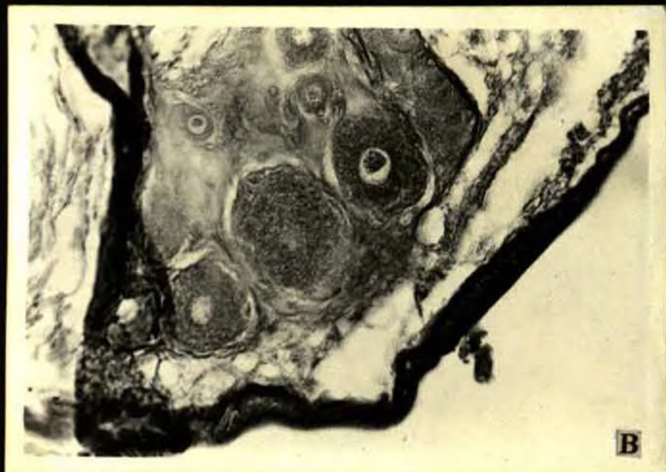
Pl. L. T.S. of brain showing giant cell type along with B type cells of neurosecretory cell group in experimental prawn, exposed to high saline water. Note the less dense cytoplasm, prominent nucleoli and vacuolization in the giant cells. Mallory's triple stain X 200.

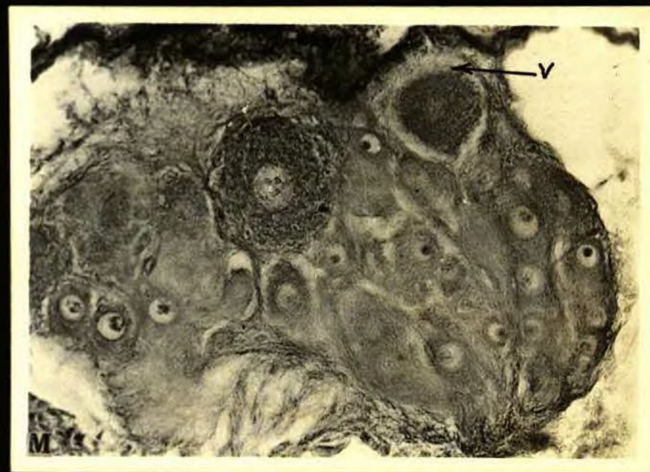
Pl. M. L.S. of T.G. showing the giant cells along with other cell types in experimental prawn exposed to high saline water. Note the less dense cytoplasm of giant cells and type A cells showing vacuoles and irregularities in cellular profile. Mallory's triple stain X 200.

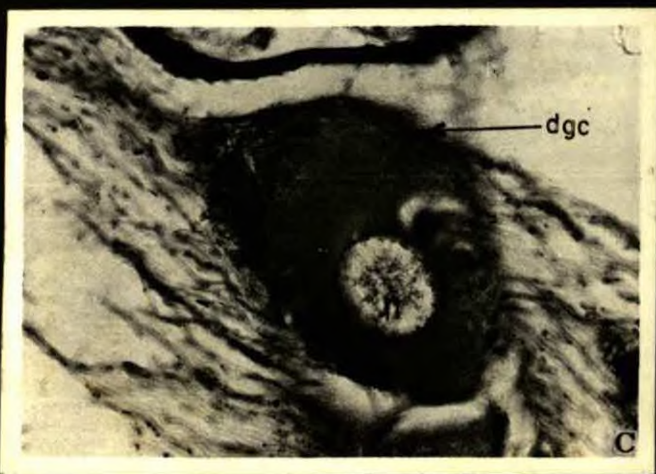
Pl. N. Another view of L.S. of T.G. showing giant cell type in experimental prawn exposed to high saline water. Note the less dense cytoplasm with vacuoles. Mallory's triple stain X 200.


dgc
A


ldc
nu
v
L


B


v
M


dgc
C

v
N

Pl. D T.S. of abdominal ganglion(A.G) showing type A cells along with other cell types in low saline acclimatized prawn. Note the dense granular cytoplasm of the cells. Mallory's triple stain x 200.

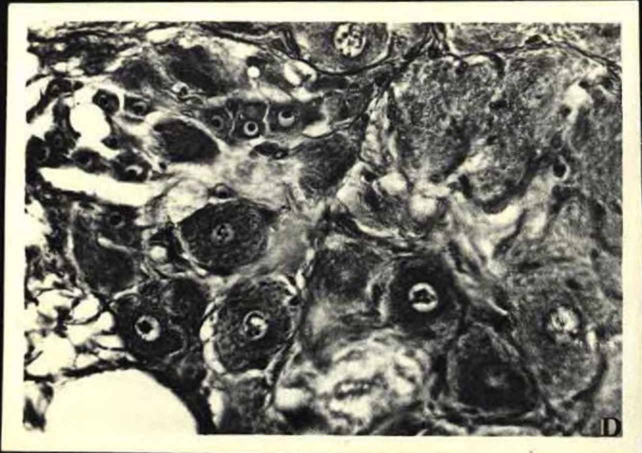
Pl. E. Another view of T.S. of A.G. showing type A, B and C cells, in the neurosecretory group of low saline acclimatized prawn. Note the granular cytoplasm of the type A cells with few vacuoles. Mallory's triple stain X 200.

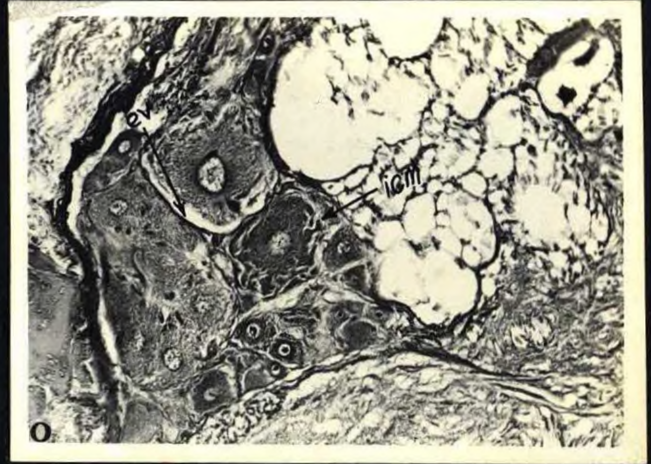
Pl. F. T.S. of brain showing giant cells with other cell types of neurosecretory cell group in high saline acclimatized prawn. Note the dense granular cytoplasm with few vacuoles and prominent nucleus in them showing no irregularities in cellular profile. Mallory's triple stain X 200.

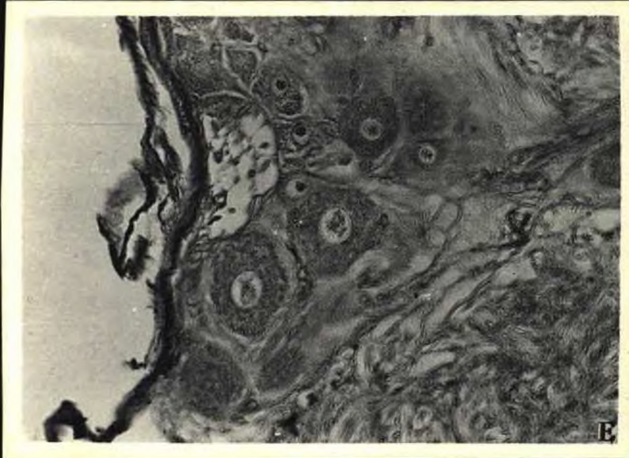
Pl. O T.S.of A.G. showing giant cells along with A,B and C type cells in the neurosecretory group in experimental prawn exposed to high saline water. Note the extensive vacuolization in giant cells and A type cells with irregularities in cellular profile. Mallory's triple stain X 100.

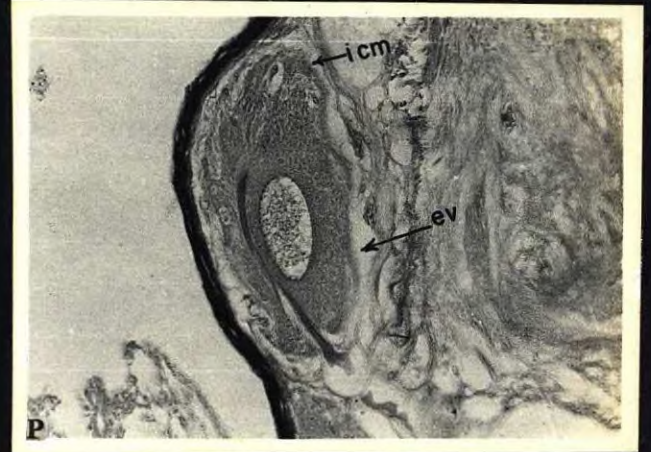
Pl. P. Another view of T.S. of A.G. showing giant cell type in experimental prawn exposed to high saline water. Note the extensive vacuolization in the cytoplasm and irregular profile of the cells showing many nucleoli in the nucleus. Mallory's triple stain X 200.

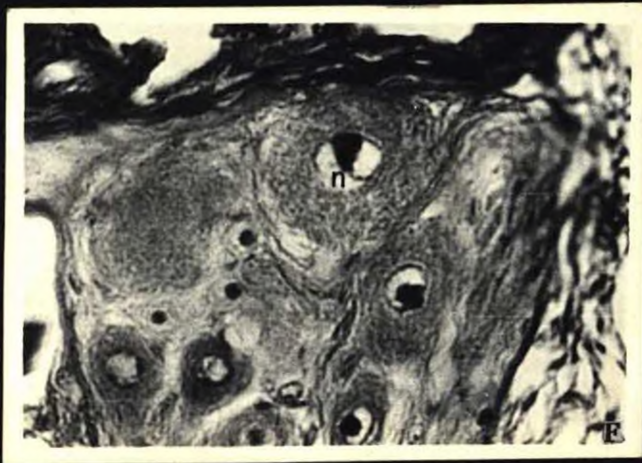
Pl. Q. T.S. of brain showing giant cell type of neurosecretory cell group in experimental prawn, exposed to low saline water. Note the less dense granular material of the cytoplasm with extensive vacuolization and showing many nucleoli in the nucleus. Mallory's triple stain X 200.

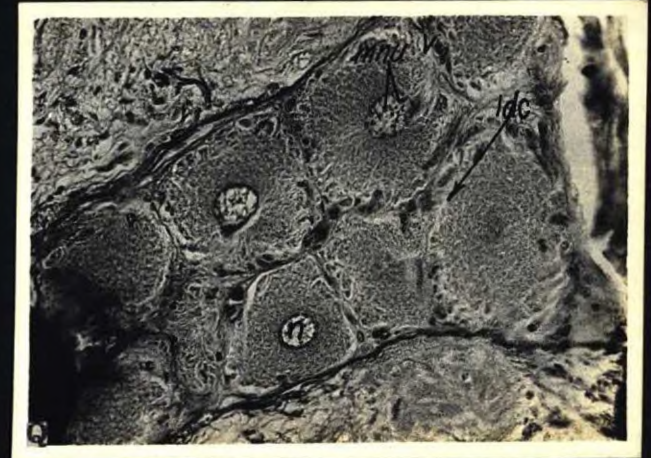

D


ev
icm
O


E


i cm
ev
P


n


ldc
Q

Pl. G. Another view of the T.S. of brain showing A, B, C and D type of cells in high saline acclimatized prawn. Note the dense granular cytoplasm and compact cells of the neurosecretory group. Mallory's triple stain X 200.

Pl. H. L.S. of T.G. showing A type of cell in high saline acclimatized prawn. Note the dense granular material in the cytoplasm and prominent nucleus. Mallory's triple stain X 200.

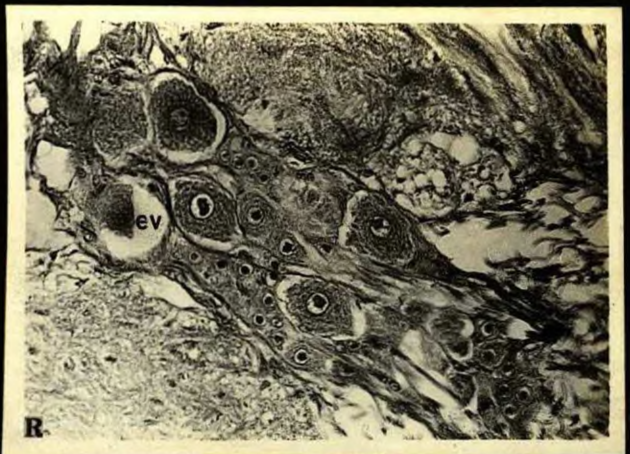
Pl. I. Another view of L.S. of T.G. showing type A, B and C cells in high saline acclimatized prawn. Note the granular dense cytoplasm with few vacuoles in them. Mallory's triple stain X 400.

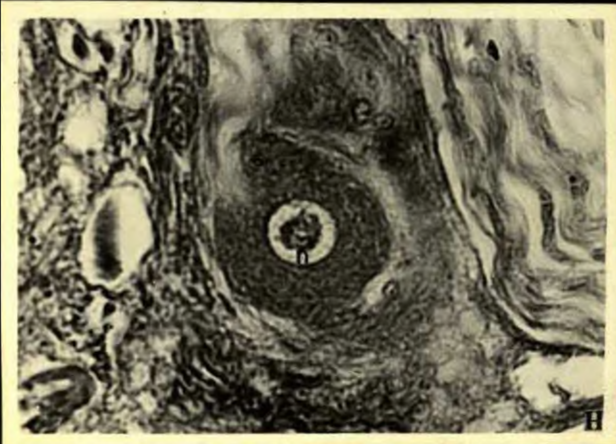
Pl. R. Another view of the T.S. of brain showing A, B, C and D type cells in experimental prawn exposed to low saline water. Note the less dense cytoplasm with extensive vacuolization in A type of cells. Mallory's triple stain X 200.

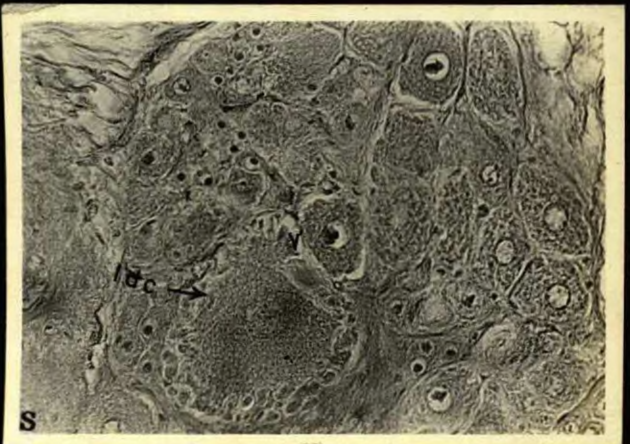
Pl. S. L.S. of T.G. showing A, B and C type cells in experimental prawn exposed to low saline water. Note the less dense granular material in the cytoplasm with extensive vacuolization in type A cells. Mallory's triple stain X 200.

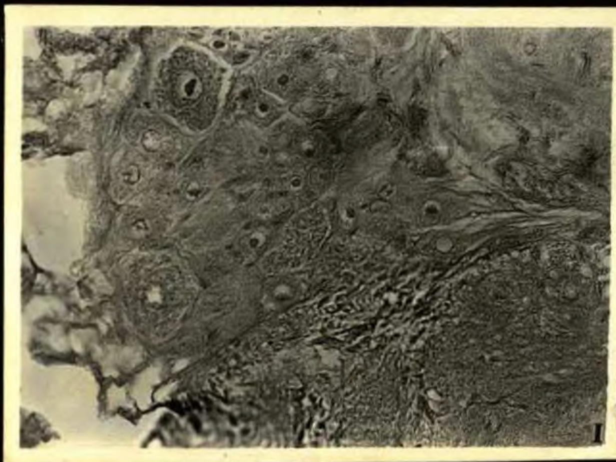
Pl. T. Another view of L.S. of T.G. showing type A cells along with other cells in experimental prawn exposed to low saline water. Note the less dense cytoplasm with extensive vacuolization and showing irregularities in cellular profile in type A cells. Mallory's triple stain X 200.

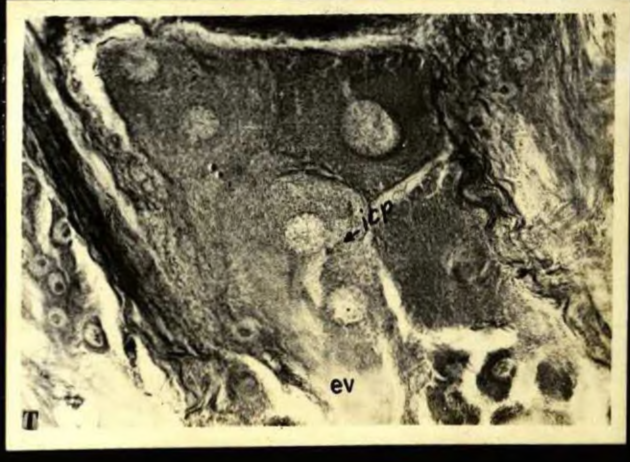
G


ev
R


n
H


v
ldc
S


I


icp
ev
T

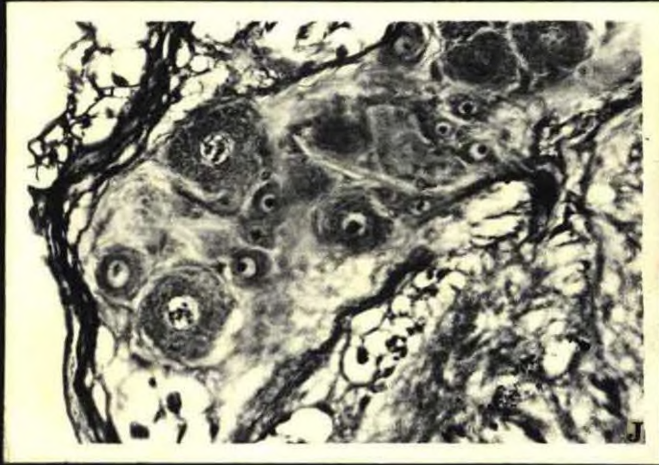
Pl. J. T.S. of A.G. showing giant cells along with other cell types in high saline acclimatized prawn. Note the dense granular cytoplasm in them. Mallory's triple stain X 200.

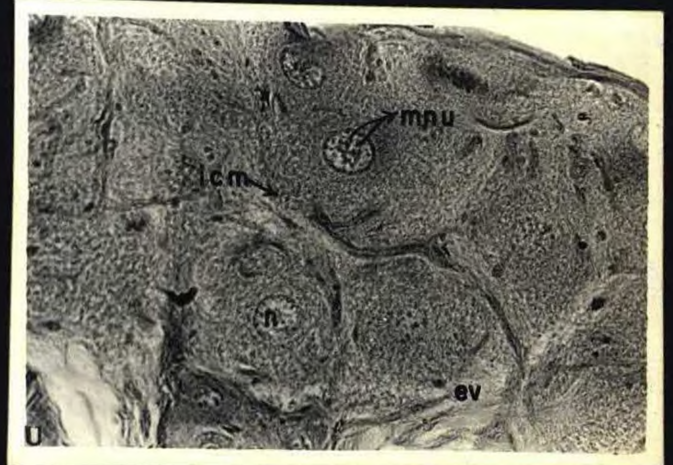
Pl. K. Another view of T.S. of A.G. showing A type of cells in high saline acclimatized prawn. Note the dense granular cytoplasm of the cells. Mallory's triple stain X 200.

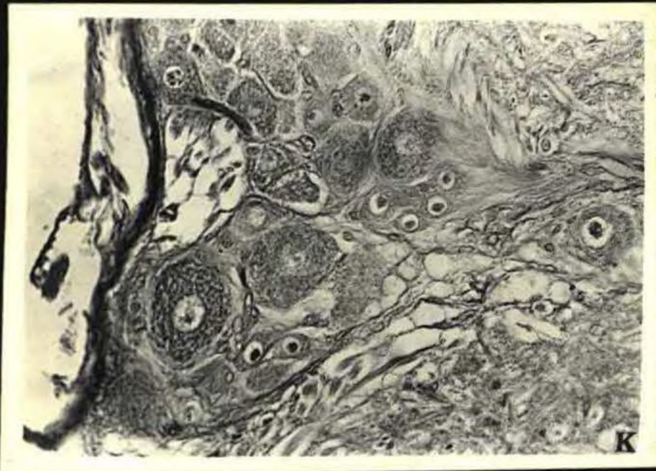
Pl. U. T.S. of A.G. showing giant cells in experimental prawn exposed to low saline water. Note the highly enlarged giant cells showing many nucleoli in the nucleus and extensive vacuolization in the cytoplasm with irregular cell margin. Mallory's triple stain x 200.

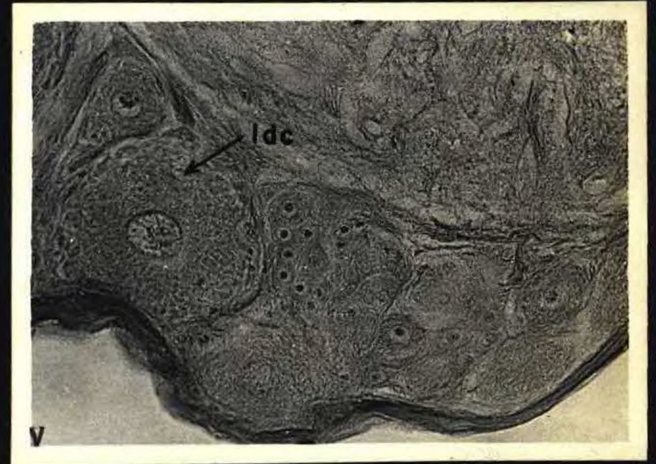
Pl. V. Another view of T.S. of A.G. showing giant cells with other cell types in the neurosecretory cell group of experimental prawn exposed to low saline water. Note the less dense granular cytoplasm showing many nucleoli in the nucleus. Mallory's triple stain X 200.

Abbreviations used in photomicrographs:
dgc - dense granular cytoplasm, ev - extensive vacuolization, icm - irregular cell margin, ldc - less dense cytoplasm, mnu - many nucleoli, icp - irregular cell profile.


J


mnu
lcm
n
ev
U


K


ldc
V

DISCUSSION

The results of the present investigation showed that the ammonia excretion rate of the prawn M. monoceros is inversely related to salinity. The findings are in agreement with observations made by Needham (1957) in C. maenas, Jeuniaux and Florkin (1961) in E. sinensis, Emerson (1967 and 1969) in euryhaline molluscs, Mangum et al. (1976) in C. sapidus, and Spaargaren et al. (1982) in P. japonicus. Haberfield et al. (1975) also reported an increase in ammonia excretion rate with decrease in salinity and suggested that lower salinity acclimation involves increased catabolism of amino acids resulting in an increased NH_4^+ excretion. Studies by Gerard and Gilles (1972) and Mangum et al. (1976) indicated an increase in the ammonia level in the blood of C. sapidus under hyposmotic stress. Vincent-Marique and Gilles (1970) also recorded high levels of ammonia in the muscle tissue of E. sinensis, on exposure to diluted media. These results may be indicative of the modifications in tissue deamination activity, as they are concomitant with changes in ammonia level in the blood and tissues. According to Mangum et al. (1976), ammonia excretion rate depends not on the ammonia present in the blood, but rather on the activity of the transport ATPase system. This effect can be explained by considering the role of ammonia in maintaining the alkali reserve of the animal at hyposmotic stress. According to Schoffeniels and Gilles (1970), intracellular isosmotic regulation mainly resulted from variations of the amino acid concentrations. These variations could either be due to synthesis or breakdown

of peptides and proteins (Gilles, 1977; Richard, 1982) or due to catabolism or synthesis of amino acids (Campbell, 1973; Schoffeniels, 1976). Jeuniaux et al. (1961) and Haberfield et al. (1975) opined that changes in ammonia excretion with salinity could be linked to the catabolism or synthesis of amino acids. However, Armitage and Morris (1982) did not observe any significant change in the amino acid concentration of Neomysis integer in response to environmental salinity. Mangum et al. (1976) and Pressley et al. (1981) reported active sodium uptake following the transfer to a hypotonic medium which was related to an increased ammonia exeetion rate and a possible reversal of this exchange (Armstrong et al., 1981) was observed following transfer to hypertonic medium. Regnault (1984) found that the rate of ammonia excretion doubled as salinity dropped from 34%$_0$ to 14%$_0$ and later reduced to 1/3rd of the value as salinity returned to 34%$_0$. Spaargaren et al. (1982) also reported high excretion rate in P. japonicus when salinity was brought down from 31 to 21%$_0$. In the present study in M. monoceros, ammonia excretion rate was found to be significantly high and showed an increasing trend when salinity dropped from 35-5%$_0$. On the other hand, in higher salinity the excretion rate reduced and showed a decreasing trend. The increase or decrease of ammonia excretion rate under different salinity conditions may possibly be related to modifications in the activity of amination - deamination sequence of the amino acids. Present investigation also showed that animals transferred to higher salinity during the first 2-3 hrs kept a high ammonia efflux rate and animals transferred to lower salinity kept a low ammonia efflux rate

during the first 2-3 hrs. Similar observations were also reported by Spaargaren _et al._ (1982). Thus the ammonia efflux is not instantaneously coupled to medium salinity, but adapts gradually, probably concurrent with internal alkali concentration (Spaargaren _et al._, 1982).

Gilles and Schoffeniels (1969), in their experiments on isolated nerve tissue of _E. sinensis_, found that ammonia undergoes significant variation. The authors found decrease in ammonia in isolated nerve during adaptation from freshwater to seawater. On the other hand, significant increase in ammonia was observed during acclimation to freshwater. The ammonia excretion rate of isolated muscle tissue was found to be high in low saline medium and decreased ammonia excretion rate was observed in high saline medium in the present study as reported by Gilles and Schoffeniels (1969). The fact that ammonia undergoes significant variation agrees with the interpretation that during hyperosmotic stress, a net synthesis of amino acids takes place from ammonia and release of ammonia from the tissues takes place under hyposmotic stress (Gilles, 1979).

Many studies on the free amino acid content of whole animals, isolated tissues and cells exposed to osmotic stresses have shown that the tissue FAA are actively regulated according to the variations of the osmolarity of the extracellular fluid (Gilles, 1979; Schoffeniels and Gilles, 1970 and 1972). Thus with an increase in salinity of the external media, there is a corresponding increase in the levels of free amino acids and a corresponding decrease in the total free amino acid level has also been observed

with a decrease in salinity. This variation has been clearly proved in many invertebrate animals and particularly in decapod crustaceans(Schoffeniels and Gilles, 1970 and 1972; Bedford and Leader, 1977; McCoid et al., 1984 and Dalla Via, 1986). The fate of amino acids in whole animals subjected to osmotic stress is mostly dependent on interactions between different tissues. Blood peptide material acting as a reservoir of amino compounds may be important in this respect. In the Chinese crab E. sinensis, under hyposmotic stress there was a transitory increase in the blood concentration of free amino acids (Spaargaren et al., 1982). On the basis of changes in total amino-nitrogen content of the blood of C. maenas withstanding osmotic stress, Siebers et al. (1972) proposed that most of the amino acids participating in the cellular volume regulation process occurring in such conditions are coming from the extracellular space. Increased efflux of amino acids from tissues to blood in euryhaline species undergoing hyposmotic stress is indicated by several studies showing that an increase in blood amino content takes place concomitant to the decrease observed at the tissue levels (Jeffries, 1966; Clark, 1968, Binns, 1969; Gerard and Gilles, 1972; Gilles, 1977). However, Boone and Claybrook (1977) reported decreased levels of FAA in the haemolymph of P. herbstii under low saline condition. In the present investigation, decreased levels of TFAA in the haemolymph was noticed under hyposmotic condition which could be related to the increased amino acid transport across the plasma membrane or due to enhanced oxidative deamination of amino acids, as corresponding increase in ammonia levels were observed in the present study.

Siebers et al. (1972) reported that during hyperosmotic stress there is a slight transitory decrease in TFAA content in the blood of C. maenas. According to the authors, this decrease might be related to an increased uptake of amino acids from the blood by various tissues. Gilles (1977) has also reported slight decrease in FAA content in the haemolymph of E. sinensis. In the present study, increased levels of TFAA was noticed in the haemolymph under hyperosmotic condition. It is interesting to note that corresponding with the increase in TFAA in the haemolymph, there was a decrease at the tissue levels after 24 hrs. This increase could be related to the efflux of amino acids from tissues to blood across the plasma membrane under hyperosmotic stress and simultaneous reduction in the ammonia excretion rate.

Several studies on the alterations in the FAA pool during adaptation to low salinity have shown that there was a decrease in tissular content of amino acids (Vincent-Marique and Gilles, 1970; Gerard and Gilles, 1972). Studies by Richard and Ceccaldi (1975) showed that FAA content in the muscle of P. kerathurus decreased while in hepatopancreas it increased, under hyposmotic stress. Boone and Claybrook (1977) also reported decreased levels of FAA in gills, muscle and hepatopancreatic tissue of P. herbstii, under low saline condition. Farmer and Reeve (1978) analysed the FAA content of whole Acartia tonsa and found the level of FAA to be reduced in hyposmotic condition. Studies by Florkin et al. (1964) and Gilles and Schoffeniels (1969) appear to support the idea that decrease in the free amino acid pool is not accomplished primarily by incorporation

of amino acids into tissue protein. Gilles and Gerard (1974) and Gerard (1975) have interpreted that the decrease in intracellular amino acid content under hyposmotic stress may be due to enhanced oxidation and an increased efflux through cell membrane. The observations made in the present study are in agreement with the findings reported by earlier workers (Florkin, 1962; Vincent-Marique and Gilles, 1970). The level of TFAA was found to decrease in the muscle and hepatopancreas, though the decrease was very prominent only during 12 and 24 hrs in the muscle tissue and during 12, 24 and 48 hrs in the hepatopancreatic tissue.

Gilles (1974) has observed increased levels of FAA in the tissues subjected to hyperosmotic shock. Baginski and Pierce (1977) have found that total amino acid pool increased in the tissues of the whole animal of M. demissus during high salinity acclimation. Gilles (1977) has reported slight decrease in the FAA content in haemolymph, and simultaneous increase of FAA in the tissues of E. sinensis. But in the present study, increased FAA level in the haemolymph, muscle and hepatopancreas was observed corresponding with a decrease in ammonia excretion rate. This increase in tissues may be due to decreased amino acid oxidation or active synthesis of peptide molecules and also decreased efflux of FAA, as reported by Gilles (1972 and 1974).

Changes in the amino acid content have been shown to occur in isolated tissues submitted to osmotic shocks. Gilles and Schoffeniels (1969) found that the concentration of amino acids in the isolated axons of E. sinensis decreased in hyposmotic condition. In fact a decrease in

amino acids in the isolated axons of E. sinensis have been reported by many workers (Gerard and Gilles, 1972; Gilles, 1972 and 1974; Gilles and Gerard 1974; Gerard, 1975). According to these authors, the decrease in the intracellular amino acid content appears to be essentially due to both an increase in the oxidation of amino acids and an increase in the passive permeability of the plasma membrane leading to an increased efflux of amino acids. Similar observations were also reported by Lang and Gainer (1969) in the muscle fibres of C. sapidus and Pierce and Greenberg (1970) in the isolated heart muscle of M. modiolus. In the present study, it was observed that corresponding with the decrease in TFAA content of the muscle and hepatopancreas, there was an increase in ammonia excretion rate. This decrease in TFAA may possibly be due to increased oxidative deamination of amino acids as reported earlier by other workers (Jeuniaux and Florkin, 1961; Florkin et al., 1964).

Florkin et al. (1964) have demonstrated that the amount of amino acids showed an increase in the muscle of E. sinensis under hyperosmotic condition. In a similar way, Bedford (1971) also showed that amino-nitrogen increased in the isolated foot muscle of the mollusc M. trifasciata submitted to hyperosmotic conditions. According to this author, the increase in amino acids is not related to a decrease in other intracellular nitrogenous compounds. However, no significant modifications in the amino acid efflux could be recorded by Gerard and Gilles (1972). In fact, Gilles (1972 and 1974) and Gilles and Gerard (1974) observed decrease in amino acid oxidation during hyperosmotic condition. Florkin and Schoffeniels (1965) have proposed that

the cation content of the cell, which would decrease or increase in concentration during adaptation to low or high salinity respectively, could modulate an enzyme or enzyme system directly involved in amino acid metabolism. In the present study also, the TFAA content of isolated muscle tissue showed a significant increase under hyperosmotic condition. Concomitant with the increase in TFAA levels the ammonia excretion rate decreased. This increase could be interpreted in terms of decreased oxidative deamination of amino acids or decreased efflux of amino acids and at the same time increased addition of amino acids to the metabolic pool (Gilles, 1979).

Experimental evidences on the effect of osmotic stress on protein modifications in crustaceans are limited. Some studies have linked the alterations of protein content with that of total free amino acids (Bedford, 1971; Venkatachari, 1974). Jeuniaux and Florkin (1961) and Binns (1969) reported that the protein content of blood increased during hyposmotic stress, while in the same condition there is an increased nitrogen excretion essentially due to increased loss of NH_3 and free amino acids. Ferraris et al. (1986) reported similar observations in P. monodon, though the blood protein content was well regulated within 24 hrs. Pequeux et al. (1979) also observed the same trends in E. sinensis, C. maenas, C. sapidus, Uca minax and Libina emarginata. Similar changes have been reported in C. maenas also by Siebers et al. (1972) and Gilles (1977) and in Astacus fluviatilis and E. sinensis by Gilles (1977). Moreover, Boone and Schoffeniels (1979) also reported increased haemocyanin synthesis in C. maenas submitted to 50% seawater. According to these authors in such conditions of acclimation

to dilute media, the increase in blood protein containing Cu^{2+} may help the animals in meeting the increased tissular oxygen demand. However, Dall (1974a,b) and Hepper (1978) opined that protein content in the haemolymph is also greatly affected by nutritional state as well as dietary source and salinity might not be an important factor in determining protein concentration in haemolymph except in unusually high or low salinities (Ferraris et al., 1986). In the present investigation also, the protein content of haemolymph was found to be increasing in low saline condition. This increase in blood proteins could be related to modifications in the activity of synthesis or related to variations in the amino acid transport that occur at the level of plasma membrane (Gilles, 1977).

Important modifications at the level of blood serum proteins during hyperosmotic conditions were also reported. Lee and Pyung (1970) and Siebers et al. (1972) observed decrease in the Cu^{2+} containing proteins in the blood of E. sinensis and C. maenas subjected to hyperosmotic stress. Significant decrease in the blood protein level was also observed in the present study. For this reverse interpretation could be considered i.e., during hyperosmotic stress, there was increased degradation or decreased synthesis of blood serum peptidic material as reported by Gilles (1977).

A few studies on the modifications of protein content in different tissues have shown that the protein content decreased under hyposmotic condition. Boone and Claybrook (1977) observed a decrease in the tissue FAA content of the mud crab P. herbestii, when the animals were acclimated to lower salinities. Florkin et al. (1964) opined that the source is either

from metabolic pool or through dietary origin, while Bedford (1971) and Venkatachari (1974) reported its mobilisation from tissue protein. In the present investigation, there was substantial increase in the protein content of haemolymph under hyposmotic condition concomitant with decrease in the protein content of muscle and hepatopancreas though the decrease was prominent only during 24 and 48 hrs. Decrease in the tissue protein observed in the present study could be due to increased catabolic process. This has been reflected on the high rate of ammonia excretion.

Studies on the protein modification in tissues under hyperosmotic conditions are lacking. Siebers (1972) found no significant change in the protein concentration in whole Oreonectus limosus during acclimation to high salinity. Thus it appears that equilibrium between protein and amino acids plays a minor role, if any, in the adjustment of amino acid pool (Gilles and Schoffeniels, 1969; Florkin et al. 1964). However, McCoid et al. (1984) observed a gra dual increase in the free amino acids in the muscle of P. vannamei, when subjected to increasing salinities. They found a maximum increase in glycine besides alanine, proline and arginine. Similar observations were also reported by Dalla Via (1986) in the juveniles of kuruma prawn P. japonicus. There was an increase in the protein content of muscle and hepatopancreas under hyperosmotic condition in the present study though the increase in muscle tissue was prominent only during 24 and 48 hrs. This increase may possibly be due to decreased oxidative deamination of amino acids or degradation of blood proteins, resulting in a transitory increase of FAA in the tissues and a concomitant rise in the protein content. It is also interesting to note that protein and TFAA content

of muscle and hepatopancreatic tissue of M. monoceros was much higher in concentrated seawater rather than in diluted water. This point has been well substantiated by Florkin and Scheer (1970).

Studies on the variation of protein content on isolated tissues throw light on the concept that the equilibrium between proteins and amino acids play only a minor role in the adjustment of the amino acid pool, during cell volume regulation process. Florkin et al. (1964) found that the amount of proline and alanine obtained from protein hydrolysis does not vary significantly under hyperosmotic condition in E. sinensis. Gilles and Schoffeniels (1969), while studying the amino acid pattern in isolated nerve tissue of E. sinensis found that intracellular metabolic mechanism involved could result from the activity of a synthesizing process and not from a modification of the steady state between amino acids and protein, since protein content did not vary significantly. Such a conclusion was also supported by Florkin et al. (1964) and Schoffeniels (1967). In the present study, the protein content of isolated muscle tissue under hyposmotic condition decreased significantly with an increase in ammonia excretion rate. This decrease could be related to increased oxidative deamination of amino acids as there was a corresponding increase in the ammonia excretion. On the other hand, protein content under hyperosmotic condition was found to increase significantly with a corresponding increase in TFAA content and decrease in the ammonia excretion rate thereby indicating decrease in oxidative deamination of amino acids.

Till recent times, there is no clear cut indication of a possible role for hormonal interaction in isosmotic regulation of the intracellular fluids. It is therefore suggested that the fate of amino acids in whole animals subjected to osmotic stress is most likely to be dependent on interactions between the different tissues. An argument favouring this view is found in the results of Duchateau and Florkin (1962), where the authors showed that removal of eyestalk gland did not alter the isosmotic regulation power of E. sinensis. It is also evident from the experiments performed on isolated nerves (Schoffeniels, 1960; Gilles and Schoffeniels, 1969) that the isosmotic regulation process is not under hormonal control since, the isolated tissue is able to regulate its amino acid level with respect to osmotic pressure of the external medium. But it is understood that with the exception of substances directly involved in molting and reproduction, hormones are produced and released from the neuroendocrine centres particularly, optic ganglion, thoracic ganglionic mass and brain. Studies correlating to changes in the appearance of neurosecretory cells with an osmoregulatory response are meagre in crustaceans.

Van den Bosch (1976) observed changes occurring in the medial neurosecretory cells in the protocerebrum of Artemia salina under hyper and hyposmotic conditions. He reported that animals kept in dilute seawater showed increase in their secretory activity. Whereas, in animals kept in concentrated saline water the neurosecretory cells diminish their rate of secretion and become filled with acidophilic granules and thus appears to become inactivated. In his work, Van den Bosch (1976) has not mentioned in detail

types of cells of different neuroendocrine masses or the individual response of different cell types to osmotic stress. The observations made on the neurosecretory cell activity in relation to osmotic responses in M. monoceros are in agreement with the findings reported by Van den Bosch (1976). In the present study, the responses of giant cells and type A, B, C and D cells of different neuroendocrine masses were observed. This is for the first time that individual neurosecretory cells of different neuroendocrine masses have been studied in response to osmotic stress. Observations made here are promising but requires further detailed studies.

SUMMARY

The present study was carried out to understand the physiological mechanism involved in the osmoregulation of the prawn, Metapenaeus monoceros. Two sets of experiments were conducted in the present investigation. In the first set, low saline acclimatized prawns were transferred to high saline medium and in the second set, high saline acclimatized prawns were transferred to low saline water. The effect of sudden salinity variation was then studied by determining the organic constituents namely, protein and TFAA in the haemolymph, muscle and hepatopancreas and also the ammonia excretion rate of the prawn. Similar experiments were also conducted on isolated muscle tissue. The changes occurring in neurosecretory cells in response to different osmotic environment were also studied. The results obtained in the present investigation are summarized below.

Ammonia

In prawns transferred from low to high saline water (35‰) the ammonia excretion rate showed a decreasing trend and the excretion rate was reversed, when prawns acclimatized to high saline water (35‰) were transferred to low saline water (5‰).

In isolated muscle tissue, ammonia excretion rate showed significant decrease and remained low till the end of 12th hr in high saline water (35‰). On the other hand, when transferred from high to low saline water

(5‰), ammonia excretion rate showed an increasing trend and remained high throughout the experimental period.

Total Free Amino Acid (TFAA)

TFAA in the haemolymph of whole prawn increased significantly when transferred to high saline water (35‰). Whereas, on transferring prawns from high to low saline water (5‰) the TFAA content decreased significantly. TFAA content in the muscle of prawns exposed to high saline water (35‰) showed a significant increase throughout the experimental period. Whereas, in prawns exposed to low saline water (5‰) significant decrease was observed only during 12 and 24 hrs of experimental duration. TFAA content in the hepatopancreas of prawns exposed to high saline water (35‰) increased significantly. Whereas, in prawns exposed to low saline water (5‰) significant decrease was more pronounced after 12th hr.

In isolated muscle tissue, TFAA content showed significant increase when exposed to high saline water (35‰). On the other hand, it decreased significantly when transferred from high to low saline water (5‰).

Protein

In the prawns transferred to high saline water (35‰), the protein content of haemolymph showed a decreasing trend and remained low during the experimental period. On the other hand, in prawns transferred from

high to low saline ($5‰$) water haemolymph protein showed an increasing trend throughout the time of experiment. The protein content of muscle in prawns transferred to high saline water ($35‰$) showed significant increase only during 24 and 48 hours. But, in prawns transferred to low saline water ($5‰$) the protein content showed a decrease which was more prominent during 24 and 48 hrs. The protein content of hepatopancreas showed an increasing trend in prawns transferred to high saline water($35‰$). Whereas, in prawns transferred to low saline water ($5‰$) protein content decreased significantly after 12 hrs and the decrease continued till 48th hr.

The protein content of isolated muscle tissue transferred to low saline water ($5‰$), however, showed a transitory increase up to 8th hr. When transferred to high saline water ($35‰$) it showed a decreasing trend throughout the period of experiment.

Neurosecretory cells

Prawns transferred from low to high saline water showed decreased activity in their neurosecretory cells when compared to prawns transferred from high to low saline water. Only giant cells and type A cells of different neuroendocrine masses exhibited response. In other cells response was not that pronounced.

Prawns transferred from high to low saline water showed increased activity in their neurosecretory cells. Here also only the giant cells and

type A cells exhibited response and response by other cell types were not that pronounced.

The results obtained in the present investigation illustrate a significant role played by TFAA, protein and ammonia excretion to fulfil the osmoregulatory needs of the animal. The variations showed increased mobilization of protein in the form of FAA from the tissues to haemolymph thereby, resulting in a decrease in the TFAA content of tissues and an increase in haemolymph protein and also accelerated catabolic activity of the amino compounds under hyposmotic condition. The possibility of reverse process during hyperosmotic stress is also predicted i.e., mobilization of protein from haemolymph to tissues resulting in a rise in the level of TFAA and protein in the tissues and a fall in the protein level in the haemolymph,concomitant with decreased catabolic activity of the amino compounds. The isolated muscle tissue also achieved cell volume regulation in a similar way by modifying the amination-deamination activity of amino compounds. Moreover, the observations made on the changes occurring in different neurosecretory cells implicate that neuroendocrine factors may have some role in the control of osmoregulation.

REFERENCES

ARMITAGE, M.E. and MORRIS, R.J. 1982. The effect of changing environmental salinity on the free amino acid composition of an estuarine population of Neomysis integer. Estuar. Coast. Shelf Sci., 14: 301-311.

ARMSTRONG, D.A. STRANGE, K. CROWE, J. KNIGHT, A. and SIMMONS, M. 1981. High salinity acclimation by the prawn Macrobrachium rosenbergii: Uptake of exogenous ammonia and changes in endogenous nitrogen compounds. Biol. Bull., 160: 349-365.

BAGINSKI, R.M. and PIERCE, S.K. 1977. The time course of intracellular free amino acid accumulation in tissues of Modiolus demissus during high salinity adaptation. Comp. Biochem. Physiol., 57A: 407-412.

BARTBERGER, C.A. and PIERCE, S.K. 1976. Relationship between ammonia excretion rate and haemolymph nitrogenous compounds of a euryhaline bivalve during low saline acclimation. Biol. Bull., 150: 1-14.

BEDFORD, J.J. 1971. Osmoregulation in Melanopsis trifasciata.-IV. The possible control of intracellular isosmotic regulation. Comp. Biochem. Physiol., 40A: 1015-1027.

BEDFORD, J.J. and LEADER, J.P. 1977. The composition of the haemolymph and muscle tissue of shore crab Hemigrapsus edwardsi exposed to different salinities. Comp. Biochem. Physiol., 10: 173.

BINNS, R. 1969. The physiology of the antennal gland of Carcinus maenas (L.). IV. The resorption of amino acids. J. Exp. Biol., 51: 29-39.

BOONE, W.R. and CLAYBROOK, D.L. 1977. The effect of low salinity on amino acid metabolism in the tissues of the common mud crab, Panopeus herbstii (Milne-Edwards). Comp. Biochem. Physiol., 52A: 99-106.

BOONE, W.R. and SCHOFFENIELS, E. 1979. Haemocyanin synthesis during hypo-osmotic stress in the shore crab, Carcinus maenas (L.). Comp Biochem. Physiol., 40A: 1015-1027.

CAMPBELL, J.W. 1973. Nitrogen excretion. Comparitive animal physiology, 3rd ed. Saunders Company, Philadelphia. pp. 279-316.

CASTILLE, F. Jr. and LAWRENCE, A. 1981. The effect of salinity on the osmotic sodium and chloride concentrations in the haemolymph of euryhaline shrimp of the genus Penaeus. Comp. Biochem. Physiol., 68A: 75-80.

CLARK, M.E. 1968. A survey of the effect of osmotic dilution on free amino acids of various polychaetes. Biol. Bull., 134: 252-260.

CROGHAN, P.C. 1961. Competition and mechanisms of osmotic adaptation. Mechanisms in Biological Competition. Symposia of Society for Experimental Biology. Great Britain. No.15. pp. 156-167.

DALL, W. 1970. Osmoregulation in the lobster Homarus americanus. J. Fish. Res. Bd. Can., 27: 1123-1130.

DALL, W. 1974a. Osmotic and ionic regulation in the western rock lobster, Panulirus longipes (Milne-Edwards). J. exp. mar. Biol. Ecol., 15: 97-125.

DALL, W. 1974b. Indices of nutritional state in the western rock lobster, Panulirus longipes (Milne-Edwards). I. Blood and tissue constituents and water content. J. exp. mar. Biol. Ecol., 8: 227-238.

DALLA VIA, G.J. 1986. Salinity responses of the juvenile penaeid shrimp Penaeus japonicus. II. Free amino acids. Aquaculture, 55: 307-316.

*DAVIS, C.W. 1978. Neuroendocrine control of sodium balance in the fiddler crab Uca pugilator. Ph.D. Thesis, University of North Carolina, Chapel Hill, North Carolina.

DENNE, L.B. 1968. Some aspects of osmotic and ionic regulation in the prawns Macrobrachium australiensis (Holothuis) and Macrobrachium equidens (dana). Comp. Biochem. Physiol., 26A: 17-30.

DIWAN, A.D. and LAXMINARAYANA, A. 1989. Osmoregulatory ability of Penaeus indicus H. Milne Edwards in relation to varying salinities. Proc. Indian Acad. Sci. (Animal Sci.)., 98(2): 105-111.

*DUCHATEAU-BOSSON, Gh and FLORKIN, M. 1955. Concentration du milieu exterieur et etat stationnaire du pool des acides amines non proteiques des muscle d' Eriocheir sinensis, Milne-Edwards. Arch. Int. Physiol. Biochim., 63: 249-251.

*DUCHATEAU-BOSSON, Gh. and FLORKIN, M. 1962. Regulation isosmotique intracellulaire chez Eriocheir sinensis apres ablation des pedoncules oculaires. Arch. Int. Physiol. Biochim., 70: 393-396.

EMERSON, D.N. 1967. Some aspects of free amino acids metabolism in the developing encysted embryos of Artemia salina the brine shrimp. Comp. Biochem. Physiol., 20: 245-261.

EMERSON, D.N. 1969. Influence of salinity on ammonia excretory rates and tissue constituents of euryhaline invertebrates. Comp. Biochem. Physiol., 29: 1115-1133.

FARMER, L. and REEVE, M.R. 1978. Role of the free amino acid pool of the copepod Acartia tonsa in adjustment to salinity change. Mar. Biol., 48: 311.316.

FERRARIS, R.P. PARADO-ESTEPA, Fe.D. LADIA, J.M. and De JESUS, E.G. 1986. Effect of salinity on the osmotic chloride, total protein and calcium concentrations in the haemolymph of the prawn Penaeus monodon (Fabricius). Comp. Biochem. Physiol., 83A: 701-708.

*FLORKIN, M. 1962. La regulation isosmotique intracellulaire chez les invertebres marins euryhalins. Bull. Acad. R. Belg. Cl. Sci., 48: 687-694.

*FLORKIN, M. DUCHATEAU-BOSSON, Gh. JEUNIAUX,Ch.and SCHOFFENIELS, E. 1964. Sur Le mechanisme de la regulation de la concentration intracellulaire en acides amines libres, chez Eriocheir sinensis, au cours de l'adaptation osmotique Arch. Int.Physiol. Biochi m., 72:892-906.

FLORKIN, M. and SCHEER, B.T. 1970. Chemical Zoology, Academic Press, New York, London. Vol. VII, pp. 49, 223, 255, 282.

FLORKIN, M. and SCHOFFENIELS, E. 1965. Euryhalinity and the concept of physiological radiation. Studies in comparative Biochemistry, Pergamon press, Oxford. pp. 640.

FLORKIN, M. and SCHOFFENIELS, E. 1969. Molecular approaches to Ecology, Academic Press, New York pp. 89-163.

GEORGE, M. and VEDAVYASA RAO, P. 1968. Observations on the development of external genitalia in some penaeid prawns. J. Mar. Biol. Ass. India., 10: 52-70.

GERARD, J.F. 1975. Volume regulation and alanine transport. Response of isolated axons of Callinectes sapidus Rathbun to hyposmotic conditions. Comp. Biochem. Physiol., 51A: 225-229.

GERARD, J.F. and GILLES, R. 1972. Modification of amino acid efflux during the osmotic adjustment of isolated axons of Callinectes sapidus. Experientia, 28: 863-864.

GILLES, R. 1972. Amino acid metabolism and isosmotic intracellular regulation in isolated surviving axons of Callinectes sapidus. Life Sci., 11: 565-572.

GILLES, R. 1973. Osmotic behaviour of isolated axons of a euryhaline and a stenohaline crustacean. Experientia, 29: 1354-1355.

*GILLES, R. 1974. Metabolisme de acids amines et controle du volume cellulaire. Arch. Int. Physiol. Biochim., 82: 423-489.

GILLES, R. 1977. Effect of osmotic stresses on the protein concentration and pattern of Eriocheir sinensis blood. Comp. Biochem. Physiol., 56A: 109-114.

GILLES, R. 1979. Intracellular organic osmotic effectors. Mechanisms of Osmoregulation in Animals, Wiley-Interscience, London, New York. pp. 111-153.

GILLES, R. and GERARD, J.F. 1974. Amino acid metabolism during osmotic stress in isolated axons of Callinectes sapidus. Life Sci., 14: 1221-1229.

GILLES, R. and SCHOFFENIELS, E. 1969. Isosmotic regulation in isolated surviving nerves of Eriocheir sinensis Milne Edwards. Comp. Biochem. Physiol., 31: 927-939.

GORNALL, A.G. BARDAWILL, C.J. and DAVID, M.M. 1949. Determination of total serum protein by means of Biuret reaction. J. Biol. Chem., 177: 751-766.

HABERFIELD, E.C. HASS, L.W. and HAMMEN, C.S. 1975. Early ammonia release by a polychaete Nereis virens and a crab Carcinus maenas in diluted seawater. Comp. Biochem. Physiol., 52A: 501-503.

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

HEPPER, B. 1978. Changes in blood serum protein levels during the moulting cycle of the lobster, Homarus gammarus (L.). J. exp. mar. Biol. Ecol., 28: 293-296.

JEFFRIES, H.P. 1966. International conditions of a diminishing blue crab population (Callinectes sapidus). Chesapeake Sci., 7: 164-170.

*JEUNIAUX, Ch. BRICTEUX-GREGOIRE, S. and FLORKIN, M. 1961. Contribution des acides amines libres a la regulation osmotique intracellulaire chez deux crustaces euryhalins, Leander serratus F. et Leander squilla L. Cah. Biol. mar., 2: 373-380.

*JEUNIAUX, Ch. and FLORKIN, M. 1961. Modification de l'excretion azotee du crabe chinois au cours de l'adaptation osmotique. Arch. Int. Physiol. Biochim., 69: 385-386.

KANESHIRO, E.S. HOLZ, G.G. and DUNHAM, P.B. 1969. Osmoregulation in a marine ciliate, Miamiensis avidus. II. Regulation of the intracellular free amino acids. Biol. Bull., 137: 161-169.

KINNE, O. 1967. Physiology of estuarine organisms with special reference to salinity and temperature: general aspects. Estuaries, Am. Ass. Advmt. Sci., Publ. No. 83; pp. 525-540.

LANG, M.A. and GAINER, H. 1969. Isosmotic intracellular regulation as a mechanism of volume control in crab muscle fibres. Comp. Biochem. Physiol., 30: 445-456.

LANGE, R. 1972. Some recent work on osmotic, ionic and volume regulation in marine animals. Oceanogr. Mar. Biol. A Rev., 10: 97-136.

LEE, B.D. and PYUNG, C. 1970. On the oxygen consumption of gill tissue of certain crabs in relation to differences in habitat. Pub. Mar. Lab. Pusan. Fish. Coll., 3: 55-56.

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.

Mc COID, V.R. MIGET, R. and FINNE, G. 1984. Effect of environmental salinity on the free amino acid composition and concentration in penaeid shrimp. J. Food Sci., 49: 327-330.

MEASURES, J.C. 1975. Role of amino acids in osmoregulation of non-halophilic bacteria. Nature, 257: 398-400.

MENON, M.K. 1933. The life histories of Decapod crustacea from Madras. Bull. Madras Govt. Mus. n.s. Nat. Hist. Sec., 3(3): pp. 45

MENON, M.K. 1951. The life history and bionomics of an Indian Penaeid prawn Metapenaeus dobsoni Miers. Proc. Indo-Pafic. Fish. Counc., 3rd meeting, Sec. II: 80-93.

NAGABHUSHANAM, R. and JYOTHI, M. 1977. Hormonal control of osmoregulation in fresh water prawn Caridina weberi. J. Anim. Morphol. Physiol., 24(1): 20-28.

NEEDHAM, A.E. 1957. Factors affecting nitrogen excretion in Carcinus maenas (Pennant). Physiologia Comp. Ocecol., 4: 209-239.

PANIKKAR, N.K. 1968. Osmotic behaviour of shrimps and prawns in relation to their biology and culture. FAO Fish. Rep., 57(2): 527-538.

PANIKKAR, N.K. and VISWANATHAN, R. 1948. Active regulation of chloride in Metapenaeus monoceros. Nature, 161: 137-138.

PEQUEUX, A. VALLOTA. A.C. and GILLES, R. 1979. Blood proteins as related to osmoregulation in crustacea. Comp. Biochem. Physiol., 64A: 433-435.

PIERCE, S.K. and GREENBERG, M.J. 1970. Free amino acid efflux from muscle hearts: a demonstration of volume regulation. Am. Zool., 10: 518.

POTTS, W.T.W. and PARRY, G. 1963. Osmotic and Ionic Regulation in Animals. Macmillan, New York. pp. 89-163, 278-329.

PRESSLEY, T.A. GRAVES, J.S. and KRALL, A.R. 1981. Amiloride sensitive ammonium and sodium transport in the blue crab. Am. J. Physiol. 241 (Regulatory Integrative Comp. Physiol.)., 10: 370-378.

RAGHAVAIAH, K. RAMAMURTHI, R. CHANDRASEKHARAM, V. and SCHEER, B.T. 1980. Neuroendocrine control of nitrogen metabolism in the Indian field crab Oziotelphusa senex senex Fabricius-I. End products and Elimination. Comp. Biochem. Physiol., 67B: 437-455.

RAMAMURTHI, R. RAGHAVAIAH, K. CHANDRASEKHARAM, V. and SCHEER, B.T. 1982. Neuroendocrine control of nitrogen metabolism in the Indian field crab Oziotelphusa senex senex Fabricius-II. Enzyme Activities. Comp. Biochem. Physiol., 71B: 223-228.

RAMAN, K.V.A. SHAKUNTALA, K. and RAVICHANDRA REDDY, S. 1981. Influence of endogenous factors on the pattern of ammonia excretion in the prawn Macrobrachium lanchesteri (de Man). Indian J. Exp. Biol., 19(1): 42-45.

REGNAULT, M. 1984. Salinity induced changes in ammonia excretion rate of the shrimp Crangon crangon over a winter tidal cycle. Mar. Ecol. Prog. Ser., 20: 119-125.

*RICHARD, P. 1982. Role biologique et ecologique des acides amines libres chez quelques crustaces Decapodes marins. These Doct. Etat, Univ. Aix-Marseille.

*RICHARD, P. and CECCALDI, H.J. 1975. Variations des acides amines libres du muscle et de l'hepatopancreas de Penaeus kerathurus (Forskal) en Function de la dessalure. Proceedings of the 9th European Marine Biology Symposium, Aberdeen University Press. pp. 451-462.

SCHOFFENIELS, E. 1960. Role des acides amines dans la regulation de la pression osmotique du milieu interieur des insectes aquatiques. Arch. Int. Physiol. Biochim., 68: 507-508.

SCHOFFENIELS, E. 1967. Cellular Aspects of Membrane Permeability. Pergamon press, London. pp. 157-185.

SCHOFFENIELS, E. 1976. Biochemical approaches to osmoregulatory process in crustacea. Perspectives in Experimental Biology, Pergamon Press, Oxford, New York. Vol. I. pp. 107-123.

SCHOFFENIELS, E. and GILLES, R. 1970. Osmoregulation in aquatic arthropods. Chemical Zoology, Academic Press, New York, London. Vol. V, pp. 255-286.

SCHOFFENIELS, E. and GILLES, R. 1972. Ionoregulation and Osmoregulation in Mollusca. Chemical Zoology, Academic Press, New York, London. Vol. VII, pp. 393-420.

*SIEBERS, D. 1972. Mechanismen der intrazellularen isosmotischen regulation der aminosaurekonzentration bei dem flusskrebs Oreconectes linosus. Z. Vergl. Physiol., 76: 97-114.

SIEBERS, D. LUCU, C. SPERLING, K.R. and EBERLEIN, K. 1972. Kinetics of osmoregulation in the crab Carcinus maenas. Mar. Biol., 17: 291-303.

SNEDECOR, G.W. and COCHRAN, W.G. 1967. Statistical methods. Iowa State University Press, Amer. Iowa, pp. 539.

SOLARZANO, L. 1969. Determination of ammonia in natural waters by phenol hypochlorite method. Limnol. Oceanogr., 14(5): 799-801

SPAARGAREN, D.H. RICHARD, P. CECCALDI, H.J. 1982. Excretion of nitrogenous products by Penaeus japonicus Bate in relation to environmental osmotic conditions. Comp. Biochem. Physiol., 72A: 673-678.

*VAN DEN BOSCH, P. 1976. Neurosecretion at regulation hydroelectrolytique chez Artemia salina. Experientia, 32: 228-229.

VENKATACHARI, S.A.T. 1974. Effect of salinity adaptation on nitrogen metabolism in the freshwater fish Tilapia mossambica. I. Tissue protein and amino acid levels. Mar Biol., 24: 57-63.

VINCENT-MARIQUE, C. and GILLES, R. 1970. Changes in the amino acid concentration in blood and muscle of Eriocheir sinensis during hypo-osmotic stress. Life Sci., 9(2): 509-512.

WEISER, W. 1965. Electrophoretic studies on blood proteins in an ecological series of isopod and amphipod species. J. mar. biol. Ass. U.K., 45: 507-523.

YEMM, E.N. and COCKING, E.C. 1955. The determination of amino acids with Ninhydrin. Analyst, 80: 209-213.

* Not referred in original.