CHAPTER 1 IV

MODULATION OF OSMOREGULATION OF THE CRAB,

E. CUNCULARIS FOLLOWING EXPOSURE TO

CARBAMATE (SEVIMO)
1.0 INTRODUCTION

Crustaceans which inhabit marine and freshwater habitat regulate osmotically and ionically in a variety of ways (Lockwood, 1967). The marine crustaceans exhibit either hyposmotic or iso-osmotic regulation in relation to the external environment (Prosser, 1973). The freshwater crustaceans are known to regulate hyperosmotically to the external environment (Lockwood, 1967).

Features important to osmotic and ionic regulation in decapod crustaceans are the permeability of the integument to salts (Gross, 1957; Nagel, 1934) and water (Rudy, 1967), active ion uptake or loss through the gills (Shaw, 1961), and occasionally through the gut (Green et al., 1959) and ion transport through the excretory organs (Dähnel and Carefoot, 1965).
The pollutants are known to interfere with a variety of physiological processes. Recently, several studies have shown that pesticides (Kinter et al., 1972; Caldwell, 1974; Roesijadi et al., 1976), metals (Thurberg et al., 1973; Roesijadi et al., 1974) and petroleum hydrocarbons (Anderson et al., 1974; Cox, 1974) modulate osmo and ionic regulatory mechanisms of the exposed crustacean species. These pollutants, in addition to the environmental physical factors, exert the stress on the osmoregulatory mechanisms of the exposed species. The mechanism of action of these pollutants differs and the response of the exposed species is also different.

DDT and PCB's are known to inhibit the Na⁺, K⁺, Mg²⁺ dependant ATPases and membrane transport in several crustaceans and fishes (Cutkomp et al., 1971; Yap et al., 1971; Cahn et al., 1977 and Miller and Kinter, 1977). Metals are known to accumulate in the gills of freshwater as well as marine decapod crustaceans and Nimmo et al., 1977) while organophosphates are known to reduce the survival ability of marine decapods in the changing salinities (Vernberg et al., 1977). Comparably there exists no literature on the effects of carbamate on the osmotic
and ionic regulation of decapod crustaceans. The use of the carbamates has been increased in recent decade due to suspended or cancelled registration of organochlorine and organophosphate pesticides. Thus the effect of carbamates on the aquatic biota should be essentially considered. It has been demonstrated that carbamates poison the animal by the inhibition of acetylcholinesterase. The other impacts of carbamate on the physiological processes of aquatic biota are not known. Thus, the present probe was designed to study the impacts of carbamate on the ionic and osmotic adjustments of the freshwater crab, *Barytelphusa cunicularis*. Being representative of the tropical freshwater eco-bio-system, *B. cunicularis* has to face to the often fluctuating environmental parameters. The osmotic and ionic adjustments are presumed to have adaptive significance (Burton, 1973).

2.0 MATERIALS AND METHODS

The locally collected *B. cunicularis* were adapted to the laboratory conditions prior to the experimentation.

For the study of osmoregulatory capacity of *B. cunicularis* parameters were selected:
1. Mortality rate of *B. cunicularis* after exposure to carbamate and different salinities.

2. Changes in blood chloride level of *B. cunicularis* after exposure to carbamate and different salinities.

3. Changes in blood chloride level of *B. cunicularis* following the endocrine manipulations and carbamate exposure.

2.1 Mortality rate of *B. cunicularis* after exposure to carbamate and different salinities:

For this experiment two lethal (LC$_{50}$/24 hr 8.913 ppm and LC$_{50}$/48 hrs 5.623 ppm) and one sublethal concentrations (2 ppm) of carbamate were selected. Two hundred and forty adult, healthy, intermolt crabs, regardless of sex were used. Three batches, each comprising 80 crabs were exposed to 8.913, 5.623 and 2 ppm for 24 hrs, 48 hrs and 7 days respectively. The crabs, after exposure to different concentrations of carbamate were directly transferred to 1, 2, 3 and 4% salinity in a batch of 10 individuals respectively.

The carbamate obtained for this study was of market grade called as sevimol. The desired concentrations of
sevimol were obtained as described in chapter - I. The salinity grades were prepared by dissolving sodium chloride in dechlorinated tap water. The mortality was recorded for each groups after exposure to different salinities over 24 and 48 hrs.

2.2 Changes in blood chloride level of *E. cunicularis* after exposure to carbamate and different salinities:

Two hundred and forty adult, intermolt crabs, regardless of sex were used. Three batches, each comprising 80 crabs were exposed to 8.913, 5.623 and 2 ppm for 24 hrs, 48 hrs and 7 days period. The crabs, after exposure to above mentioned carbamate concentrations were transferred to 1, 2, 3 and 4% salinity in a batch of 10 individuals respectively.

The blood chloride level was estimated after 24 hrs and 48 hrs of exposure to the different salinity grades in the normal and carbamate exposed crabs. The blood chloride level was estimated with classical conway microdiffusion method with the help of conway unit as described by Conway (1950).
2.3 Changes in blood chloride level of *B. cunicularis* following the endocrine manipulation and carbamate exposure:

Adult, intermolt crabs, regardless of sex were selected for this experiment. Eighty eyestalk ablated crabs were exposed to 3 ppm carbamate concentration for 24 hrs. 4 batches, each comprising 10 eyestalk ablated crabs and which are exposed to above carbamate concentration were transferred to 1, 2, 3 and 4% salinity. Simultaneously the control groups were maintained using the eyestalk ablated crabs in normal tap water. The blood chloride level of both groups (control and experimental) was estimated after 24 and 48 hrs of exposure to different salinity grades.

3.0 RESULTS

3.1 Mortality rate of *B. cunicularis* after exposure to carbamate and different salinities:

No mortality occurred for 24 and 48 hrs when normal crabs were exposed to 1 and 2% salinity. 20% mortality was recorded for normal crabs after 24 hrs of exposure to 4% salinity while no mortality was recorded in 3% salinity for 24 hrs. 20 and 80% mortality was recorded for normal crab after 48 hrs of exposure to 3 and 4% salinity respectively (Table 1).
Table 1: Mortality rate in percent of carbamate (sevImol) pre-exposed freshwater crab, *Barytelphusa cunicularis* after exposure to different salinities at different time intervals.

<table>
<thead>
<tr>
<th>Carbamate pre-exposure concentrations and exposure periods</th>
<th>8.913 ppm (24 hrs)</th>
<th>5.613 ppm (48 hrs)</th>
<th>2 ppm (7 days)</th>
<th>Normal crabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity exposure period and salinity grades</td>
<td>24 hrs</td>
<td>48 hrs</td>
<td>24 hrs</td>
<td>48 hrs</td>
</tr>
<tr>
<td>1%</td>
<td>Nil</td>
<td>20</td>
<td>Nil</td>
<td>30</td>
</tr>
<tr>
<td>2%</td>
<td>Nil</td>
<td>20</td>
<td>Nil</td>
<td>50</td>
</tr>
<tr>
<td>3%</td>
<td>Nil</td>
<td>20</td>
<td>Nil</td>
<td>60</td>
</tr>
<tr>
<td>4%</td>
<td>40</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>
Crabs exposed to all concentrations of sevimol (8.913, 5.623 and 2.00 ppm) survived up to 3% salinity for 24 hrs exposure period. While 40, 30 and 60% mortality was recorded in 4% salinity respectively for same exposure period (Table 1).

The sevimol exposed crabs could not survive in different salinities after 48 hrs of exposure period. 20% mortality rate was recorded up to 3% salinity for crabs exposed to 8.913 ppm concentration of sevimol. 30, 50 and 60% mortality was recorded in 1, 2 and 3% salinity grades respectively for crabs exposed to 5.623 ppm concentration of sevimol for 48 hrs. Crabs exposed to 2 ppm concentration of sevimol showed 20, 20 and 40% mortality in 1, 2 and 3% salinity respectively after 48 hrs. 100% mortality was observed for all sevimol concentrations exposed crabs after exposure to 4% salinity for 48 hrs (Table 1).

3.2 Blood chloride level of *B. cunicularis*:

3.2.1 Effect of salinities on blood chloride level of normal *B. cunicularis*:

The blood chloride level of normal crab increased with increasing salinities after 24 hrs and 48 hrs. The blood chloride level was increased 27.85 and 25.42% in 1% salinity after 24 and 48 hrs of exposure. In 2%
salinity the blood chloride level increased up to 187.50 and 209.17% after 24 and 48 hrs of exposure period respectively. In 3% salinity the blood chloride level decreased when compared to 2% salinity at both exposure period. In 4% salinity the blood chloride level significantly increased up to 174.46 and 219.60% for 24 and 48 hrs of exposure period respectively (Table 2, Fig. 1,2).

3.2.2 Effect of carbamate exposure on blood chloride level of E. cunicularia in relation to salinity changes:

The blood chloride level of crabs exposed to sevimore concentration was decreased when compared to normal crabs. The rate of chloride uptake was reduced more in the crabs exposed to 8.913 ppm of sevimore for 24 hrs compared to the crabs exposed to 5.623 ppm sevimore for 48 hrs. The reduction in chloride uptake in crabs exposed to 5.623 ppm concentration of sevimore for 48 hrs was minimum. In the prolonged carbamate exposure (2 ppm) of the crabs the chloride uptake was decreased from 29.45% to 56.40% in different salinities (1, 2, 3 and 4% salinity) after 24 hrs. The crabs
Table - 2: Percent increase in the blood chloride level of carbamate (sevimol) pre-exposed freshwater crab, *Barytelphusa cunicularis* after the salinity exposure compared to normal base control crabs

<table>
<thead>
<tr>
<th>Carbamate pre-exposure concentrations and exposure periods</th>
<th>8.913 ppm</th>
<th>5.623 ppm</th>
<th>2 ppm</th>
<th>Normal crab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs</td>
<td>48 hrs</td>
<td>24 hrs</td>
<td>48 hrs</td>
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<tr>
<td>Salinity exposure period and salinity grades</td>
<td></td>
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<tr>
<td>1%</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>24 hrs</td>
<td>18.57</td>
<td>27.60</td>
<td>18.57</td>
<td>49.78</td>
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<tr>
<td>24 hrs</td>
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<td>3%</td>
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</tr>
<tr>
<td>24 hrs</td>
<td>38.00</td>
<td>48.80</td>
<td>101.10</td>
<td>289.32</td>
</tr>
<tr>
<td>48 hrs</td>
<td></td>
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<tr>
<td>4%</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hrs</td>
<td>121.39</td>
<td>-</td>
<td>128.57</td>
<td>-</td>
</tr>
<tr>
<td>48 hrs</td>
<td></td>
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</tbody>
</table>


exposed to 2 ppm concentration of sevimol showed increased in blood chloride uptake after 48 hrs of exposure to different grades of salinity (Table 3, Fig. 1,2).

The rate of chloride uptake, however, in relation to salinity in sevimol exposed crabs was considerably decreased compared to normal crabs. The crabs exposed to 2 ppm sevimol concentration for 7 days showed decrease of blood chloride level in different salinities after 24 hrs while the blood chloride level of these crabs increased in different salinities after 48 hrs. Similarly, the crabs exposed to lethal concentrations (8.913 and 5.623 ppm) of sevimol showed increase of blood chloride level after 48 hrs of salinity exposure when compared to the blood chloride level of these crabs after 24 hrs of salinity exposure (Table 2, Fig. 1,2).

3.2.3 Changes in blood chloride level of *B. cunicularis* following the endocrine manipulations and carbamate exposure:

3.2.3.1 Effect of eyestalk ablation on blood chloride regulation in relation to salinity changes:

The blood chloride level of eyestalk ablated crabs increased up to 2% salinity but later it
Table - 3: Percent reduction in the blood chloride level of carbamate (sevimol) pre-exposed freshwater crab, *Barytelphusa cunicularis* after exposure to different salinities compared to the normal crabs exposed to different salinities.

<table>
<thead>
<tr>
<th>Salinity exposure period and salinity grades</th>
<th>6.913 ppm 24 hrs</th>
<th>5.623 ppm 48 hrs</th>
<th>2 ppm 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs</td>
<td>48 hrs</td>
<td>24 hrs</td>
</tr>
<tr>
<td>1%</td>
<td>07.26</td>
<td>01.71</td>
<td>15.36</td>
</tr>
<tr>
<td>2%</td>
<td>45.12</td>
<td>36.37</td>
<td>47.60</td>
</tr>
<tr>
<td>3%</td>
<td>42.67</td>
<td>34.76</td>
<td>16.45</td>
</tr>
<tr>
<td>4%</td>
<td>19.33</td>
<td>-</td>
<td>16.72</td>
</tr>
</tbody>
</table>
Figure 1: Blood chloride level of carbamate (sevimol) pre-exposed freshwater crab, *Barytelphusa cunicularis* after exposure to different salinities for 24 hrs.
Figure 2: Blood chloride level of carbamate (sevmol) pre-exposed freshwater crab, Barytelphusa cunicularia after exposure to different salinities for 48 hrs.
decreased. The blood chloride level was increased for 1% salinity after 48 hrs and for 2% salinity after 24 hrs. However, the blood chloride level of eyestalk ablated crabs decreased after 24 hrs in 4% salinity and after 48 hrs in 3% salinity (Fig. 3,4).

3.2.3.2: Effect of carbamate exposure on blood chloride level of eyestalk ablated crabs:

The sevimol exposure increased the blood chloride level of eyestalk ablated crabs with the salinities. The blood chloride level was increased more in 3% salinity after 48 hrs. Carbamate exposed eyestalk ablated crabs showed linear and consistant increase in blood chloride level after exposure to different salinity grades for 24 hrs (Fig. 3,4).

4.0 DISCUSSION

Mechanism of ionic and osmotic regulation has been well understood in case of the decapod crustaceans (Lockwood, 1967). Decapod crustaceans inhabit different environments including marine to the freshwater habitat. Freshwater decapods are known to regulate their osmo-ionic concentration as do the marine ones (Warren, 1971).
Figure 3: Blood chloride level of endocrine manipulated and carbamate (sevimol) pre-exposed freshwater crab, *Barytelphusa cunicularis* after exposure to different salinities for 24 hrs.
BLOOD C (mg/100 ml)

CONCENTRATION OF NaCl (%)
Figure 4: Blood chloride level of endocrine manipulated and carbamate (sevimol) pre-exposed freshwater crab, *Barytelphusa cumircularis* after exposure to different salinities for 48 hrs.
In the freshwater aquatic ecosystem organisms are subjected to numerous varying environmental factors. The impact of a particular pollutant may be different during the periods of extreme fluctuations in the normal environmental factors. In general, multiple factor interaction has been shown to be reducing the survival of the exposed species (Vernberg and Vernberg, 1972; Vernberg et al., 1974). Many studies with the marine decapods have demonstrated that the safe concentration of a particular organochlorine or organophosphate became lethal to the species at the periods of reduced salinities. Nimmo and Bahner (1974) have demonstrated in the adult shrimps that PCB's were more toxic in the reduced salinities. Vernberg et al. (1977) have shown that fiddler crab, Uca pugilator were more sensitive towards Arochlor compound at the reduced salinities and higher temperatures. Likewise, Caldwell (1974) has observed for the crabs, Cancer magister and Hemigrapsus nudus that they were more sensitive to the methoxychlor at reduced salinities. The freshwater crab, B. cunicularis showed more sensitivity towards carbamate in the increasing salinities.
However, the sensitivity was time dependant than the concentration of carbamate. The above contention is evident from the fact that crabs exposed to LC$_{50}$/24 hr concentration of carbamate for 24 hrs could tolerate salinity upto 3% compared to the crabs B. cunicularis preexposed to the LC$_{50}$/48 hrs concentration for 48 hrs and sublethal concentration of carbamate for seven days. The increased sensitivity towards the carbamate at higher salinities may be due to the ionic imbalance of the interior milieu. This contention is supported by the fact that the carbamate preexposed crabs could not elevate their blood chloride level as do the normal crabs exposed to higher salinities.

Inorganic ions are the major constituents that contribute to the ionic molarity of the internal body fluids of the aquatic organisms (Kinne, 1971). Chloride ions which are the major anions are often used as the indicator of total ionic concentration of body fluids (Prosser, 1973). A variety of mechanisms are adapted by the freshwater crabs to maintain osmotic and ionic balance of their interior milieu (Lockwood, 1967).
Uptake of different ions from the surrounding environment is one of the key factors as the freshwater crabs are known to hyper-regulate their osmionic concentrations in relation to the external environment (Robertson, 1960). Further, the ionic uptake is associated with the conservation of the ions through the excretion. Similarly, the ionic regulation is assisted with changes in the permeability to the water uptake and loss which is reflected in the body volume regulation of the crabs (Subrahmanyam, 1979). The tissue sequestration of ions from the hemolymph is also one of the major mechanisms to have a buffering between the hemolymph and the tissues of the animals.

The active site of ionic gain and loss in the freshwater crab, *E. cunicularis* is the gill (Diwan, 1971). The ionic conservation or retention is achieved through the antennary gland (Warren, 1971). The major ions that are taken up from the external medium via the gill system are Na\(^+\) and Cl\(^-\). Particularly the chloride uptake is coupled with the Na\(^+\) uptake and NH\(_4\)\(^+\) loss from the body (Subrahmanyam, 1979). It is a known fact that Na\(^+\) uptake is Na\(^+\) K\(^+\) Mg\(^{++}\) ATPase dependant
(Whittam and Wheeler, 1970) and chloride uptake on carbonic anhydrase enzyme in this crab (Krishnamoorthy and Virabhadrachari, 1969).

Pollutants are known to interfere with the ionic regulation of the exposed decapods either the marine or the freshwater one. Nimmo and Bahner (1974) have observed that when brown shrimp, *Penaeus aztecus* was exposed to PCB at low salinities it depressed hemolymph levels of $\text{Na}^+$, $\text{Cl}^-$, $\text{Ca}^{++}$ and total ions, although osmotic concentration did not differ from the control group. The juvenile shrimp, *Palaemonetes pugio* exposed to PCB, showed the reduction of hemolymph chloride level (Roesijadi et al., 1976). Eisler and Edmunds (1966) reported alternation of blood and tissue levels of ions in puffer, *Sphaeroides maculatus* exposed to organochlorine pesticide, endrin. The toxicity of organochlorine pesticides and related compounds such as PCBs, to aquatic animals has been attributed in part to osmoregulatory dysfunction (Grant and Merle, 1970; Janicki and Kinter, 1971 and Kinter, et al., 1972).

Anderson et al. (1973) have measured the chloride concentration of blood of grass shrimp, *Palaemonetes*
pugio, exposed to 10 ppb of Arochlor 1254. They reported that the chloride level of the blood in exposed shrimp was slightly less than that of the control groups after 24 hrs exposure period. The brown shrimp, Penaeus aztecus exposed to 20% water soluble factor (WSF) and then transferred to 30% salinity showed that after 24 hrs and 48 hrs exposure animals had significantly lower blood chloride level than that of the control animals (Anderson et al., 1973).

However, some workers have observed that chloride concentration of blood of animals does not change when animals are exposed to pollutional stress. In adult grass shrimp, Palaemonetes pugio there was a little or no effect of PCBs on hemolymph chloride and osmotic concentrations, chloride space and chloride exchange kinetics, although exposure concentrations approached the 96 hrs LD50 values (Roesijadi et al., 1976). Roesijadi et al. (1974) have observed that in Petrolisthes ornatus mercury exposure did not affect the chloride regulatory ability of crabs. Exposure of the crabs, Carcinus maenas and Cancer irroratus to copper and cadmium altered the osmoregulatory ability in different ways. The osmoconcentration
of both the crabs, *C. maenas* and *C. irroratus* decreased when they were exposed to copper and the blood was essentially iso-osmotic to the medium. With cadmium exposure, however, osmo-concentration of *C. maenas* was elevated while no effect was observed with *C. irroratus* (Thurberg *et al.*, 1973).

Comparatively, it appears from this study that carbamate (sevimol) though it induces histopathological changes in the gill tissue (See chapter - V) does not totally interfere with the ionic regulation of the crab, *B. cunicularis*. This is more pertinent, since the sevimol pre-exposed crab, *B. cunicularis* when exposed to different salinities showed a fair degree of ionic regulation. However, the elevation of the blood chloride levels was not the degree of normal crabs after exposure to the increasing salinities.

However, above discussion does not explains that why there was a decrease in the blood chloride levels of pesticide exposed crabs after exposure to increasing salinities. Surprisingly, even after exposure to LC$_{50}$ concentration of 24 hrs and 48 hrs of carbamate (Sevimol)
*B. cunicularis* could regulate their chloride level, however, the elevation of chloride level was less, comparing to the normal crabs exposed to different salinities. The decrease in blood chloride level in the blood of sevimol exposed crab may be due to the following reasons:

Sevimol must be slowing down the uptake of chloride ions from the external medium either due to gill damage and/or due to inhibition of the enzyme systems. The damage in gill structure of crab, *B. cunicularis* was observed when crabs were exposed to different concentrations of sevimol for the different periods (See chapter - V). Devarooroo (1981) has observed changes in gill architecture of crab, *B. cunicularis* exposed to different salinities.

It is believed that the ATPase is involved in the transport of ions. The change in ATPase enzyme activity due to pesticidal stress may cause the disturbances in the osmoregulatory ability of organisms. The inhibition of ATPase enzyme has been observed due to exposure to pollutants (Yap et al., 1971, Davis et al., 1972; Burg and Green 1973 and Caldwell, 1974).
The inhibition of ATPase enzyme would inhibit Na\(^+\) uptake which in turn would decrease the Cl\(^-\) uptake as Na\(^+\) and Cl\(^-\) uptake in crab, *B. cunicularis* is known to be coupled (Subrahmanyan, 1979).

The next possibility is that sevimol may be some how enhancing the tissue imbibing of the Cl\(^-\) ions which is essential for the ionic homeostasis between blood and tissues. However, it is difficult to understand that through the what mechanism sevimol is increasing tissue uptake of Cl\(^-\) ions. This could be one of the detoxifying mechanism as carbamate also damage the tissues rich with lipid moities such as hepatopancreas and gonads in *B. cunicularis* (See Chapter - V, VI). Thus, the distribution of carbamate in different organs may invite the disturbances in the ionic homeostasis which would naturally lead to the rebalancing of the ions.

The fourth possibility is that sevimol may be reducing the water loss after exposure to higher salinities in crab, *B. cunicularis*. Subrahmanyan (1979) has observed that the crab, *Oziotelphusa senex senex* lost weight considerably when they were adapted to the
higher salinities. Thus, interfering with water movement from the body fluids to outside could also result in dilution of interior milieu which would naturally reduce the ionic concentration of the blood of crab, *B. cunicularis*. This is consistent with the observations of Payne et al. (1978) who have observed decreased chloride level of plasma in the oil treated fish, *Tautogolabrus adspersus* due to hydration.

The last and most important impact of the sevimol may be on the resorption of ions or the retention of the ions in body fluids. It is known that the ions are absorbed from the excretory system of crustaceans to regulate ionic concentration (Warren, 1971). The blood chloride level of the crab, *B. cunicularis* would decrease if the antennary glands are inhibited to resorb the Cl⁻ ions from the urine.

In any eventuality the interference of carbamate (Sevimol) with the ionic regulation would make survival of *B. cunicularis* difficult in the osmolabile environment.

Many of the pesticides are known to interfere with the endocrine functioning in various animals
groups (Balazas, 1975; Samaranayaka, 1976). Maddrell and Reynolds (1972) have demonstrated that insecticides induce the release of hormones in the insects. Among decapod crustaceans Nagabhushanam et al. (1979) have demonstrated effect of Arochlor 1242 on the eyestalk neurosecretory system of the crab, Uca pugilator. Fingerman et al. (1981) have demonstrated that DDT exposure brings out the release of hyperglycemic hormone from the eyestalks in the freshwater crab, Barytelphusa querini. Since, blood chloride level in B. cunicularis are hormonally regulated, it was thought proper to study if carbamate is modulating blood chloride levels via the neuroendocrine system. Additionally carbamate acts on the acetyl cholinesterase that controls the amount of acetylcholine in the central nervous system. Fingerman et al. (1974) have demonstrated that several neurotransmitters are involved in the release of different neurohormones in the decapod crustaceans. Inhibition of the enzyme by carbamate or their metabolites causes accumulation of acetylcholine and disruption of normal neurotransmission (Koelle, 1963; O'Brien, 1967 and Karczmar, 1970). Thus, it will not be improper at this stage to expect the action of carbamate on the neuroendocrine factor that modulates the blood chloride regulation in B. cunicularis.
The eyestalk ablated crabs after exposure to salinity showed increase in the blood chloride level upto 2% salinity which was decreased in the 3% and 4% salinities. This indicates that the factor that probably induces chloride uptake does not exist in the extra eyestalk nerve centres. Similar are the result for the eyestalk ablated carbamate pre-exposed crabs. Possibly, carbamate is not acting via the neuroendocrine centers but it has direct impact on the different chloride regulatory mechanisms of *E. cunicularia*.